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Protective effects of plasma alpha-tocopherols on the risk of inorganic arsenic-related urothelial carcinoma

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ABSTRACT

Arsenic plays an important role in producing oxidative stress in cultured cells. To investigate the interaction between high oxidative stress and low arsenic methylation capacity on arsenic carcinogenesis, a case–control study was conducted to evaluate the relationship among the indices of oxidative stress, such as urinary 8-hydroxydeoxyquanine (8-OHdG), as well as plasma micronutrients and urinary arsenic profiles on urothelial carcinoma (UC) risk. Urinary 8-OHdG was measured using high-sensitivity enzyme-linked immunosorbent assay kits. The urinary arsenic species were analyzed using high-performance liquid chromatography and hydride generator-atomic absorption spectrometry. Plasma micronutrient levels were analyzed using reversed-phase high-performance liquid chromatography. The present study showed a significant protective effect of plasma alpha-tocopherol on UC risk. Plasma alpha-tocopherol levels were significantly inversely related to urinary total arsenic concentrations and inorganic arsenic percentage (InAs%), and significantly positively related to dimethylarsinic acid percentage (DMA%). There were no correlations between plasma micronutrients and urinary 8-OHdG. Study participants with lower alpha-tocopherol and higher urinary total arsenic, higher InAs%, higher MMA%, and lower DMA% had a higher UC risk than those with higher alpha-tocopherol and lower urinary total arsenic, lower InAs%, lower MMA%, and higher DMA%. These results suggest that plasma alpha-tocopherol might modify the risk of inorganic arsenic-related UC.

1. Introduction

Bladder cancer is the most common malignancy of the urinary tract and it was estimated that 1900 new cases would be diagnosed and 747 deaths would occur in Taiwan in 2005 according to the data from Department of Health, the Executive Yuan. Cigarette smoking and carcinogens in drinking water could have a direct effect on urinary tract cancer development (Negri and La, 2001). Inorganic arsenic is recognized as a potent human carcinogen. Epidemiological evidences showed that exposure arsenic in drinking water were associated with skin cancer, liver cancer and bladder cancer (Chiou et al., 1995;Hsueh et al., 1997). Huang et al. reported a significant association between insufficient arsenic methylation and the development of urothelial carcinoma (UC) conducted from the cohort study in the arseniasis hyperendemic area (Huang et al., 2008). Further data showed that smokers in Argentina and the US with a high monomethylarsonic acid percentage (%MMA) had a high odds ratio of bladder cancer (Steinmaus et al., 2006).

Oxidative stress resulting from the disturbance of the pro-oxidant/antioxidant balance might be related to oncogenic stimulation (Valko et al., 2006). DNA lesions induced by reactive oxygen species (ROS), including 8-hydroxydeoxyquanine (8-OHdG), could be involved in the etiology of cancer (Beevi et al., 2007). Urinary 8-OHdG is one of the most abundant markers of DNA damage, which is increased in cancer patients, compared to the general population (Howard et al., 1998). Plasma retinol, alpha-tocopherols, lycopene, and beta-carotenes are the major lipophilic antioxidant constituents of fruits and vegetables. Dietary intake of fruits and vegetables has been associated with increased levels of plasma lipophilic micronutrients in epidemiological

Abbreviations: UC, urothelial carcinoma; InAs, inorganic arsenic; iAs^{3+}, inorganic arsenite; iAs^{5+}, inorganic arsenate; DMA^{5+}, dimethylarsinic acid; MMA^{5+}, monomethylarsonic acid; % InAs, percentage of InAs; % DMA^{5+}, percentage of DMA5+; % MMA^{5+}, percentage of MMA5+.

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studies (Briviba et al., 2008). Exposure to antioxidant supplement or tomato extract could effectively attenuate oxidative stress, acute inflammation, tumor cell proliferation, cell cycle progression, and induce the expression of apoptotic Bax protein in in-vitro studies (Amir et al., 1999;Jamshidzadeh et al., 2008;Tang et al., 2008). The extent of DNA damage could be altered by reducing the TNF-α level, DNA-protein cross-links, DNA stand breaks, and inhibiting the activation of caspase cascade in rats treated with arsenic along with alpha-tocopherol (Ramanathan et al., 2005;Kadirvel et al., 2007). Until now, epidemiological studies evaluating the association between major lipophilic antioxidants and bladder cancer risk have reported conflicting findings (Zeegers et al., 2001;Michaud et al., 2002;Hung et al., 2006).

There is evidence that dietary supplementation could improve oxidative disturbances, and that plasma micronutrient levels could prevent cancer incidences (Woodside et al., 2005). However, the association between plasma micronutrients and arsenic-related DNA damage is still unclear (Chung et al., 2006; Hsueh et al., 1997; Verret et al., 2005). Therefore, the objective of the present study is to investigate whether plasma micronutrients could modify UC risk resulting from altered DNA damage associated with urinary arsenic concentrations.

2. Materials and methods

2.1. Study participants

We conducted a hospital-based case–control study. Study protocols have been described in detail elsewhere (Pu et al., 2007). Briefly, 170 UC cases and 402 healthy control participants were recruited from September 2002 to April 2006. All UC cases were diagnosed with histological confirmation and pathological verification. This was done using routine urological practices, including endoscopic biopsy or surgical resection of urinary tract tumors, followed by histopathological examination by board-certified pathologists. Healthy control participants who had no prior history of cancer were matched to UC patients in terms of age ±5 years and gender. The majority of study participants (>80%) lived in Taipei city and drank tap water from Taipei Water Department of the Taipei City Government. The average arsenic concentration of tap water is 0.7 μg/L, but ranges from non-detectable to 4.0 μg/L. The Research Ethics Committee of National Taiwan University Hospital, Taipei, Taiwan, approved the study. All participants provided informed consent before the questionnaire, interview, and bio-specimen collection. The study was consistent with the World Medical Association Declaration of Helsinki.

2.2. Questionnaire, interview, and biological specimen collection

Well-trained interviewers collected detailed information on demographics and socioeconomic characteristics, lifestyle factors, such as cigarette smoking and alcohol consumption, residential and occupational history, and personal and family histories of disease through a structured questionnaire. Spot urine samples were immediately transferred to a −20 °C freezer until required for urinary arsenic profile and 8-OHdG analyses. In addition, blood specimens were centrifuged at 3000 rpm for 10 min to separate plasma and stored at −80 °C until micronutrient analyses.

