

Population-Based Case–Control Study of Chinese Herbal Products Containing Aristolochic Acid and Urinary Tract Cancer Risk

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Background Consumption of Chinese herbs that contain aristolochic acid (eg, Mu Tong) has been associated with an increased risk of urinary tract cancer.

Methods We conducted a population-based case–control study in Taiwan to examine the association between prescribed Chinese herbal products that contain aristolochic acid and urinary tract cancer. All patients newly diagnosed with urinary tract cancer (case subjects) from January 1, 2001, to December 31, 2002, and a random sample of the entire insured population from January 1, 1997, to December 31, 2002 (control subjects), were selected from the National Health Insurance reimbursement database. Subjects who were ever prescribed more than 500 pills of nonsteroidal anti-inflammatory drugs and/or acetaminophen were excluded, leaving 4594 case patients and 174701 control subjects in the final analysis. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by using multivariable logistic regression models for the association between prescribed Chinese herbs containing aristolochic acid and the occurrence of urinary tract cancer. Models were adjusted for age, sex, residence in a township where black foot disease was endemic (an indicator of chronic arsenic exposure from drinking water [a risk factor for urinary tract cancer]), and history of chronic urinary tract infection. Statistical tests were two-sided.

Results Having been prescribed more than 60 g of Mu Tong and an estimated consumption of more than 150 mg of aristolochic acid were independently associated with an increased risk for urinary tract cancer in multivariable analyses (Mu Tong: at 61–100 g, OR = 1.6, 95% CI = 1.3 to 2.1, and at >200 g, OR = 2.1, 95% CI = 1.3 to 3.4; aristolochic acid: at 151–250 mg, OR = 1.4, 95% CI = 1.1 to 1.8, and at >500 mg, OR = 2.0, 95% CI = 1.4 to 2.9). A statistically significant linear dose–response relationship was observed between the prescribed dose of Mu Tong or the estimated cumulative dose of aristolochic acid and the risk of urinary tract cancer ($P < .001$ for both).

Conclusions Consumption of aristolochic acid–containing Chinese herbal products is associated with an increased risk of cancer of the urinary tract in a dose-dependent manner that is independent of arsenic exposure.

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Aristolochic acid nephropathy—a progressive form of renal interstitial fibrosis—was first reported in a group of young Belgian patients with end-stage renal disease in 1993 and was thought to be caused by the use of Chinese herbal medicines that contained aristolochic acid (1–3). Aristolochic acid has been shown to be associated with urothelial cancer in studies of clinical cases around the world, in animal models, and by the detection of aristolochic acid–DNA adducts in kidney and ureteral tissues (4–6). In 2002, the International Agency for Research on Cancer classified herbal remedies that contain plant species of the genus *Aristolochia*, which contain a high level of aristolochic acid, as carcinogenic in humans (ie, group 1 carcinogens) (7). Consequently, many countries have since banned the use of herbs containing aristolochic acid, including Taiwan, in November of 2003 (8–10). Before this time,

however, products containing these herbs were widely prescribed in Taiwan.

In Taiwan, there have been case reports of renal failure associated with the use of Chinese herbal products (11,12) and herbs that contain aristolochic acid (13). However, to our knowledge, there have been no reports of urinary tract cancer associated with the use of herbs or herbal products containing aristolochic acid. In March of 1995, Taiwan established the National Health Insurance (NHI) program, which covers more than 96% of Taiwanese residents (14). The NHI routinely reimburses enrollees for the cost of prescribed medicines, including Chinese herbal products containing aristolochic acid, which were widely prescribed before the ban in 2003. We used the NHI reimbursement database to conduct a population-based case–control study to examine the association

CONTEXT AND CAVEATS

Prior knowledge

Consumption of Chinese herbs that contain aristolochic acid has been associated with an increased risk of urinary tract cancer.

Study design

A population-based case-control study in Taiwan to examine the association between prescribed Chinese herbal products that contain aristolochic acid and urinary tract cancer. The analysis controlled for chronic arsenic exposure in drinking water (a risk factor for urinary tract cancer).

Contribution

Prescribed aristolochic acid-containing Chinese herbal products were associated with an increased risk of urinary tract cancer in a dose-dependent manner that was independent of arsenic exposure.

Implications

Products that contain any amount of aristolochic acid may carry substantial risk for urinary tract cancer, and continued surveillance of herbs or Chinese herbal products that might be adulterated with aristolochic acid-containing herbs is recommended.

