行政院國家科學委員會專題研究計畫成果報告

無機砷暴露，抗氧化維生素、脂質及宿主易感受性對動脈粥狀硬化危險性交互作用之分子流行病學研究

Arsenic exposure, null genotypes of glutathione S-transferase M1, T1 and P1, and risk of carotid atherosclerosis among residents in Lanyang Basin of Taiwan

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中文摘要

本研究的主要目的在探討經由飲用含砷井水與頸動脈粥狀硬化之相關性。此外，研究中也評估人類易感受基因 GSTM1、T1、P1 及 P53 與砷暴露參予所造成的影響。研究對象共 605 人，其中包含男性 289 人、女性 326 人。在調整年齡、性別後發現飲水砷濃度≧100 μg/L 及 50.1-99.9 μg/L 這兩組都有顯著的頸動脈粥狀硬化疾病罹病危險性。此外，調整年齡、性別後研究對象之 GSTP1 為 W/M 及 M/M 基因型者其罹患頸動脈粥狀硬化的 OR 值為 2；P53 為 W/M 及 M/M 基因型者其調整後的 OR 值為 1.9，且均達統計上的顯著差異。GSTP1 之基因型為 W/M 及 M/M 且含砷井水濃度為≧100 μg/L 及 50.1-99.9 μg/L 之研究對象的頸動脈粥狀硬化罹病危險性分別為 4.1 及 3.2 且具統計上的顯著差異。P53 基因型為 W/M、M/M 且飲用水含砷井水濃度為≧50 μg/L 以上者罹患頸動脈粥狀硬化的罹病危險性為對照組的 3 倍，由此可見環境中砷暴露與人類易感受基因與頸動脈粥狀硬化之間確有明顯的交互作用存在。

關鍵詞：砷、麩氨基硫化合物、粥狀動脈硬化

Abstract

The specific aim of this study is to elucidate the association between arsenic exposure through drink well water and carotid atherosclerosis. In addition, the joint effect between human susceptible genes including GSTM1, T1, P1, and P53 and arsenic exposure is also evaluated. A total of 605 study subjects included 289 men and 326 women were recruited in this study. A significant age-sex-adjusted odds ratios of risk of carotid atherosclerosis were observed both in exposure groups with arsenic concentration in well water greater than 100 and ranged from 50.1 to 99.9 ug/L. In addition, a significant higher age-sex-adjusted odds ratio of 2.0 for the development of carotid atherosclerosis was observed among study subjects with genotypes of W/M and M/M of GSTP1 and 1.9 for study subjects with genotypes of W/M and M/M of P53. Significant age-adjusted odds ratios of 4.1 and 3.2 for the development of carotid atherosclerosis were also found for arsenic concentrations in well water of 50.1-99.9 and >100 ug/L , respectively, among study subjects with W/M and M/M genotypes of GSTP1. A significant three times risk for development carotid

atherosclerosis was also observed among study subjects who drank well water with arsenic content greater than 50 ug/L and with W/M or M/M genotype of P53 compared with referent group.