2.3. Urinary arsenic profiles and 8-OHdG measurement

Levels of urinary arsenic profiles including iAs^{3+}, iAs^{5+}, MMA^{5+}, and DMA^{5+} were measured by high-performance liquid chromatography (HPLC) equipped with a hydride generator and atomic absorption spectrometer (HG-AAS) (Hsueh et al., 1998a). Standard reference material SRM 2670 urine was obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), which contains 480 ± 100 μg/L of inorganic arsenic. SRM 2670 was used as a quality standard and analyzed with the urine samples. The mean value of arsenic in our SRM 2670 sample was 507 ± 17 μg/L (n = 4). Recovery rates of the four arsenic species were calculated using the following formula: ([(sample spiked standard solution concentration) − sample concentration]/standard solution concentration × 100). Recovery rates for iAs^{3+}, DMA^{5+}, MMA^{5+}, and iAs^{5+} ranged from 93.8 to 102.2%, with detection limits of 0.02, 0.08, 0.05, and 0.07 μg/L, respectively. Urinary 8-OHdG was analyzed using a competitive enzyme-linked immunosorbent assay (ELISA) kit (Japan Institute for the Control of Aging, Fukuroi, Japan), described in detail elsewhere (Chung et al., 2008). The detection range of the ELISA assay was 0.5–200 ng/mL and the intra-and inter-assay coefficients of variance (CV) were 9.8% and 6.7%, respectively. Urinary arsenic species are stable at least 6 months when stored at −20 °C (Chen et al., 2002). Therefore, the urine assays of the urinary arsenic species and 8-OHdG were performed within 6 months after collection.

2.4. Determination of plasma micronutrient levels

Plasma concentrations of retinol, alpha-tocopherol, lycopene, and beta-carotene were analyzed using reversed-phase HPLC (Hitachi Inc, Tokyo, Japan) according to the protocol described previously (Hsueh et al., 1998b). The mobile phase concentration of methanol: acetonitrite:chloroform was 47:47:6, and multi-wavelength monitoring was carried out during the experiment. Micronutrient levels were detected at 325 nm for retinol, 280 nm for alpha-tocopherol, and 466 nm for lycopene and beta-carotene. Plasma specimens for each case control set were thawed from a −80 °C refrigerator in dim light at room temperature and assayed on the same day to ensure that temporal variability in the laboratory assays would affect cases and controls equally. All laboratory personnel were unaware of the disease status of the subjects whose serum samples were tested. Recovery rates were 96.1% for retinol, 94.6% for alpha-tocopherol, 88.4% for

Table 1
Demographic characteristics and lifestyle for 170 UC cases and 402 controls.

Variables	UC cases (n = 170)	Control (n = 402)	OR[a] (95% CI)	P value
Age (years) (mean ± SE)	62.14 ± 1.08	61.50 ± 0.71	1.00 (0.99–1.02)	0.65
Gender				
Male	123 (72.35)	277 (68.91)	1.16 (0.78–1.73)	0.46
Female	47 (27.65)	125 (31.09)	1.00	
Education				
Elementary school or below	66 (38.82)	72 (18.05)	1.00[b]	
High school	67 (39.41)	138 (34.59)	0.45 (0.29–0.72)	<0.01
College or above	37 (21.76)	189 (47.37)	0.17 (0.10–0.29)	<0.01
Paternal ethnicity				
Fukien Taiwanese	124 (72.94)	217 (54.11)	1.00[b]	
Hakka Taiwanese	16 (9.41)	45 (11.22)	0.65 (0.35–1.20)	0.17
Mainland Chinese	30 (17.65)	137 (34.16)	0.34 (0.21–0.55)	<0.01
Cumulative cigarette smoking (pack-years)	17.71 ± 2.00	9.99 ± 0.97	1.02 (1.01–1.03)	<0.01
0	78 (49.06)	255 (65.89)	1.00	
>0	81 (50.94)	132 (34.11)	2.42 (1.53–3.82)	<0.01
Alcohol drinking				
Never	99 (58.24)	206 (51.37)	1.00	
Occasional	29 (17.06)	130 (32.42)	0.44 (0.27–0.71)	<0.01
Regular	42 (24.71)	65 (16.21)	1.20 (0.73–1.96)	0.47
Pesticide usage				
No	142 (84.02)	386 (96.50)	1.00	
Yes	27 (15.98)	14 (3.50)	5.25 (2.67–10.33)	<0.01

SE: standard error.
[a] Adjusted by age and gender.
[b] p<0.01 for trend test.

lycopene, and 99.4% for beta-carotene. Furthermore, we used alpha-tocopherol acetate as an internal control to minimize systematic error and the CV was <5%.

2.5. Statistical analysis

All analyses were performed using SAS 9.0 (SAS, version 9.0, Cary, NC). Total arsenic concentration (μg/L) was the sum of iAs^{3+}, iAs^{5+}, MMA^{5+}, and DMA^{5+} levels. The relative proportion of urinary arsenic species (iAs^{3+} %, iAs^{5+} %, MMA^{5+} %, and DMA^{5+} %) was calculated by dividing each arsenic species level by the total arsenic concentration. Inorganic arsenic percentage (InAs%) was the sum of iAs^{3+} % and iAs^{5+} %. Differences between cases and controls were tested by Student's t-test in continuous variables. A Log10-transformation was applied to the factors with an abnormal distribution before statistical analyses. A multiple linear regression model was used to estimate the effects of potential factors, including various arsenic species and urinary 8-OHdG, on plasma micronutrients. Tertile values of urinary arsenic profile, plasma micronutrient, and 8-OHdG levels of the controls were used as cutoff points for the trend test. Multiple logistic regression models were used to estimate the multivariate adjusted odds ratio (OR) and the 95% confidence interval (CI). Barr et al. (2005) reported that the significant predictors of urinary creatinine concentrations include age, sex, race, body mass index and fat-free mass. In addition, one study of 1650 adults in Bangladesh revealed that urinary creatinine concentrations are significantly correlated with plasma folate concentrations, particularly among males (Gamble and Liu, 2005). Therefore, we treated urinary creatinine as a separate variable in the regression model. For the joint effect analysis, the cutoff points for total arsenic, plasma micronutrient, and 8-OHdG levels were the respective medians of the controls. Finally, we estimated both the additive model (synergy index) and the multiplicative model to evaluate the joint effects of plasma micronutrients, as well as various arsenic species, on UC risk. An observed synergy index value that departs substantially from the expected additive null (i.e., synergy index not equal to 1) suggests an additive interaction effect (Hosmer and Lemeshow, 1992). The binary interaction terms were calculated by multiplying the indicators for two explored risk factors and then added to the main effect models; the p values were also calculated.