Limitations

Not all of the diagnoses were confirmed by histopathology reports. Subjects may have taken additional nephrotoxic herbs or agents that were not prescribed. Actual intakes of the prescribed herbal products recorded in the database were not validated. Smoking history was not taken into account.

From the Editors

between having been prescribed Chinese herbal products that contain substantial amounts of aristolochic acid, including Guan Mu Tong and Guang Fangchi, and the risk of urinary tract cancer. In addition to smoking, chronic exposure to arsenic in drinking water, which causes an endemic peripheral vascular disease called black foot disease in specific areas of Taiwan, has been documented to be associated with an increased incidence of bladder cancer (15–17). We conducted this case-control study in Taiwan to examine the association between urinary tract cancers and having been prescribed Chinese herbal products that contain aristolochic acid and a potential dose-response relationship. In particular, we controlled for the potential confounding effect of arsenic exposure.

Materials and Methods

Study Population and Data Collection

This study was initiated after approval by the review board of the Committee on Chinese Medicine and Pharmacy, Department of Health, Taiwan. It was designed as a population-based case-control study to investigate associations between having been prescribed Chinese herbal products and the occurrence of urinary tract cancer in Taiwan between January 1, 2001, and December 31, 2002. All data were obtained from the NHI reimbursement database. The National Health Research Institutes of Taiwan (in Chunan, Taiwan) anonymized and maintained the NHI reim-

bursement data as files suitable for research (18). The identification numbers of all individuals with reimbursement data in the NHI database were encrypted to protect the privacy of the individuals. These files provided detailed demographic data (including birth date and sex) and information regarding health-care services provided for each patient, including all payments for outpatient visits, hospitalizations, and prescriptions, as well as where each patient lived. The data for each outpatient visit or hospitalization contained up to five diagnoses that were coded according to the *International Classification of Diseases, Ninth Revision (ICD-9)* classification (19), all drugs prescribed and the doses (ie, conventional medicines, including generic and commercial brands of acetaminophen [paracetamol] and nonsteroidal anti-inflammatory drugs, as well as Chinese herbal products), and the date of each prescription. During the study period (ie, from January 1, 1997, to December 31, 2002), all prescribed medications were covered under the NHI of Taiwan and no drug could be dispensed at a pharmacy without a doctor's prescription.

To select potential case subjects for this study, we first obtained the NHI catastrophic illness registry files for all patients who were diagnosed with urinary tract cancer or end-stage renal disease from January 1, 1997, to December 31, 2002. Because all patients who are registered as having a catastrophic illness are exempt from all copayments, their data are very comprehensive. A diagnosis of urinary tract cancer or end-stage renal disease made by doctors and officials of the NHI is usually accurate: Urinary tract cancer must be proven by tissue pathology and is classified as cancer of the upper urinary tract, which includes the renal pelvis and ureter (*ICD-9* codes 189.1 and 189.2, respectively) or bladder cancer (*ICD-9* code 188). The database contained 20777 prevalent cases of urinary tract cancer that were diagnosed from January 1, 1997, to December 31, 2002. Within this population, we identified 5995 patients who were newly diagnosed with urinary tract cancer from January 1, 2001, to December 31, 2002, to allow at least 4 years between January 1, 1997, and the date of diagnosis to give sufficient time for case subjects to accumulate sufficient doses of herbal products to induce urinary tract cancer. The average incidence rate of urinary tract cancer for these newly diagnosed patients was 105 per million person-years.

The control group consisted of a 200000-person random sample of the entire insured population in Taiwan (approximately 22.5 million persons) from January 1, 1997, to December 31, 2002, and was representative of the insured population in terms of sex and age (18). We excluded control subjects with incomplete data for age or sex ($n = 152$) and those with any diagnosis related to urinary tract cancer ($n = 145$).

Most epidemiological studies have found the analgesic phenacetin to be a risk factor for urinary tract cancer (20), whereas the available evidence for acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) as risk factors for urinary tract cancer are inconclusive and discrepant (21,22). Phenacetin was banned by the Department of Health of Taiwan in 1986 and has not been prescribed ever since. Because the use of 600–1000 pills of acetaminophen, NSAIDs, or mixed analgesics has been associated with an increased risk of renal damage or renal cancer in previous studies (23,24), to prevent confounding of our results by analgesic use, we excluded subjects who were ever prescribed more than a total of 500 pills of acetaminophen and/or NSAIDs (1401 case subjects and

25 002 control subjects), which left 4594 new urinary tract cancer case subjects and 174 701 control subjects in the final analysis.