key words: Arsenic, glutathione , S-transferase , atherosclerosis

1. Introduction

Arsenic is widely distributed in nature and mainly transported in the environment by water. The main source of arsenic exposure for the general population is through ingestion of water containing high level of inorganic arsenic and of seafood contains high level of organic arsenic. However, organic arsenic is less toxic than inorganic arsenic. (World Health Organization, 1981; US Public Health Service, 1989). The tentative MCL for arsenic in drinking water set by the US Environmental Protection Agency is 0.05 mg/L. Inorganic arsenic has also been well documented as one of the major risk factors for BFD, a unique peripheral vascular disease identified in the endemic area of arseniasis located on the southwest coast of Taiwan where residents had used high-arsenic artesian well water for more than 50 years (Tseng, 1968; Chen et al., 1988a). The pathological types of BFD include arteriosclerosis obliterans (70％) and thromboangiitis obliterans (30％) which developed from severe underlying systemic arteriosclerosis (Tseng et al., 1961; Yeh and How, 1963). The atherogenic effects of arsenic has also been well documented. Ingested inorganic arsenic through drinking water has been related to the development of peripheral vascular disease in Poland, Chile, Mexico, Argentina, Japan and Xinjiang, China (World Health Organization, 1981; Chen and Lin, 1994; Engel et al., 1994; Cebrian et al., 1994; Borgono et al., 1977; Hotta et al., 1989; Wang and Huang, 1994) and among Moselle vintners exposed to inorganic arsenic through contaminated wine (Grobe, 1976). Current studies have also reported long-term exposure to arsenic in drinking water was significantly associated with risk for development ischemic heart disease, cerebrovascular disease and peripheral vascular disease in Taiwan, showing a dose-response relationship(Wu et al., 1989; Chen and Wang, 1990; Tseng et al., 1996; Chen, et al., 1996; Chiou 1997a). In addition, the association between ingestion arsenic through drinking water and hypertension and diabetes mellitus has also been reported in Taiwan and Bangladesh (Lai et al., 1992; Chen et al., 1995; Rahman et al., 1998, 1999). Glutathione (GSH) S-transferases (GST) are a large family of phase II detoxification enzymes that catalyze the conjugation of reduced GSH to a wide spectrum of hydrophobic and electrophilic compounds. There are four subclasses of GST in mammalian cells, namely alpha, mu, pi and theta (Board et al., 1990). Many studies have suggested that GSH might be involved in the initial reduction of arsenate to arsenite and the subsequent oxidative methylation. GSH is necessary enzyme for arsenic methylation, perhaps through the formation of arsenite that is the preferred arsenic form for methylation, or through conjugation with arsenic (Buchet and Lauwerys, 1985, 1987; Thompson, 1993). Humans with null genotypes of GSTM1, T1, and P1 have been considered to be a high risk group of cancers due to their GSH deficiency. The specific aim of this study is to evaluate the synergistic effects of arsenic exposure through drinking water and genetic polymorphisms of GST M1, T1, and P1 on the risk of carotid atherosclerosis.

2. Results and Discussion

A total of 605 study subjects included 289 men and 316 women were recruited in

this study. As shown in Table 1, compared with youngest age group, the significant odds ratios of 5.9 and 2.3 were observed for the oldest and middle age groups, respectively. Significant risk for occurrence of carotid atherosclerosis was also observed among hypertension patients with age-sex-adjusted odds ratio of 2.4. In addition, men, alcohol drinkers, and patients with diabetes mellitus also had higher but not significant risk for the development of carotid atherosclerosis. Table 2 showed that a significant age-sex-adjusted odds ratios of risk of carotid atherosclerosis were observed both in exposure groups with arsenic concentration in well water greater than 100 and ranged from 50.1 to 99.9 ug/L. Moreover, study subjects with cumulative arsenic exposure greater than 1.0 mg/L-year also had significant age-sex-adjusted risk of developing carotid atherosclerosis. As illustrated in Table 3, a significant higher age-sex-adjusted odds ratio of 2.0 for the development of carotid atherosclerosis was observed among study subjects with genotypes of W/M and M/M of GSTP1 and 1.9 for study subjects with genotypes of W/M and M/M of P53. Significant age-adjusted odds ratios of 4.1 and 3.2 for the development of carotid atherosclerosis were also found for arsenic concentrations in well water of 50.1-99.9 and >100 ug/L , respectively, among study subjects with W/M and M/M genotypes of GSTP1.. However, study subjects with null genotype of GSTM1 and T1 did not have higher risk of carotid atherosclerosis. Table 4 illustrated that study subjects who drank well water contained arsenic level greater than 50 ug/L and with W/M or M/M genotype of GSTP1 had 2.7 folds risk of developing carotid atherosclerosis compared with those who drank well water contained arsenic concentration equal and less than 50 ug/L as referent group. Significant age-adjusted odds ratios of 4.1 and 3.2 for the development of carotid atherosclerosis were also found for arsenic concentrations in well water of 50.1-99.9 and >100 ug/L , respectively, among study subjects with W/W genotype of GSTP1. A significant three times risk for development carotid atherosclerosis was also observed among study subjects who drank well water with arsenic content greater than 50 ug/L and with W/M or M/M genotype of P53 compared with referent group.

The atherogenic effects of ingestion inorganic arsenic through drinking water have been well documented. A serious studies carried out in Taiwan have reported the significant association between long-term exposure to arsenic in drinking water and the risk for the development of atherosclerotic vascular diseases such as ischemic heart disease, cerebrovascular disease, and peripheral vascular disease, showing a dose-response relationship(Wu et al., Chen, 1990; Tseng et al., 1996; Chen, et al., 1996; Chiou 1997). GST are a large family of phase II detoxification enzymes that catalyze the conjugation of reduced GSH to a wide spectrum of hydrophobic and electrophilic compounds. They play important roles in protection mechanisms against chemical carcinogenesis. Many studies have suggested that GSH might be involved in the initial reduction of arsenate to arsenite and the subsequent oxidative methylation. In this study, a significant high risk of developing carotid atherosclerosis was observed among study subjects with W/M or M/M genotypes of GSTP1 and P53. Humans with null genotypes of GST P1 and P53 have been considered to be a high risk group of carotid atherosclerosis due to their GSH deficiency.