3. Results

The sociodemographic characteristics of UC cases and controls are shown in Table 1. Participants who had higher educational levels and whose paternal ethnicity was Mainland Chinese had a significantly lower UC risk than those with lower educational levels and whose paternal ethnicity was Fukien Taiwanese. Occasional alcohol drinkers had a significantly lower UC risk than non-drinkers. Cigarette smokers and pesticide users had a significantly higher UC risk than non-smokers and non-users, respectively.

Mean ± standard error (SE) of urinary arsenic profiles, 8-OHdG, and plasma micronutrients stratified by UC status are shown in Table 2. With trend analysis on exposure strata in tertiles, high total arsenic, high MMA%, low DMA%, and low plasma alpha-tocopherol

Table 2
Urinary arsenic profiles and 8-OHdG and plasma micronutrients concentrations of UC 170 cases and 402 controls.

Variables	UC cases (n = 170) No. (%)	Control (n = 402) No. (%)	Crude OR (95% CI)	P value	OR[a] (95% CI)	P value
Total arsenic (μg/g creatinine) (Mean ± SE)	37.67 ± 2.98	21.10 ± 0.79	1.04 (1.03–1.05)	≤ 0.01	1.03 (1.01–1.04)	< 0.01
< 12.15	13 (7.65)	134 (33.33)	1.00[&]		1.00[&]	
12.15-22.10	36 (21.18)	134 (33.33)	2.77 (1.41–5.46)	≤ 0.01	2.80 (1.26–6.21)	0.01
≥ 22.10	121 (71.18)	134 (33.33)	9.31 (5.01–17.31)	≤ 0.01	6.71 (3.14–14.35)	< 0.01
InAs% (Mean ± SE)	7.18 ± 0.58	6.94 ± 0.48	1.00 (0.98–1.02)	0.77	1.00 (0.98–1.03)	0.72
< 2.86	44 (25.88)	134 (33.33)	1.00[&]		1.00	
2.86-6.03	52 (30.59)	134 (33.33)	1.18 (0.74–1.89)	0.48	1.61 (0.91–2.84)	0.10
≥ 6.03	74 (43.53)	134 (33.33)	1.68 (1.08–2.62)	0.02	1.15 (0.66–2.00)	0.62
MMA% (Mean ± SE)	13.19 ± 0.99	7.53 ± 0.36	1.07 (1.05–1.09)	≤ 0.01	1.08 (1.05–1.12)	≤ 0.01
< 3.29	33 (19.41)	134 (33.33)	1.00[&]		1.00[&]	
3.29-9.08	35 (20.59)	134 (33.33)	1.06 (0.62–1.81)	0.83	1.67 (0.86–3.23)	0.13
≥ 9.08	102 (60.00)	134 (33.33)	3.09 (1.95–4.90)	≤ 0.01	5.56 (3.09–10.00)	< 0.01
DMA% (Mean ± SE)	79.63 ± 1.14	85.52 ± 0.58	0.97 (0.95–0.98)	≤ 0.01	0.97 (0.96–0.99)	< 0.01
≥ 91.46	34 (20.00)	134 (33.33)	1.00[&]		1.00[&]	
83.23-91.46	47 (27.65)	134 (33.33)	1.38 (0.84–2.28)	0.21	1.91 (1.03–3.54)	0.04
< 83.23	89 (52.35)	134 (33.33)	2.62 (1.65–4.16)	≤ 0.01	2.60 (1.49–4.55)	< 0.01
8-OHdG (ng/mg creatinine) (Mean ± SE)	7.48 ± 0.97	5.95 ± 0.21	2.00 (1.08–3.68)	0.03	1.71 (0.80–3.63)	0.17
< 3.81	56 (32.94)	134 (33.33)	1.00		1.00	
3.81-6.52	51 (30.00)	134 (33.33)	0.91 (0.58–1.43)	0.68	1.22 (0.71–2.11)	0.47
≥ 6.52	63 (37.06)	134 (33.33)	1.13 (0.73–1.73)	0.59	1.00 (0.57–1.73)	0.99
Retinol (μg/mL) (Mean ± SE)	1.10 ± 0.04	1.09 ± 0.02	1.06 (0.72–1.55)	0.77	1.00 (0.63–1.58)	0.99
< 0.87	64 (37.65)	139 (34.58)	1.00		1.00	
0.87-1.15	50 (29.41)	132 (32.84)	0.82 (0.53–1.28)	0.38	0.90 (0.52–1.54)	0.70
≥ 1.15	56 (32.94)	131 (32.59)	0.93 (0.60–1.43)	0.74	0.97 (0.58–1.64)	0.91
Alpha-tocopherol (μg/mL) (Mean ± SE)	10.35 ± 0.44	12.42 ± 0.28	0.92 (0.89–0.96)	≤ 0.01	0.94 (0.90–0.98)	0.01
< 9.47	89 (52.35)	139 (34.58)	1.00[&]		1.00[&]	
9.47-13.60	52 (30.59)	132 (32.84)	0.62 (0.41–0.93)	0.02	0.73 (0.44–1.22)	0.23
≥ 13.60	29 (17.06)	131 (32.59)	0.35 (0.21–0.56)	≤ 0.01	0.48 (0.27–0.84)	0.01
Lycopene (μg/dL) (Mean ± SE)	9.02 ± 0.82	11.19 ± 0.60	0.98 (0.97–1.00)	0.05	1.00 (0.98–1.02)	0.82
< 4.24	74 (43.53)	139 (34.58)	1.00[&]		1.00	
4.24-12.46	59 (34.71)	132 (32.84)	0.84 (0.55–1.27)	0.41	1.07 (0.64–1.78)	0.80
≥ 12.46	37 (21.76)	131 (32.59)	0.53 (0.34–0.84)	0.01	0.93 (0.53–1.64)	0.80
Beta-carotene (μg/dL) (Mean ± SE)	24.68 ± 2.22	22.17 ± 1.16	1.00 (1.00–1.01)	0.28	1.01 (1.00–1.02)	0.06
< 9.49	51 (30.00)	139 (34.58)	1.00		1.00	
9.49-23.94	58 (34.12)	132 (32.84)	1.20 (0.77–1.87)	0.43	1.30 (0.75–2.28)	0.35
≥ 23.94	61 (35.88)	131 (32.59)	1.27 (0.82–1.97)	0.29	1.49 (0.86–2.57)	0.15