Exposure Assessment

According to standard prescriptions recommended by the Committee on Chinese Medicine and Pharmacy in Taiwan (25), Chinese herbal products produced before new regulations were promulgated in November of 2003 might include the following herbs containing aristolochic acid: Ma Dou Ling (*Fructus Aristolochiae*), Tian Xian Teng (*Caulis Aristolochiae*), Xi Xin (*Asarum heterotropoides*), Guan Mu Tong (*Aristolochia manshuriensis*), Guang Fangchi (*Aristolochia fangchi*), and Qing Mu Xiang (*Radix Aristolochiae*). Of these, Guan Mu Tong and Guang Fangchi were once sold under the names of Mu Tong (*Akebia* species) and Fangchi (*Stephania* species), respectively. Several studies revealed that 89.2%–100% of Fangchi preparations contained Guang Fangchi (26–28), and more than 84% of Mu Tong preparations contained Guan Mu Tong (29). These herbs were taken as single products or were components of mixed herbal formulas that are recommended by ancient Chinese medicine books (eg, Mu Tong in the Long Dan Xie Gan mixture). Because the prescription data from the NHI database can be linked directly to the actual drug(s) prescribed by means of the product number, we were able to identify all subjects who had taken these Chinese herbal products. In addition, each pharmaceutical company in Taiwan has published and submitted the detailed composition of each of its products to the Committee on Chinese Medicine and Pharmacy to be approved for registration. By using this information, we determined the original amounts of herbs, in grams, for each mixture of Chinese herbal products, and the total dose of each aristolochic acid-containing herb (eg, Mu Tong, Fangchi, and Xi Xin) during the exposure period from January 1, 1997, to the date of diagnosis of urinary tract cancer or December 31, 2002, if censored, was summed for each subject included in this study. To allow a minimal induction time for an exposed subject to develop urinary tract cancer, we calculated the cumulative dose for each herb prescribed to an individual up to 1 year before the diagnosis of urinary tract cancer. We also calculated the estimated cumulative dose of aristolochic acid for each subject using the following estimates obtained in previous studies: The estimated average doses of aristolochic acid per 1 g of Guan Mu Tong, Guang Fangchi, and Xi Xin were 2.59, 2.04, and 0.042 mg, respectively (26,28–31).

The reimbursement database also listed the townships in which all subjects lived. We identified subjects who lived in the four townships in Taiwan that have been reported to be areas endemic for black foot disease—Pu-Tai and Yi-Chu in Chiayi County and Hsueh-Chia and Pei-Men in Tainan County (16,17)—and controlled for this factor as a surrogate for arsenic exposure. Because aristolochic acid is also associated with end-stage renal disease (1,2), we also linked the files of patients with urinary tract cancer to the catastrophic illness registry for end-stage renal disease to determine if there were overlapping case subjects with exposures to aristolochic acid and arsenic.

According to the Committee on Chinese Medicine and Pharmacy (25), Mu Tong is usually prescribed for the treatment of hepatitis, urinary tract infection, rhinitis, dysmenorrhea, and eczema. Recurrent or chronic urinary tract infection associated with

schistosomiasis or prolonged indwelling catheters in patients with spinal cord injury is associated with an increased risk of bladder cancer (15,32), whereas urinary tract infection from other causes has not shown a consistent association with risk of bladder cancer (15,33). Hence, we defined patients with chronic urinary tract infection as those who had such a diagnosis at least 12 times up to 1 year before the diagnosis of urinary tract cancer, and we controlled for this potential confounder during the risk-estimate analysis.