Self-evaluation of this study

A significant high risk of developing carotid atherosclerosis was observed among study subjects with W/M or M/M genotypes of GSTP1 and P53. Humans with null genotypes of GST P1 and P53 have been considered to be a higher risk group of carotid atherosclerosis due to their GSH

deficiency. These findings derived from the study have given important evidences of atherogeneicity of arsenic. In addition, the joint effect between arsenic exposure and human susceptible genes on carotid atherosclerosis has also been found in this study. The hypothesis of this study has been well tested and proved.

3. References

1.Board, P., Coggan, M., Johnson, P., Ross, V., Suzuki, T. and Webb, G. (1990) Genetic heterogeneity of the human glutathione transferase: a complex of gene families. Pharmac. Ther., 48:357-369.

2.Borgono, J.M., Vicent, P.,Venturino, H. and Infante, A. (1977) Arsenic in the drinking water of the city of Antofagasta: Epidemiological and clinical study before and after the installation of the treatment plant. Environ Health Perspect., 19:103-105.

3.Buchet, J.P. and Lauwerys, R. (1985) Study of inorganic arsenic methylation by rat liver in vitro: relevance for the interpretation of observations in man, Arch. Toxicol., 57:125-129.

4.Buchet, J.P. and Lauwerys, R. (1987) Study of factors influencing the in vivo methylation of inorganic arsenic in rats, Toxico. Appl. Pharmacol., 91:65-74.

5.Cebrian, M. E., Albores, A., Garcia-Vargas, G.., Del Razo, L. M. and Ostrosky-Wegman, P. (1994) chronic arsenic poisoning in humans: The case of Mexico. In: Advance in Environmental Science and Technology, Vol 27, Arsenic in the Environmental, Part II: Human Health and Ecosystem Effects, Nriagu, J. O. (ed.), pp. 93-107, John Wiley & Sons Inc, New York.

6.Chen, C. J. and Lin, L. J. (1994) Hunan carcinogenicity and atherogenicity induced by chronic exposure to inorganic arsenic. In: Advance in Environmental Science and Technology, Vol 27, Arsenic in the Environmental, Part II: Human Health and Ecosystem Effects, Nriagu, J. O. (ed.), pp. 109-131, John Wiley & Sons Inc, New York.

7.Chen, C. J. and Wang C. J. (1990) Ecological correlation between arsenic level in well water and age-adjusted mortality from malignant neoplasms. Cancer Res., 50:5470-5474.

8.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.

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

10.Chen, C.J., Wu, M.M., Lee, S.S., Wang, J.D., Cheng, S.H. and Wu, H.Y. (1988a) Atherogenicity and carcinogenicity of high-arsenic artesian well water: multiple risk factors and related malignant neoplasms of blackfoot disease, Artheriosclerosis, 8: 452-60.

11.Chiou, H. Y., Huang, W. I., Su, C. L., Chang, S. F., Hsu, Y. H. and Chen, C. J. (1997) Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke, 28: 717-723.

12.Engel, R. E., Hopenhayn-Rich C., Receveur O. and Smith, A. H. (1994) vascular effects of chronic arsenic exposure: A review. Epidemiol. Rev., 16:184-209.

13.Grobe, J.W. (1976) Peripheral circulatory disorders and acrocyanosis with arsenic [in German]. Berufs Dermatosen, 24:78-84.

14.Hotta, N. (1989) clinical aspects of chronic poisoning due to environmental and occupational pollution in and around small refining spot. Jpn. J. Constit. Med., 53:49-70.

15.Lai, M.S., Hsueh, Y.M., Chen C. J., Shyu, M. P., Chen, S. Y., Kuo, T. L., Wu, M. M. and Tai, T. Y. (1992) ingested inorganic