SE: standard error.
[&] $p<0.01$ for trend test. Cutoff points for urinary arsenic profile, 8-OHdG and plasma micronutrients were the respective tertiles of the controls.
[a] Adjusted by age, gender, education, paternal ethnicity, cigarette smoking, alcohol drinking, pesticide use and urinary creatinine levels.

were found to be significantly positively associated with the OR of UC in a dose–response relationship regardless of unadjustment or adjustment for covariates (Table 2). There was a significant dose–response relationship between plasma lycopene and UC risk under unadjusted analysis. However, this significant association did not exist when we adjusted for other confounders. Urinary 8-OHdG was significantly different between UC cases and controls, but it was not related to the UC odds ratio for the trend test, regardless of unadjustment or adjustment for covariates.

A multivariate linear regression was used to explore the potential factors affecting plasma micronutrients in the control group (Table 3). For the urinary arsenic profile, plasma retinol and alpha-tocopherol were significantly negatively correlated with InAs% ($p = 0.02$ and 0.01, respectively). Alpha-tocopherol significantly increased with increasing DMA% ($p = 0.05$). This finding suggests that the preferred arsenic methylation profile is related to higher alpha-tocopherol. Further, the levels of alpha-tocopherol and beta-carotene were significantly increased with increasing age. For males, the levels of alpha-tocopherol and beta-carotene were significantly lower than those for females. For lifestyle risk factors, the levels of lycopene and beta-carotene were significantly decreased in people who had higher cumulative cigarette smoking. These results indicated that the elderly, females and non-smokers have higher antioxidants than younger people, males and smokers. No associations between the habits of alcohol drinking or pesticide use and plasma micronutrients were observed in our results.

Further analyses were carried out to assess the joint effects of each plasma micronutrient and total arsenic levels on the urinary 8-OHdG levels in the control group (Fig. 1). Trend analysis revealed progressively increased 8-OHdG through exposure to no risk factor (low total arsenic and high lycopene or low total arsenic and high beta carotene), either one of the factors, or both of the two risk factors (p values for trends were 0.01 and 0.02, respectively) after adjustment for age, gender education, paternal ethnicity, cigarette smoking, alcohol drinking, and pesticide use in multivariate linear regression.

The joint effect of the arsenic methylation profile and plasma alpha-tocopherol on UC risk are shown in Table 4. The significantly increased UC risk was most pronounced in participants who had high total arsenic and low plasma alpha-tocopherol ($p \leq 0.01$ for trend test). However, this interaction was statistically significant only in the multiplicative model, but not in the synergy index. The same patterns were shown in the joint effect of InAs%, MMA%, or DMA%, and plasma alpha-tocopherol on UC risk. However, all synergy index and multiplicative models were insignificant.

4. Discussion

To our knowledge, this is the first study to evaluate whether plasma alpha-tocopherols could modify UC risk related with poor arsenic methylation in a population without obvious exposure to arsenic. Among these lipophilic antioxidants, we only found the protective predictor of plasma alpha-tocopherols on UC risk. Then, plasma alpha-tocopherol levels were inversely associated with total arsenic and InAs%, and positively associated with DMA% in a multivariate linear regression model. Lower plasma alpha-tocopherol might modify the risk of inorganic arsenic-related UC.

Since 2000, the allowable arsenic levels in drinking water have decreased from 50 to 10 μg/L in Taiwan. According to the Taipei Water Department of the Taipei City Government, the average arsenic concentration in Taipei tap water is 0.7 μg/L (range: non-detectable to 4.0 μg/L). In the early 1910s, there were two endemic areas of long-term exposure to arsenic from drinking water in Taiwan, including southwestern and northeastern areas. Until the mid-1970s, a public water supply system was generally utilized and artesian well water was no longer used in daily life (Chen et al., 2002). Distinct from previously exposed areas, the majority of our participants (≥80%) lived in Taipei City. Compared to the arsenic-contaminated southwestern area in Taiwan (Hsueh et al., 1997), the sums of urinary $iAs^{3\pm}$, $iAs^{5\pm}$, $MMA^{5\pm}$and $DMA^{5\pm}$ of study participants in the present study were low (mean value of total arsenic: approximately 70 μg/L vs. 25 μg/L, respectively). We randomly collected drinking water from 37 UC cases and measured the total arsenic levels; the mean ± standard error was 17.14 ± 0.55 μg/L greater than the standard level. In addition to drinking water, arsenic species were recently detected in agricultural rice paddy soils in the Guandu Plain of northern Taiwan (Chiang et al., 2010). Although we do not know the exact exposure source, we still observed a significantly increased risk of UC in those with an unfavorable urinary arsenic profile, including higher InAs%, higher MMA% or lower DMA% (Pu et al., 2007).

The standard concentration of arsenic in drinking water and the risk of low doses of arsenic have been discussed throughout the world (Schoen et al., 2004; Snow et al., 2005). Arsenic-induced cytotoxicity caused by high arsenic exposure and mediated through reactive oxygen species was fully evidenced in *in-vitro* and human studies (Shi

Table 3
Multivariate analysis of the risk factors for plasma micronutrients in the control group.