Statistical Analyses

We used univariate and multivariable logistic regression models to assess the independent association of various risk factors with new occurrences of urinary tract cancer in case patients and control subjects. Potential risk factors included age, sex, residence in a township where black foot disease was endemic, history of chronic urinary tract infection, and cumulative doses of prescribed aforementioned Chinese herbs containing aristolochic acid before the diagnosis of urinary tract cancer. For each potential risk factor, the odds ratio (OR) for the occurrence of urinary tract cancer and the 95% confidence interval (CI) were estimated. We constructed two logistic regression models for two different types of exposure assessment: prescribed dosages of Chinese herbs (model 1) and different estimated dosages of aristolochic acid as risk factors (model 2). A Mantel extension test for linear trend was conducted by the Mantel–Haenszel method for the adjusted odds ratios of developing urinary tract cancer under different prescribed doses of Mu Tong, Fangchi, and Xi Xin for model 1 and by estimated doses of aristolochic acid for model 2. On the basis of our previous study on prescribed Chinese herbal products and chronic kidney disease (34), we initially classified the doses of Mu Tong, Fangchi, and Xi Xin by increments of 1, 30, 60, 100, and 200 g. Then, we combined several categories together to simplify the table according to the tendency of increased risk. We also tried to directly apply the number of grams of Mu Tong or milligrams of aristolochic acid as a continuous variable to fit the model and presented the increased risks in 30-g increments of doses of Mu Tong or 100-mg increments of aristolochic acid. χ^2 tests for category variables (sex, having end-stage renal disease history) and t tests for continuous variables (age) were used to compare differences in clinical features among urinary tract cancer patients who consumed more than 60 g of Mu Tong and among urinary tract cancer patients who lived in a township where black foot disease was endemic. All the above analyses were conducted using the SAS statistical software (version 9.2; SAS Institute Inc, Cary, NC). All statistical tests were two-sided.

Results

In logistic regression model 1, factors that were independently associated with an increased risk for a new occurrence of urinary tract cancer after adjustment for other risk factors were being male (OR = 1.7, 95% CI = 1.6 to 1.8), older age, residence in a township where black foot disease was endemic (OR = 4.4, 95% CI = 3.4 to 5.8), having history of chronic urinary tract infection (OR = 1.6, 95% CI = 1.3 to 2.1), and having been prescribed more than 60 g of Mu Tong (for 61–100 g, OR = 1.6, 95% CI = 1.3 to 2.1; for 101–200 g, OR = 2.0, 95% CI = 1.4 to 2.7; for >200 g, OR = 2.1, 95% CI = 1.3 to 3.4) (Table 1). In logistic regression model 2, we

replaced the variables of prescribed Chinese herbs with estimated doses of aristolochic acid and found that estimated aristolochic acid doses greater than 150 mg were independently associated with an increased risk for occurrence of urinary tract cancer after adjustment for all other risk factors (for 151–250 mg, OR = 1.4, 95% CI = 1.1 to 1.8; for 251–500 mg, OR = 1.6, 95% CI = 1.2 to 2.1; for >500 mg, OR = 2.0, 95% CI = 1.4 to 2.9). A statistically significant ($P < .001$) linear dose–response relationship was present between the risk of developing urinary tract cancer and the prescribed dose of Mu Tong and the estimated intake of aristolochic acid. There was also a linear dose–response trend when we applied the exposure variable as a continuous variable for every 30-g increment of prescribed Mu Tong or 100-mg increment of estimated aristolochic acid (Table 1), and there was no statistically significant interaction between residence in a township where black foot disease was endemic and having been prescribed aristolochic acid–containing herbs. More than 100 g of Fangchi (OR = 3.1, 95% CI = 2.1 to 4.5) or more than 300 g of Xi Xin (OR = 2.4, 95% CI = 1.6 to 3.8) was statistically significantly associated with an increased crude odds ratio for urinary tract cancer. However, the adjusted odds ratios were not statistically significant for subjects who were prescribed high cumulative doses of Fangchi or Xi Xin. The numbers of prescriptions for Ma Dou Ling, Tian Xian Teng, or Qing Mu Xiang were very small, and none of these herbs was statistically significantly associated with the risk of urinary tract cancer (data not shown).

The case patients were further stratified into two subgroups: those diagnosed with cancer of the upper urinary tract (43%; $n = 1985$) and those diagnosed with bladder cancer (57%; $n = 2609$). In a multivariable logistic regression model adjusted for age and sex, there was a statistically significant association between residing in a township where black foot disease was endemic or having been prescribed more than 60 g of Mu Tong and an increased risk for the occurrence of bladder cancer (Table 2). There was a statistically significant ($P < .001$) linear dose–response relationship between having been prescribed more than 60 g of Mu Tong or estimated consumption of more than 150 g of aristolochic acid and risk of occurrence of bladder cancer.