arsenic and prevalence of diabetes mellitus. Am. J. Epidemiol., 139:484-492.
16.Rahman, M., Tondel, M., Ahmad, S. K. and Axelson, O. (1998) diabetes mellitus associated with arsenic exposure in Bangladesh. Am. J. Epidemiol., 148:198-203.
17.Rahman, M., Tondel, M., Ahmad, S. K. Chowdhury, I. A., Faruquee, M. H. and Axelson, O. (1999) Hypertension and arsenic exposure in Bangladesh. Hypertension, 33:74-78.
18.Thompson, D.J. (1993) A chemical hypothesis for arsenic methylation in mammals, Chem.-Biol. Interact., 88: 89-114.
19.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, 120:125-133.
20.Tseng, W. P., Chen, W. Y., Sung, J. L. and Chen, J. S. (1961) A clinical study of blackfoot disease in Taiwan: An endemic peripheral vascular disease. Memoirs College Med. Natl. Taiwan Univ., 7:1-17.
21.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.
22.U.S. Public Health Service (1989) Toxicological profile for arsenic, U.S. Public Health Service, Washington DC, pp. 9-67.
23.Wang, L. F. and Huang, J. Z. (1994) Chronic arsenicism from drinking water in some areas of Xinjiang, China. In: Advance in Environmental Science and Technology, Vol 27, Arsenic in the Environmental, Part II: Human Health and Ecosystem Effects, Nriagu, J. O. (ed.), pp. 156-172, John Wiley & Sons Inc, New York.
24.World Health Organization (1981) Environmental Health Criteria 18: Arsenic, WHO, Geneva, pp. 18-22.
25.Wu, M. M., Kuo, T. L., Hwang, Y. H. and Chen, C. J. (1989) Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. Am. J. Epidemiol., 103:1123-1131.
26.Yeh, S. and How, S. W. (1963) A pathological study on the blackfoot disease in Taiwan. Reports Inst. Pathol. Natl. Taiwan Univ., 14:25-73.

Table 1. Sociodemographic characteristics, life style and disease status of carotid atherosclerosis patients and controls

Variable	Case	Control	OR (95%CI)[a]	OR (95%CI)[b]
	No(%)	No(%)		
Age				
<55	25(8.9)	85(26.2)	1.0	
55-64.9	105(37.5)	153(47.1)	2.3(1.4-3.9) **	
65+	150(53.6)	87(26.7)	5.9(3.5-9.8) ***	
Sex				
Woman	127(45.4)	189(58.2)	1.0	1.0
Man	153(54.6)	136(41.8)	1.7(1.2-2.3) ***	1.4(1.0-1.9) §
BMI (kg/m^2)				
<20	47(17.4)	51(16.3)	1.0	1.0
20-25.9	127(47.0)	134(42.8)	1.0(0.6-1.6)	1.3(0.8-2.2)
26+	96(35.6)	127(40.8)	0.8(0.5-1.3)	1.3(0.8-2.4)
Cigaratte smoking				
No	157(56.1)	223（68.6）	1.0	1.0
Yes	123(43.9)	102（31.4）	1.7（1.2-2.4） **	1.1（0.6-1.9）
Alcohol drinking				
No	215(76.8)	276（85.2）	1.0	1.0
Yes	65(23.2)	48（14.8）	1.7（1.2-2.6） **	1.3（0.8-2.1）
Hypertension				
No	149(76.4)	243（87.7）	1.0	1.0
Yes	46(23.6)	34（12.3）	2.2（1.4-3.6） **	2.4（1.4-4.0） **
Diabetes Mellitus				
No	179(91.8)	255（92.1）	1.0	1.0
Yes	16(8.2)	22（7.9）	1.0（0.5-2.0）	1.1（0.5-2.2）

a: crude odds ratio b:age-sex-adjusted odds ratio §: $0.05<p<0.1$ *: $0.01<p<0.05$ **: $0.001<p<0.01$ *** :$p<0.001$

Table 2 Age-sex-adjusted odds ratio (OR) and 95% confidence interval (CI) of carotid atherosclerosis by various arsenic exposure indices

Variable	Case	Control	OR (95%CI)[a]	OR (95%CI)[b]
	No(%)	No(%)		
Arsenic concentration in well water（μg/L）				
≤50	50（17.9）	115（35.4）	1.0	1.00
50.1-99.9	92（32.9）	84（25.8）	2.5（1.6-3.9）***	2.13（1.04-4.32）***
100+	138（49.2）	126（38.8）	2.5（1.02-4.02）***	2.13（1.04-4.32）***
Cumulative arsenic exposure (mg/L-year）				
<1.0	58（20.7）	101（31.1）	1.0	1.0
1.0-6.9	150（53.6）	156（48.0）	1.7（1.1-2.5）**	1.8（1.2-2.8）**
7.0+	72（25.7）	68（20.9）	1.8（1.2-2.9）**	1.9（1.1-3.0）**
Duration of drinking well water（year）				
<30	52（18.6）	57（71.03）	1.0	1.0
30-49	127（45.4）	167（47.1）	0.8（0.5-1.3）	0.9（0.5-1.4）
50+	101（36.0）	101（33.1）	1.1（0.7-1.7）	0.8（0.5-1.4）

a: crude odds ratio b:age-sex-adjusted odds ratio

§: 0.05<p<0.1 ＊:0.01<p<0.05 **: 0.001<p<0.01 *** :p<0.001

Table 3 Age-sex-adjusted odds ratio (OR) and 95% confidence interval (CI) of carotid atherosclerosis by genetic polymorphisms of GSTM1, T1 , P1 and P53