Variables	Retinol (μg/mL)		α-tocopherol (μg/mL)		Lycopene (μg/dL)		β-carotene (μg/dL)	
	β (SE)	p	β (SE)	p	β (SE)	p	β (SE)	p
Age	0.0005 (0.001)	0.49	0.003 (0.001)	<0.01	0.001 (0.002)	0.70	0.004 (0.001)	0.01
Gender (male vs. female)	0.04 (0.02)	0.06	−0.06 (0.02)	0.02	0.05 (0.07)	0.47	−0.13 (0.05)	0.01
Education								
Elementary school or below								
High school	−0.02 (0.02)	0.42	0.04 (0.03)	0.14	−0.04 (0.07)	0.57	0.03 (0.05)	0.59
College or above	−0.03 (0.03)	0.21	0.07 (0.03)	0.01	0.02 (0.07)	0.78	0.04 (0.06)	0.45
Cumulative cigarette smoking	0.01 (0.02)	0.71	−0.01 (0.02)	0.56	−0.19 (0.06)	<0.01	−0.11 (0.05)	0.02
Alcohol drinking								
Never								
Occasional	−0.02 (0.02)	0.37	−0.01 (0.02)	0.71	0.05 (0.06)	0.36	0.02 (0.05)	0.66
Regular	0.02 (0.03)	0.45	−0.02 (0.03)	0.53	−0.04 (0.08)	0.62	−0.09 (0.06)	0.12
Pesticide usage (yes vs. no)	−0.005 (0.03)	0.89	−0.04 (0.04)	0.30	−0.15 (0.10)	0.12	0.01 (0.07)	0.85
Total arsenic levels	0.002 (0.03)	0.94	−0.06 (0.03)	0.05	−0.04 (0.08)	0.65	0.10 (0.06)	0.08
InAs%	−0.05 (0.02)	0.02	−0.07 (0.02)	0.01	−0.10 (0.06)	0.10	−0.09 (0.05)	0.07
MMA%	−0.04 (0.02)	0.07	−0.03 (0.02)	0.20	−0.04 (0.07)	0.54	−0.02 (0.05)	0.69
DMA%	0.16 (0.12)	0.17	0.24 (0.12)	0.05	0.15 (0.33)	0.64	0.05 (0.25)	0.83
8-OHdG	−0.03 (0.03)	0.32	0.005 (0.03)	0.88	−0.02 (0.09)	0.79	0.07 (0.07)	0.28

All models included age, gender, education, paternal ethnicity, cigarette smoking, alcohol drinking, pesticide use, and total arsenic level.

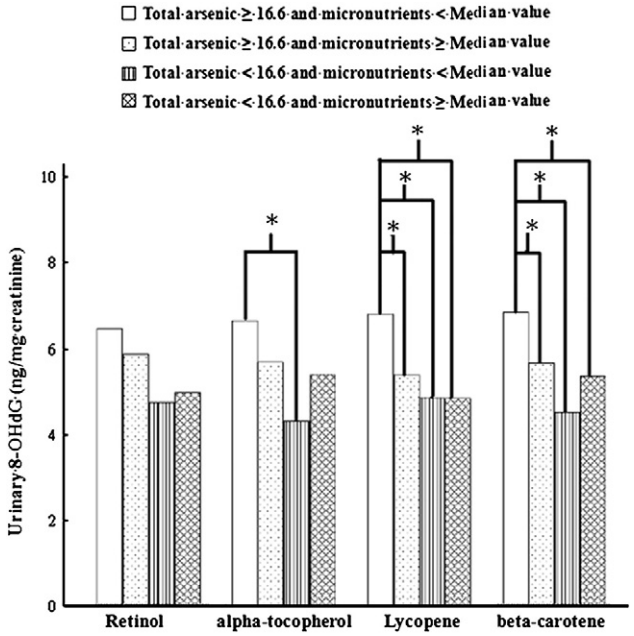

Fig. 1. The joint effect of plasma micronutrients and total arsenic levels on the urinary 8-OHdG levels in the control group. * $p \leq 0.05$ for multiple linear regression.

et al., 2004; Basu et al., 2004; Kubota et al., 2006). Moore et al. (1997) demonstrated that chromosome damage in the form of micronuclei in human exfoliated bladder cells are induced by high arsenic exposure. The effects of low dose arsenic exposure should be further evaluated. Our previous study found a significantly positive association between urinary arsenic profiles (high total arsenic, high iAs^{3+}%, high MMA%, and low DMA%) and UC risk (Pu et al., 2007); we also found a significant increase in 8-OHdG levels related to urinary arsenic species (total arsenic, iAs^{3+}, MMA, and DMA) in low arsenic exposure areas (Chung et al., 2008). However, only a weak association between urinary 8-OHdG levels and arsenic-associated UC risk was observed. In addition, one study demonstrated that low levels of arsenic provoked a cellular adaptive oxidative stress response through increasing antioxidant levels (Fu et al., 2010). Therefore, we further evaluated the joint effect of urinary arsenic profile and plasma micronutrient levels on UC risk in the present study. Our study showed that participants who had lower total arsenic and higher lycopene (or beta-carotene) had lower urinary 8-OHdG levels than those with higher total arsenic and lower lycopene (or beta-carotene) ($p<0.01$ for trend test) in the control group. However, we did not find any association between plasma lipophilic antioxidants and urinary 8-OHdG level in multivariate linear regression models, which is consistent with the findings of Kimura et al. (2006).