We next examined the clinical features of urinary tract cancer case subjects who were prescribed more than 60 g of Mu Tong ($n = 118$) or who lived in a township where black foot disease was endemic ($n = 88$) (Table 3). In general, urinary tract cancer case subjects who were prescribed more than 60 g of Mu Tong were younger, had a higher male to female ratio, and more often experienced end-stage renal disease before the occurrence of urinary tract cancer than those who lived in a township where black foot disease was endemic. In fact, none of the case subjects who lived in a township where black foot disease was endemic had end-stage renal disease before the occurrence of urinary tract cancer. The fact that only two case subjects with urinary tract cancer (one with upper urinary tract cancer and one with bladder cancer) lived in a township endemic for black foot disease and were ever prescribed more than 60 g of Mu Tong indicates that these two risk factors are independent.

Discussion

This population-based study is the first study to our knowledge to document a linear dose–response relationship between prescribed

Chinese herbal products containing aristolochic acid and the risk of urinary tract cancer after controlling for confounding by age, sex, living in a township endemic for black foot disease (a surrogate of arsenic contamination in the water supply), and history of chronic urinary tract infection. This study has a number of strengths that deserve attention. Because the NHI reimbursement database collects all prescription information prospectively, we can rule out the possibility of recall bias for the intake doses of various Chinese herbal products. Because we included all patients newly diagnosed with urinary tract cancer in Taiwan from 1997 to 2002, and because the control subjects in this study were selected from a simple random sampling of the insured general population, we can also rule out the possibility of selection bias. In fact, our estimate of 105 new urinary tract cancer cases per million person-years in this study is practically the same as the 106 cases per million person-years calculated from 2001 data of the Taiwanese National Cancer Registry (35). Because the Belgian patients who were diagnosed with urinary tract cancer after using aristolochic acid–containing Fangchi for an average of 15 months and 9 months after stopping the use of Fangchi (4,5), we allowed a minimum induction time of 1 year and calculated the cumulative dose up to 1 year before diagnosis. Thus, this study design allowed us to detect most, if not all, cases of aristolochic acid–associated urinary tract cancer. Moreover, to prevent potential confounding of our results by analgesic nephropathy, we excluded case and control subjects with more than a moderate consumption (ie, 500 pills) of analgesics. Finally, we categorized subjects according to their residence in townships endemic for black foot disease (a surrogate of high arsenic exposure), and, by controlling for the potential confounding effects of this risk factor in multivariable logistic regression models, we were able to clearly separate the carcinogenic effect of arsenic exposure from that of exposure to Chinese herbal products. We further demonstrated that distinctive clinical features are associated with exposure to arsenic and Chinese herbal products, indicating no confounding effect of each on the other. Thus, we concluded that a dose–response relationship exists between aristolochic acid–associated Chinese herbal products and urinary tract cancer.

Black foot disease is a peripheral vascular disease that has been endemic to the coastal region of Taiwan for the past 60 years, is related to the water derived from artesian wells containing arsenic, and has been documented to be associated with an increased incidence of bladder cancer (16,17). Our multivariable logistic regression models corroborated the fact that living in a township where black foot disease was endemic was independently associated with an increased risk of cancer of the bladder and upper urinary tract. In addition, comparison of clinical features of patients with these cancers indicated that no patients with urinary tract cancer associated with black foot disease (or arsenic exposure) developed end-stage renal disease before the occurrence of cancer. In fact, there were only two cases of urinary tract cancer who were simultaneously associated with having been prescribed more than 60 g of Mu Tong and with living in a township endemic for black foot disease. Hence, these findings indicate that exposure to more than 60 g of Mu Tong and to arsenic are two independent risk factors for urinary tract cancer.

In this study, the association between the cumulative doses of aristolochic acid–containing herbs and the risk of occurrence

Table 1. Frequency distributions of various risk factors and crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for new occurrence of urinary tract cancer from multivariable logistic regression models*

