Research Article

Cancer Epidemiology, Biomarkers & Prevention

Risk Assessment of Upper Tract Urothelial Carcinoma Related to Aristolochic Acid

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Abstract

Background: Aristolochic acid is a toxin found in plants of the genus *Aristolochia*, to which humans can be exposed either through certain Chinese herbal medicines or through inadvertent commingling with food crops. Our objective was to estimate cumulative exposures of aristolochic acid associated with increased risk of end-stage renal disease (ESRD), and to conduct a systematic review and meta-analysis on aristolochic acid-induced upper tract urothelial carcinoma (UUC).

Methods: Using epidemiologic studies on aristolochic acid-related disease from multiple different regions of the world, a systematic review was conducted in which relative risks (RR), HRs, and ORs were derived or extracted directly, and a meta-analysis was conducted. One study was used to estimate a benchmark dose lower confidence limit (BMDL) for aristolochic acid-related ESRD.

Results: Mean values for risk ratios, ORs, RRs, or HRs, of UUC caused by aristolochic acid ranged from 1 to 49. A meta-analysis of these studies resulted in a pooled OR of 5.97 [95% confidence interval (CI), 2.78–12.84] for this aristolochic acid-related cancer. The obtained BMDL for aristolochic acid-related ESRD was 0.42 g cumulative aristolochic acid exposure.

Conclusions: Aristolochic acid exposure is significantly associated with an increased risk of UUC, and there is a dose-dependent relationship between cumulative aristolochic acid exposure and ESRD risk.

Impact: Individuals who use certain Chinese herbal medicines may significantly increase their risk of developing UUC and/or ESRD, as would individuals who are inadvertently exposed to aristolochic acid through commingling of *Aristolochia* plants with harvested food crops. *Cancer Epidemiol Biomarkers Prev; 22(5); 812–20. ©2013 AACR.*

Introduction

Aristolochic acid is a toxin found in plants of the genus *Aristolochia*. For millennia, *Aristolochia* plants have been used for medicinal purposes, particularly for women in childbirth to ease labor and delivery. Since then, these plants have been used by populations worldwide to treat diverse health conditions. Nearly 200 years ago, the toxicity of certain *Aristolochia* species was recognized in humans and animals (1). However, *Aristolochia* plants containing aristolochic acid are still commonly used in certain herbal medicines, particularly specific Chinese herbal medicines that have been used worldwide (2–4). There may also be inadvertent dietary exposure to aristolochic acid in certain parts of the world. Indeed, a recent commentary by researchers in the International Agency for Research on Cancer (IARC) states that aristolochic acid-associated cancer may have global proportions (5).

In the 1990s, aristolochic acid was identified as the agent causing severe renal diseases in Belgian women who had consumed weight-loss supplements containing *Aristolochia fangchi*. In these women, rapidly progressive renal interstitial fibrosis led ultimately to chronic renal failure, and about 5% of the women developed end-stage renal disease (ESRD). Nearly all the patients with ESRD who had consumed aristolochic acid had developed either upper tract urothelial carcinoma (UUC) or urothelial dysplasia; an unusually high rate for patients with ESRD in general (2–5). Yet, these well-studied cases likely represent only a small proportion of the total number of aristolochic acid nephropathy (AAN) cases worldwide. Nearly a million kilograms of *Aristolochia* plants are harvested each year in China alone for medicinal purposes (1). In Asian nations, they are often marketed under the medicinal names *Guan mutong* (*MuTong, mu tong*) or *Guang fangchi* (*fangji*). All herbal medicines suspected to contain aristolochic acid are listed in IARC Monograph 100A (6). IARC has classified plants containing aristolochic acid as a Group 1 human carcinogen (6).

In addition, certain populations worldwide may suffer inadvertent aristolochic acid exposure. Aristolochic acid-specific DNA adducts were found in renal tissue from

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patients with Balkan endemic nephropathy (BEN; ref. 6). BEN is a chronic, progressive renal disease found in rural communities along tributaries of the Danube River in Serbia, Bosnia, Croatia, Bulgaria, and Romania, and is strongly associated with UUC. Because of the unusual epidemiology of BEN (occurrence only in particular farming villages, and familial but not inherited pattern of disease), scientists had for decades speculated that an environmental agent could be causing the disease. Various agents, including aristolochic acid, ochratoxin A (a nephrotoxic mycotoxin found in multiple agricultural commodities), heavy metals, selenium deficiency, and Pliocene coal deposits leaching aromatic hydrocarbons into well water, have all been postulated in the last 50 years as causing BEN. Most have been ruled out, such as heavy metals and selenium deficiency (7). Because of BEN's similarities in pathophysiology to AAN in the herbal medicine cases in Belgium, aristolochic acid was suspected to be a cause of BEN. Recently, aristolactam-DNA (AL-DNA) adducts were found in the renal cortex of patients with BEN (8), lending support to the hypothesis that aristolochic acid is the causal agent of this disease. As Balkan populations do not typically consume Chinese herbal medicines, the exposure route is believed to be consumption of bread in which seeds from the weed *Aristolochia clematitis* had commingled with wheat grain (9).