Variable	Case	Control	OR (95%CI)[a]	OR (95%CI)[b]
	No(%)	No(%)		
GSTM1				
Non-null	136（48.7）	139（42.8）	1.0	1.0
Null	143（51.3）	186（57.2）	0.8（0.6-1.1）	0.9（0.5-1.0）
GSTT1				
Non-null	134（48.0）	133（40.9）	1.0	1.0
Null	145（52.0）	192（59.1）	0.8（0.5-1.0）§	0.7（0.5-1.0）§
GSTP1				
W/W	178（63.8）	248（76.4）	1.0	1.0
W/M	95（34.1）	71（21.8）	1.8（1.3-2.6）***	2.0（1.4-3.0）***
M/M	6（2.1）	6（1.8）		
P53				
W/W	53（19）	91（28）	1.0	1.0
W/M	175（62.7）	175（53.8）	1.7（1.1-2.4）*	1.9（1.3-2.9）**
M/M	51（18.3）	59（18.2）		

a: crude odds ratio b:age-sex-adjusted odds ratio

§: 0.05<p<0.1 * :0.01<p<0.05 **: 0.001<p<0.01 *** :p<0.001

Table 4 Age-sex-adjusted odds ratio (OR) and 95% confidence interval (CI) of carotid atherosclerosis by genetic polymorphisms of GSTP1,and various arsenic exposure indices

Variable	GSTP1					
	W/W			W/M or M/M		
	case	control	OR[a](95%CI)	case	control	OR[a](95%CI)
Arsenic concentration in well water（μg/L）						
≤50	29	85	1.0	20	31	1.0
50.1-99.9	60	66	2.7(1.5-4.9)***	32	18	4.1(1.-10.0)**
100+	89	97	2.7(1.6-4.5)***	49	29	3.2(1.4-7.1)**
Cumulative arsenic exposure（mg/L-year）						
<1.0	39	78	1.0	18	24	1.0
1.0-6.9	93	120	1.6(1.0-2.6)§	57	36	2.4(1.1-5.4)*
7.0+	46	50	1.7(1.0-3.1)§	26	18	2.1(0.8-5.1)
Duration of drinking well water（year）						
<30	33	43	1.0	19	14	1.0
30-49	84	126	0.9(0.5-1.5)	43	41	0.8(0.4-2.0)
50+	61	79	0.7(0.4-2.6)	39	23	1.0(0.4-2.6)

a: age-sex-adjusted odds ratio

§: 0.05<p<0.1 ＊:0.01<p<0.05 **: 0.001<p<0.01 *** :p<0.001

Wild/Wild: W/W; Wild/Mutant or Mutant/Mutant: W/M or M/M

Table 5 Age-sex-adjusted odds ratio (OR) and 95% confidence interval (CI) of carotid atherosclerosis by genetic polymorphisms of P53,and various arsenic exposure indices

Variable	P53					
	W/W			W/M or M/M		
	case	control	OR[a](95%CI)	case	control	OR[a](95%CI)
Arsenic concentration in well water（μg/L）						
≤50	19	40	1.0	30	75	1.0
50.1-99.9	15	26	1.3(0.5-3.0)	77	58	3.8(2.1-6.8)***
100+	19	25	1.5(0.7-3.5)	119	101	3.1(1.8-5.3)***
Cumulative arsenic exposure（mg/L-year）						
<1.0	18	35	1.0	39	67	1.0
1.0-6.9	24	40	1.2(0.5-2.5)	126	115	2.1(1.2-3.4)**
7.0+	11	16	1.3(0.5-3.3)	61	52	2.1(1.2-3.8)*
Duration of drinking well water（year）						
<30	10	15	1.0	42	42	1.0
30-49	27	47	0.8(0.3-2.1)	100	119	0.9(0.5-1.6)
50+	16	29	0.8(0.3-2.3)	84	73	1.0(0.5-1.4)

a: age-sex-adjusted odds ratio

§: 0.05<p<0.1 * :0.01<p<0.05 **: 0.001<p<0.01 *** :p<0.001

Wild/Wild: W/W; Wild/Mutant or Mutant/Mutant: W/M or M/M