Risk factor	Case subjects, N = 4594	Control subjects, N = 174701	Crude OR (95% CI)	Model 1		Model 2	
				Adjusted OR (95% CI)†	P	Adjusted OR (95% CI)‡	P
Sex							
Female	1566	83671	1.0 (Referent)	1.0 (Referent)	<.001	1.0 (Referent)	<.001
Male	3028	91030	1.8 (1.7 to 1.9)	1.7 (1.6 to 1.8)		1.7 (1.6 to 1.8)	
Age, y							
<40	199	115789	1.0 (Referent)	1.0 (Referent)	<.001	1.0 (Referent)	<.001
40–59	1194	42260	16.4 (14.1 to 18.9)	16.2 (14.0 to 18.9)		16.1 (13.9 to 18.8)	
60–74	1932	11308	99.3 (85.8 to 115)	96.3 (83.1 to 112)		95.5 (82.4 to 111)	
75–99	1269	5344	138 (119 to 161)	135 (116 to 158)		135 (116 to 157)	
Residence in township where black foot disease was endemic							
No	4506	174151	1.0 (Referent)	1.0 (Referent)	<.001	1.0 (Referent)	<.001
Yes	88	550	6.2 (4.9 to 7.8)	4.4 (3.4 to 5.8)		4.4 (3.4 to 5.8)	
Chronic UTI							
No	4511	174091	1.0 (Referent)	1.0 (Referent)	<.001	1.0 (Referent)	<.001
Yes	83	610	5.3 (4.2 to 6.6)	1.6 (1.3 to 2.1)		1.6 (1.3 to 2.1)	
Mu-Tong, total amount prescribed, g							
0	3987	149464	1.0 (Referent)	1.0 (Referent)	.016	NA	
1–60	489	22354	0.8 (0.7 to 0.9)	1.0 (0.9 to 1.2)		1.0 (Referent)	
61–100	50	1485	1.3 (0.95 to 1.7)	1.6 (1.3 to 2.1)		1.6 (1.3 to 2.1)	
101–200	46	1003	1.7 (1.3 to 2.3)	2.0 (1.4 to 2.7)		2.0 (1.4 to 2.7)	
> 200	22	395	2.1 (1.4 to 3.2)	2.1 (1.3 to 3.4)		2.1 (1.3 to 3.4)	
Each 30-g increase†	NA	NA	1.1 (1.01 to 1.1)	1.1 (1.06 to 1.15)		1.1 (1.06 to 1.15)	
Fangchi, total amount prescribed, g							
0	3927	150456	1.0 (Referent)	1.0 (Referent)		1.0 (Referent)	
1–60	623	23456	1.0 (0.9 to 1.1)	0.9 (0.8 to 1.0)	.689	0.9 (0.8 to 1.0)	.121
61–100	15	427	1.3 (0.8 to 2.2)	0.7 (0.4 to 1.2)	.293	0.7 (0.4 to 1.2)	.180
> 100	29	362	3.1 (2.1 to 4.5)	1.3 (0.9 to 2.0)	<.001	1.3 (0.9 to 2.0)	.181
Xi-Xin, total amount prescribed, g							
0	3680	139385	1.0 (Referent)	1.0 (Referent)		1.0 (Referent)	
1–100	839	33072	1.0 (0.9 to 1.04)	1.1 (1.003 to 1.2)	.303	1.1 (1.003 to 1.2)	.044
101–300	54	1917	1.1 (0.8 to 1.4)	0.7 (0.4 to 1.2)	.641	0.7 (0.4 to 1.2)	.246
> 300	21	327	2.4 (1.6 to 3.8)	1.3 (0.9 to 2.0)	<.001	1.3 (0.9 to 2.0)	.412
Aristolochic acid, estimated total consumption, mg							
0	3274	121820	1.0 (Referent)	1.0 (Referent)		1.0 (Referent)	
1–150	1151	48869	0.9 (0.8 to 0.9)	0.9 (0.8 to 0.9)	<.001	1.0 (0.96 to 1.1)	.348
151–250	69	2032	1.3 (0.99 to 1.6)	1.3 (0.99 to 1.6)	.59	1.4 (1.1 to 1.8)	.012
251–500	64	1403	1.7 (1.3 to 2.2)	1.7 (1.3 to 2.2)	<.001	1.6 (1.2 to 2.1)	<.001
> 500	36	577	2.3 (1.7 to 3.3)	2.3 (1.7 to 3.3)	<.001	2.0 (1.4 to 2.9)	<.001
Each 100-mg increase†	NA	NA	1.1 (1.03 to 1.1)	1.1 (1.03 to 1.1)	<.001	1.1 (1.06 to 1.13)	<.001

* NA = not applicable; UTI = urinary tract infection.

† Estimation of OR based on continuous variable for every 30-g increase of Mu Tong or 100-mg increase of aristolochic acid.

‡ Logistic regression models for different dosages of Chinese herbs (model 1) and different estimated dosages of aristolochic acid as risk factors (model 2) were adjusted for age, sex, residence in a township where black foot disease was endemic, and history of chronic UTI.