Alpha-tocopherol present in the cell membrane could disrupt lipid peroxyl radical chains, thus preventing further lipid peroxidation (Kadirvel et al., 2007). In addition, alpha-tocopherol was interrelated with reduced glutathione (GSH); ascorbic acid could improve cellular thiol status as well as maintain DNA and proteins in native form through the recycling processes (Meister, 1994b). During the process, tocopheroxyl radicals are transformed by accepting free radicals and then reduced to alpha-tocopherol under reaction with ascorbic acid. Sequentially, the dehydroascorbic acid formed in this reaction is reduced to ascorbic acid with the oxidation of reduced GSH (Meister, 1994a; Brigelius-Flohe and Traber, 1999). Therefore, the antioxidant protection of alpha-tocopherol might be a result of neutralizing ROS directly or regulating GSH recycling indirectly or another enzymatic mechanism (Ramanathan et al., 2002). Exposure to inorganic arsenic in drinking water or further arsenic methylation in the human body might be involved in ROS production (Lynn et al., 2000;Nishikawa et al., 2002;Pi et al., 2002;Wei et al., 2005). In animal models, the administration of alpha-tocopherol and ascorbic acid to arsenic-exposed rats significantly reduced oxidative stress and produced cell apoptosis by directly eliminating free radicals (Lynn et al., 2000; Ramanathan et al., 2005;Kadirvel et al., 2007). In our data, 12.42 ± 0.28 μg/mL of alpha-tocopherol in healthy controls was similar to 14.3 ± 0.09 μg/mL reported by Badjatia et al., and was lower than 27.48 ± 12.92 μg/mL reported by Liang et al.; these studies also reported the same significant protection on UC risk (Liang et al., 2008; Badjatia et al., 2010). Alpha-tocopherol affects the expression and

Table 4
Ad justed odds ratios and 95% confidence intervals (CI) for the interaction of urinary total arsenic and plasma micronutrients on UC risk.

Plasma alpha-tocopherol (μg/mL)	Urinary arsenic species	Cases/controls	OR[a] (95% CI)	Synergy index (95% CI)	p[b]
≥11.21	Total arsenic<16.60	5/100	1.00	0.97 (0.55–1.69)	0.05
≥11.21	Total arsenic≥16.60	25/101	5.17(1.74–15.38)		
<11.21	Total arsenic<16.60	42/97	6.85(2.407–19.55)		
<11.21	Total arsenic≥16.60	98/104	10.70(3.89–29.47)		
			$P_{trend}<0.01$		
≥11.21	InAs%<4.32	26/108	1.00	1.49 (0.22–10.20)	0.55
≥11.21	InAs%≥4.32	47/93	1.86 (0.96–3.59)		
<11.21	InAs% ≤ 4.32	21/89	0.77 (0.37–1.66)		
<11.21	InAs%>4.32	76/112	1.94 (1.03–3.68)		
			P_{trend}–0.18		
≥11.21	MMA%<6.10	14/104	1.00	1.42 (0.75–2.70)	0.54
≥11.21	MMA%≥6.10	32/97	2.65 (1.15–6.11)		
<11.21	MMA% ≤ 6.10	33/93	5.28 (2.29–12.19)		
<11.21	MMA%>6.10	91/108	9.43 (4.29–20.74)		
			$P_{trend}<0.01$		
≥11.21	DMA%≥88	16/101	1.00	2.29 (0.91–5.74)	0.55
≥11.21	DMA%<88	26/100	1.66 (0.74–3.71)		
<11.21	DMA%≥88	31/96	2.47 (1.14–5.38)		
<11.21	DMA%<88	97/105	5.88 (2.82–12.23)		
			$P_{trend}<0.01$		

Total arsenic (μg/g creatinine).
Cutoff points for urinary arsenic profile and plasma alpha-tocopherol were the respective medians of the controls.
[a] Adjusted by age, gender, education, paternal ethnicity, cigarette smoking, alcohol drinking, pesticide use and urinary creatinine levels.
[b] p represents statistical interaction as a departure from joint effects of multiplicative model.

activity of the immune and inflammatory cells by inhibiting the activity of protein kinase C, an important cell-signaling molecule (Sampayo-Reyes and Zakharyan, 2006). Additionally, glutathione is critical to the metabolism of the arsenic methylation, specifically the initial reduction of iAs^{5+} to iAs^{3+} (Zakharyan et al., 1999). Perhaps, alpha-tocopherol diminishes arsenic toxicology through interaction with glutathione to decrease UC risk. The joint effect of alpha-tocopherol and glutathione on UC risk needs further investigation.

Previous studies demonstrated conflicting findings regarding pesticide use and human cancers (Rusiecki et al., 2004; Kang et al., 2008; Koutros et al., 2009; Chrisman et al., 2009). The United States Agricultural Health Study constructed a prospective cohort of 57,311 licensed pesticide applicators in the United States, enrolled between 1993 and 1997, and evaluated the incidence of cancer developed through 2004. For heterocyclic aromatic amine pesticide users, the study found significant trends in risk with increasing lifetime exposure for bladder cancer and colon cancer (Koutros et al., 2009). However, moderate to weak correlations were observed for bladder cancer in pesticide sales in Brazil (Chrisman et al., 2009). The index of pesticide exposure in our study is a simple yes-or-no variable, and we evaluated it as a confounder to adjust in the multivariate logistic model. Future study may need to explore the association between pesticide use and bladder cancer. In addition, the carcinogens in cigarette smoke, including polycyclic aromatic hydrocarbons, aromatic amines, and other chemicals, are proposed to be involved in ROS production and oxidative damage (Pryor, 1997). In our previous work, we found a significant joint effect of cigarette smoking and arsenic methylation capacity on UC risk (Pu et al., 2007); we did not prove the association between cigarette smoking and oxidative stress, including urinary 8-OHdG levels (Chung et al., 2008). However, lycopene and beta-carotene were significantly inversely related with cumulative cigarette smoking in this study.

The present study attempted to clarify the important role of oxidative stress on arsenic-related UC risk based on biochemical levels. We used the indices of general oxidative stress evaluation, including plasma micronutrients and urinary 8-OHdG, which resulted from environmental exposure. In the present study, we could not clarify the roles of plasma micronutrients on the association between urinary 8-OHdG and UC risk. The role of ROS-related DNA repair enzymes in arsenic carcinogenesis needs further exploration. The accuracy of one-spot evaluation of plasma antioxidants may be in doubt. However, the values might be reliable if all subjects reported no accompanying changes in lifestyle. In addition, the specimen collection and measurement of urinary arsenic and plasma micronutrients were completed at the same time in this study, but they may be an effect modifier of each other on UC risk. Plasma samples were collected since cancer diagnosis, which might underestimate the association between plasma micronutrients and UC risk if UC patients improved their dietary habits after diagnosis. However, we still observed a significant inverse relationship between alpha-tocopherol and UC risk. The information of food frequency in Chinese dietary was not precise and recall bias should be also considered. Therefore, we used the concentrations of plasma micronutrients as a better predictor to evaluate individual nutrition status. We have no information about medication that may affect plasma micronutrient levels, which was a study limitation. Because of the small sample size, statistical significance should be interpreted with caution. Prevalent UC cases were recruited in this study; however, we cannot exclude the findings about the association between plasma micronutrients or arsenic and its various metabolites and UC, and the fact that these associations might be the result of and not the cause for UC. In conclusion, our results might suggest a potential protective effect of alpha-tocopherol on UC, particularly in study participants with inefficient arsenic methylation capability.