Table 2. Adjusted odds ratios (OR) for the development of upper urinary tract cancer and bladder cancer by multivariable logistic regression models*

Variable	Upper UTC			Bladder cancer		
	n = 1985	Adjusted OR (95% CI)	P	n = 2609	Adjusted OR (95% CI)	P
Residence in township endemic for black foot disease						
No	1953	1.0 (Referent)		2553	1.0 (Referent)	
Yes	32	3.8 (2.6 to 5.6)	<.001	56	5.0 (3.6 to 6.9)	<.001
Chronic UTI						
No	1965	1.0 (Referent)		2546	1.0 (Referent)	
Yes	20	0.9 (0.6 to 1.4)	.585	63	2.3 (1.7 to 3.0)	<.001
Mu-Tong, total amount prescribed, g						
0	1698	1.0 (Referent)		2289	1.0 (Referent)	
1–60	239	1.1 (0.9 to 1.3)	.244	250	1.0 (0.8 to 1.2)	.762
61–100	22	1.5 (0.9 to 2.3)	.084	28	1.7 (1.1 to 2.6)	.011
101–200	19	1.8 (1.1 to 2.9)	.026	27	2.2 (1.4 to 3.4)	<.001
> 200	7	1.3 (0.6 to 3.0)	.530	15	2.8 (1.6 to 5.1)	<.001
Fangchi, total amount prescribed, g						
0	1684	1.0 (Referent)		2243	1.0 (Referent)	
1–60	282	0.9 (0.8 to 1.0)	.093	341	1.0 (0.8 to 1.1)	.610
61–100	6	0.6 (0.3 to 1.4)	.250	9	0.7 (0.4 to 1.5)	.394
>100	13	1.3 (0.7 to 2.4)	.350	16	1.3 (0.8 to 2.3)	.306
Xi-Xin, total amount prescribed, g						
0	1556	1.0 (Referent)		2124	1.0 (Referent)	
1–100	391	1.1 (1.0 to 1.3)	.087	448	1.1 (0.96 to 1.2)	.211
101–300	27	1.0 (0.6 to 1.5)	.859	27	0.7 (0.5 to 1.1)	.173
>300	11	1.8 (0.9 to 3.5)	.099	10	0.9 (0.4 to 1.8)	.706

* ORs adjusted for age, sex, residence in a township where black foot disease was endemic, and history of chronic UTI. CI = confidence interval; UTC = urinary tract cancer; UTI = urinary tract infection.

of urinary tract cancer seemed to be dose-dependent. According to the annual reports of National Laboratory of Food and Drug in Taiwan, 1 g of Guan Mu Tong and Guang Fangchi was estimated to contain 2.59 and 2.04 mg of aristolochic acid, respectively (26,28,29). Thus, a cumulative dose of 60 g of Mu Tong and of 100 g of Fangchi in Chinese herbal products contains approximately 155 and 204 mg, respectively, of aristolochic acid, which is slightly higher than the 147-mg aristolochic acid dose reported in the Belgian reports (2,4). In this study, we observed a consistent dose-response relationship between the estimated intake of aristolochic acid (or prescribed dose of aristolochic acid-containing Mu Tong) and urinary tract cancer, which suggests that aristolochic

acid may be the agent responsible for carcinogenicity. In this study, more than 100 g of Fangchi was statistically significantly associated with an increased crude odds ratio for urinary tract cancer; however, the association did not reach statistical significance after adjustment for other risk factors, probably because of the small number of case subjects. By contrast, Xi Xin contains only minute amounts of aristolochic acid, or approximately 0.009–0.042 mg aristolochic acid per g, which is approximately 1:50 to 1:200 of the amount in *Aristolochia fangchi* (30,31). At the prescribed median daily dose of 0.9 g for Xi Xin in this study (34), exposure to more than 155 mg of aristolochic acid would take longer than 10 years. Thus, we were unable to detect any

Table 3. Clinical features of patients with urinary tract cancer who were prescribed more than 60 g of Mu Tong or lived in an area endemic for black foot disease*

Feature	Upper UTC			Bladder cancer		
	Prescribed >60 g of Mu Tong (n = 48)	Resident of black foot disease-endemic area (n = 32)	P	Prescribed >60 g of Mu Tong (n = 70)	Resident of black foot disease-endemic area (n = 56)	P
Mean age, y (SD)	60.6 (12.3)	66.2 (10.3)	.303†	63.4 (11.9)	69.5 (9.5)	.098†
Male to female ratio	1.1	0.8	.430†	1.5	1.4	.889†
ESRD before UTC occurrence, %	6.3	0	.213‡	17.1	0	.001‡
Resident of black foot disease-endemic area, %	2.1	100	NA	1.4	100	NA
Prescribed >60 g of Mu Tong, %	100	3.1	NA	100	1.8	NA

* ESRD = end-stage renal disease; NA = not applicable; UTC = urinary tract cancer.

† Two-sided t test.

‡ Two-sided χ^2 test.

association between prescription of Chinese herbal products containing Xi Xin (*Asarum heterotropoides*) and urinary tract cancer. However, careful attention should still be paid regarding the use of Chinese herbal products containing Xi Xin, and long-term follow-up should be provided for subjects who regularly consume these products.

In this study, 43% of urinary tract cancer cases were upper urinary tract cancer, which is similar to rates reported by the National Cancer Registry (35) and in a previous clinical report of pathology-confirmed urinary tract cancer cases in Taiwan (36). These rates are much higher than those in other countries, in which less than 10% of all urinary tract cancer cases are upper urinary tract cancer (37). In this study, prescription of Chinese herbal products was associated with urothelial cancers that occurred in all parts of the urinary tract, similar to what was reported in a recent case series of Belgian women who received kidney transplants for end-stage aristolochic acid nephropathy in which 44.7% had upper urinary tract cancer and 39.5% had bladder cancer (38). Thus, we suggest that aristolochic acid induces urothelial cancers in the upper urinary tract and bladder with approximately equal tendency.

There are some limitations to this study. First, because patient identities were not available from the NHI reimbursement database, we were unable to obtain any histopathology reports to confirm the diagnoses. However, because the approval for registering urinary tract cancer as a catastrophic illness is based on pathology and/or cytology evidence and is followed by a full waiver of copayment, such a diagnosis is made only after very serious consideration and is always accurate. The accuracy of diagnosis of urinary tract cancer from the NHI data is corroborated by the high agreement between the calculated incidence rate from this study and that from the National Cancer Registry of Taiwan, in which 95% of bladder cancers and 91%–92% of upper urinary tract cancers have histopathologic proof (35). Second, we were unable to contact the patients directly about their use of herbs because of anonymization of their identification numbers; therefore, we could not rule out that subjects might have taken additional nephrotoxic herbs or agents that were not prescribed. However, because the NHI system has comprehensive coverage and the copayment for prescriptions is universally 50 NT\$ (new Taiwan) (approximately equal to US \$1.5), which is generally less than the cost of herbs sold in Taiwan's markets, the likelihood that subjects purchased other aristolochic acid-containing herbs, nephrotoxic drugs, or alternative medicines without a prescription is low. Third, we were unable to validate the actual intake of the prescribed herbal products recorded in the database. Because 95% of the dosing frequencies for Chinese herbal products were only for 1 week (39), a large cumulative dose indicates that the patient continued receiving the same prescription for a long period of time and implies that the patient actually consumed the prescribed medication. Even if the patient did not take all of the prescribed medications, our findings would underestimate the effect of aristolochic acid-related Chinese herbal products. Fourth, because the reimbursement data did not include smoking history, we were unable to control for this factor in the model construction. Because the smoking rates for men and women in Taiwan in the last two decades ranged from 47% to 62% and from 2.3% to 5.3%, respec-

tively, (40), we assumed that sex was, in part, a surrogate variable for smoking.

In conclusion, having been prescribed more than 60 g of Mu Tong or more than 150 mg aristolochic acid from Chinese herbal products was associated with an increased risk of developing urinary tract cancer. The linear dose–response relationship between the estimated level of aristolochic acid and urinary tract cancer might be useful for considering a complete ban, or for establishing limits, on the consumption of herbal products and/or herbs that contain low amounts of aristolochic acid (41). Care must still be taken for prescribing doses less than 60 g of Mu Tong or 150 mg of aristolochic acid because there might be no threshold dose under a linear dose–response relationship and given recent evidence for an increased risk of chronic kidney disease associated with a prescribed dose of 30–60 g of Mu Tong (34). In this study, from 2001 to 2002, there were 118 new cases of urinary tract cancer in Taiwan associated with the ingestion of more than 60 g of the Chinese herb Mu Tong, which represents 3% of all new patients with urinary tract cancer. In addition to a ban on products that contain any amount of aristolochic acid, we also recommend continued surveillance of herbs or Chinese herbal products that might be adulterated with aristolochic acid-containing herbs. Finally, patients with a history of aristolochic acid nephropathy or consumption of Mu Tong or Fangchi before they were banned should be monitored regularly for urinary cancer.

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