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References

Amir H, Karas M, Giat J, Danilenko M, Levy R, Yermiahu T, et al. Lycopene and 1, 25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. Nutr Cancer 1999;33:105–12.
Badjatia N, Satyam A, Singh P, Seth A, Sharma A. Altered antioxidant status and lipid peroxidation in Indian patients with urothelial bladder carcinoma. Urol Oncol 2010;28:360–7.
Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect 2005;113:192–200.
Basu A, Ghosh P, Das JK, Banerjee A, Ray K, Giri AK. Micronuclei as biomarkers of carcinogen exposure in populations exposed to arsenic through drinking water in West Bengal, India: a comparative study in three cell types. Cancer Epidemiol Biomarkers Prev 2004;13:820–7.
Beevi SS, Rasheed MH, Geetha A. Evidence of oxidative and nitrosative stress in patients with cervical squamous cell carcinoma. Clin Chim Acta 2007;375:119–23.
Brigelius-Flohe R, Traber MG. Vitamin E: function and metabolism. FASEB J 1999;13:1145–55.
Briviba K, Bub A, Moseneder J, Schwerdtle T, Hartwig A, Kulling S, et al. No differences in DNA damage and antioxidant capacity between intervention groups of healthy, nonsmoking men receiving 2, 5, or 8 servings/day of vegetables and fruit. Nutr Cancer 2008;60:164–70.
Chen YC, Amarasiriwardena CJ, Hsueh YM, Christiani DC. Stability of arsenic species and insoluble arsenic in human urine. Cancer Epidemiol Biomark Prev 2002;11:1427–33.
Chiang KY, Lin KC, Lin SC, Chang TK, Wang MK. Arsenic and lead (beudantite) contamination of agricultural rice soils in the Guandu Plain of northern Taiwan. J Hazard Mater 2010;181:1066–71.
Chiou HY, Hsueh YM, Liaw KF, Horng SF, Chiang MH, Pu YS, et al. Incidence of internal cancers and ingested inorganic arsenic: a seven-year follow-up study in Taiwan. Cancer Res 1995;55:1296–300.
Chrisman JR, Koifman S, de Novaes SP, Moreira JC, Koifman RJ, Meyer A. Pesticide sales and adult male cancer mortality in Brazil. Int J Hyg Environ Health 2009;212:310–21.
Chung JS, Haque R, Guha Mazumder DN, Moore LE, Ghosh N, Samanta S, et al. Blood concentrations of methionine, selenium, beta-carotene, and other micronutrients in a case-control study of arsenic-induced skin lesions in West Bengal, India. Environ Res 2006;101:230–7.
Chung CJ, Huang CJ, Pu YS, Su CT, Huang YK, Chen YT, et al. Urinary 8-hydroxydeoxyguanosine and urothelial carcinoma risk in low arsenic exposure area. Toxicol Appl Pharmacol 2008;226:14–21.
Fu J, Woods CG, Yehuda-Shnaidman E, Zhang Q, Wong V, Collins S, Sun G, Andersen ME, Pi J. Low-level arsenic impairs glucose-stimulated insulin secretion in pancreatic beta cells: involvement of cellular adaptive response to oxidative stress. Environ Health Perspect 2010;118:864–70.
Gamble MV, Liu X. Urinary creatinine and arsenic metabolism. Environ Health Perspect 2005;113:A442–3.
Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;3:452–6.
Howard DJ, Ota RB, Briggs LA, Hampton M, Pritsos CA. Environmental tobacco smoke in the workplace induces oxidative stress in employees, including increased production of 8-hydroxy-2′-deoxyguanosine. Cancer Epidemiol Biomark Prev 1998;7:141–6.
Hsueh YM, Chiou HY, Huang YL, Wu WL, Huang CC, Yang MH, et al. Serum beta-carotene level, arsenic methylation capability, and incidence of skin cancer. Cancer Epidemiol Biomark Prev 1997;6:589–96.
Hsueh YM, Huang YL, Huang CC, Wu WL, Chen HM, Yang MH, et al. Urinary levels of inorganic and organic arsenic metabolites among residents in an arseniasis-hyperendemic area in Taiwan. J Toxicol Environ Health 1998a;54:431–44.
Hsueh YM, Wu WL, Huang YL, Chiou HY, Tseng CH, Chen CJ. Low serum carotene level and increased risk of ischemic heart disease related to long-term arsenic exposure. Atherosclerosis 1998b;141:249–57.
Huang YK, Huang YL, Hsueh YM, Yang MH, Wu MM, Chen SY, et al. Arsenic exposure, urinary arsenic speciation, and the incidence of urothelial carcinoma: a twelve-year follow-up study. Cancer Causes Control 2008;19:829–39.
Hung RJ, Zhang ZF, Rao JY, Pantuck A, Reuter VE, Heber D, et al. Protective effects of plasma carotenoids on the risk of bladder cancer. J Urol 2006;176:1192–7.
Jamshidzadeh A, Baghban M, Azarpira N, Bardbori AM, Niknahad H. Effects of tomato extract on oxidative stress induced toxicity in different organs of rats. Food Chem Toxicol 2008;46:3612–5.
Kadirvel R, Sundaram K, Mani S, Samuel S, Elango N, Panneerselvam C. Supplementation of ascorbic acid and alpha-tocopherol prevents arsenic-induced protein

oxidation and DNA damage induced by arsenic in rats. Hum Exp Toxicol 2007;26: 939–46.
Kang D, Park SK, Beane-Freeman L, Lynch CF, Knott CE, Sandler DP, Hoppin JA, Dosemeci M, Coble J, Lubin J, Blair A, Alavanja M. Cancer incidence among pesticide applicators exposed to trifluralin in the Agricultural Health Study. Environ Res 2008;107:271–6.
Kimura S, Yamauchi H, Hibino Y, Iwamoto M, Sera K, Ogino K. Evaluation of urinary 8-hydroxydeoxyguanine in healthy Japanese people. Basic Clin Pharmacol Toxicol 2006;98:496–502.
Koutros S, Lynch CF, Ma X, Lee WJ, Hoppin JA, Christensen CH, Andreotti G, Freeman LB, Rusiecki JA, Hou L, Sandler DP, Alavanja MC. Heterocyclic aromatic amine pesticide use and human cancer risk: results from the U.S. Agricultural Health Study. Int J Cancer 2009;124:1206–12.
Liang D, Lin J, Grossman HB, Ma J, Wei B, Dinney CP, et al. Plasma vitamins E and A and risk of bladder cancer: a case-control analysis. Cancer Causes Control 2008;19:981–2.
Lynn S, Gurr JR, Lai HT, Jan KY. NADH oxidase activation is involved in arsenite-induced oxidative DNA damage in human vascular smooth muscle cells. Circ Res 2000;86: 514–9.
Meister A. Glutathione, ascorbate, and cellular protection. Cancer Res 1994a;54:1969–75.
Meister A. Glutathione-ascorbic acid antioxidant system in animals. J Biol Chem 1994b;269: 9397–400.
Michaud DS, Pietinen P, Taylor PR, Virtanen M, Virtamo J, Albanes D. Intakes of fruits and vegetables, carotenoids and vitamins A, E, C in relation to the risk of bladder cancer in the ATBC cohort study. Br J Cancer 2002;87:960–5.
Moore LE, Smith AH, Hopenhayn-Rich C, Biggs ML, Kalman DA, Smith MT. Micronuclei in exfoliated bladder cells among individuals chronically exposed to arsenic in drinking water. Cancer Epidemiol Biomark Prev 1997;6:31–6.
Negri E, La VC. Epidemiology and prevention of bladder cancer. Eur J Cancer Prev 2001;10:7-14.
Nishikawa T, Wanibuchi H, Ogawa M, Kinoshita A, Morimura K, Hiroi T, et al. Promoting effects of monomethylarsonic acid, dimethylarsinic acid and trimethylarsine oxide on induction of rat liver preneoplastic glutathione S-transferase placental form positive foci: a possible reactive oxygen species mechanism. Int J Cancer 2002;100: 136–9.
Pi J, Yamauchi H, Kumagai Y, Sun G, Yoshida T, Aikawa H, et al. Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water. Environ Health Perspect 2002;110:331–6.
Pryor WA. Cigarette smoke radicals and the role of free radicals in chemical carcinogenicity. Environ Health Perspect 1997;105(Suppl. 4):875–82.
Pu YS, Yang SM, Huang YK, Chung CJ, Huang SK, Chiu AW, et al. Urinary arsenic profile affects the risk of urothelial carcinoma even at low arsenic exposure. Toxicol Appl Pharmacol 2007;218:99-106.
Ramanathan K, Balakumar BS, Panneerselvam C. Effects of ascorbic acid and alpha-tocopherol on arsenic-induced oxidative stress. Hum Exp Toxicol 2002;21:675–80.
Ramanathan K, Anusuyadevi M, Shila S, Panneerselvam C. Ascorbic acid and alpha-tocopherol as potent modulators of apoptosis on arsenic induced toxicity in rats. Toxicol Lett 2005;156:297–306.
Rusiecki JA, De RA, Lee WJ, Dosemeci M, Lubin JH, Hoppin JA, Blair A, Alavanja MC. Cancer incidence among pesticide applicators exposed to atrazine in the Agricultural Health Study. J Natl Cancer Inst 2004;96:1375–82.
Sampayo-Reyes A, Zakharyan RA. Inhibition of human glutathione S-transferase omega by tocopherol succinate. Biomed Pharmacother 2006;60:238–44.
Schoen A, Beck B, Sharma R, Dube E. Arsenic toxicity at low doses: epidemiological and mode of action considerations. Toxicol Appl Pharmacol 2004;198:253–67.
Shi H, Hudson LG, Ding W, Wang S, Cooper KL, Liu S, Chen Y, Shi X, Liu KJ. Arsenite causes DNA damage in keratinocytes via generation of hydroxyl radicals. Chem Res Toxicol 2004;17:871–8.
Snow ET, Sykora P, Durham TR, Klein CB. Arsenic, mode of action at biologically plausible low doses: What are the implications for low dose cancer risk? Toxicol Appl Pharmacol 2005;207:557–64.
Steinmaus C, Bates MN, Yuan Y, Kalman D, Atallah R, Rey OA, et al. Arsenic methylation and bladder cancer risk in case-control studies in Argentina and the United States. J Occup Environ Med 2006;48:478–88.
Tang FY, Shih CJ, Cheng LH, Ho HJ, Chen HJ. Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. Mol Nutr Food Res 2008;52:646–54.
Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
Verret WJ, Chen Y, Ahmed A, Islam T, Parvez F, Kibriya MG, et al. A randomized, double-blind placebo-controlled trial evaluating the effects of vitamin E and selenium on arsenic-induced skin lesions in Bangladesh. J Occup Environ Med 2005;47:1026–35.
Wei M, Arnold L, Cano M, Cohen SM. Effects of co-administration of antioxidants and arsenicals on the rat urinary bladder epithelium. Toxicol Sci 2005;83:237–45.
Woodside JV, McCall D, McGartland C, Young IS. Micronutrients: dietary intake v. supplement use. Proc Nutr Soc 2005;64:543–53.
Zakharyan RA, yala-Fierro F, Cullen WR, Carter DM, Aposhian HV. Enzymatic methylation of arsenic compounds. VII. Monomethylarsonous acid (MMAIII) is the substrate for MMA methyltransferase of rabbit liver and human hepatocytes. Toxicol Appl Pharmacol 1999;158:9-15.
Zeegers MP, Goldbohm RA, van den Brandt PA. Are retinol, vitamin C, vitamin E, folate and carotenoids intake associated with bladder cancer risk? Results from the Netherlands Cohort Study. Br J Cancer 2001;85:977–83.