Although epidemiologic data for aristolochic acid-related diseases are limited in comparison with those for other dietary toxins such as aflatoxin (10–12), several key studies have assessed not only the increased risk of a particular disease due to aristolochic acid exposure, but have also correlated disease incidence with different cumulative aristolochic acid doses. The aims of this article were to review these epidemiologic studies, to estimate aristolochic acid doses associated with nontrivial risk of ESRD, and to conduct a systematic review and meta-analysis on the studies that estimate risk of aristolochic acid-related UUC.

Materials and Methods

We conducted a literature search in June 2012 on articles in the PubMed database concerning the effects of aristolochic acid exposure in human populations. Search terms used without restriction included combinations of: aristolochic acid, guang fangchi/fangji, guan mutong/mu tong, ESRD, BEN, Chinese herb(s) nephropathy, urothelial carcinoma, transitional cell carcinoma, and dose–response. From the retrieved articles, we reviewed reference lists to identify further relevant studies. These studies provided the basis for our dose–response assessment and meta-analysis. The systematic review and meta-analysis were conducted and reported in adherence to PRISMA standards for meta-analyses (13).

For the dose–response assessment, we identified one epidemiologic study that found at least 4 different cumulative doses of aristolochic acid and quantified their corresponding effects in the humans who consumed those doses. To these data, we applied benchmark dose (BMD) modeling to estimate a point of departure for aristolochic acid risk assessment purposes. BMD modeling is a methodology applied in dose–response assessment to find the lowest dose of a toxin expected to have biologic significance. Compared with the former method of using no observed effect levels as a point of departure, BMD modeling involves finding a statistical model that best fits the entire dose–response curve; then, from that model, identifying the dose corresponding to a certain proportion (often 10%) of adverse response in the study population. This particular dose is the BMD. The lower bound of the confidence interval (CI) around that dose, the benchmark dose lower confidence limit (BMDL), is often used in regulatory risk assessments as the point of departure from which to calculate a reference dose or tolerable daily/weekly intake for human exposure to a substance. We used the United States Environmental Protection Agency Benchmark Dose Software (BMDS v.2.2) to fit statistical models for the dose–response data in one study on aristolochic acid toxicity. Multiple statistical models can be fit in BMDS. We chose the model that had the lowest Akaike information criterion score and the highest P value (corresponding to best statistical fit) to estimate the BMDL.

For the systematic review and meta-analysis, studies were included if they met the following criteria: (i) human subjects, (ii) aristolochic acid, plants containing aristolochic acid, or herbal medicines containing aristolochic acid as the exposure of interest, (iii) UUC as the outcome of interest, and (iv) relative risk (RR), OR, or HR estimates, or data to calculate these. The following data were extracted from each study: authors; publication year; study design, location, and period; participants' gender/ages; number of participants; number of UUC cases; adjusted RRs/ORs/HRs; and variables adjusted for analysis. Because RR and OR can be used interchangeably when the disease is relatively rare (<15%; UUC rates are lower than this in the populations studied), and HRs were estimated from Cox proportional hazard models and used to approximate RRs (14), we calculated a summary OR for aristolochic acid-related UUC.

If the study examined the association between aristolochic acid exposure and UUC at various exposure levels, we chose the ORs reflecting the highest levels of aristolochic acid exposure for the meta-analysis (12). The data synthesis was conducted using both fixed-effects and random-effects models; if heterogeneity is present, the random effect model is considered more appropriate, as variation among studies can be taken into account (15, 16). Heterogeneity among the studies was assessed by the Cochran Q statistic and I^2 statistic. Publication bias was also assessed.

Results

Literature search and study characteristics

Ten studies were found in which human exposure to aristolochic acid was associated with adverse health effects. The step-by-step process of our literature search

is presented in Fig. 1. From 98 results, we excluded animal studies, chemical methodologic studies, *in vitro* studies, and review articles. Using the eligibility criteria described above, 6 studies were selected. Four more relevant studies were identified from the authority reports (17, 18). Eight of the 10 studies reported ORs, RR, or HRs, or data from which these risk metrics could be calculated for aristolochic acid-related UUC. These 8 studies were included in the meta-analysis.

Table 1 provides an overview of the eligible studies. The 8 studies (19–26) on aristolochic acid exposure and UUC risk (1 cohort, 5 case-control, and 2 survival analyses) were published between 2004 and 2012. Four studies were conducted in Taiwan, 3 in China, and 1 across Croatia, Bosnia, and Serbia. Four ORs and the corresponding CI were calculated using the data provided in the articles (20, 24–26). One of the case–control studies investigated the risk of UUC at 2 aristolochic acid exposure levels (151–250 mg; >250 mg; ref. 23). AL-DNA adducts and *TP53* mutation spectra served as biomarkers of exposure in 2 molecular epidemiologic studies (25, 26). We used the ORs from AL-DNA adducts in our meta-analysis, as AL-DNA adducts have been shown to serve as a specific biomarker of effect for aristolochic acid-induced UUC, as well as a robust biomarker of aristolochic acid exposure (26). Other important characteristics of the eligible studies, including the study periods, numbers of participants, numbers of UUC events, measure of exposure, and covariates adjusted in the analysis are also listed in Table 1.

The techniques used to detect, identify, and quantify AL-DNA adducts include ^{32}P-postlabeling and mass spectroscopy analyses. Nortier and colleagues (4) used the nuclease P1 enrichment version of the ^{32}P-postlabeling method to detect and quantify DNA adducts formed by aristolochic acid, whereas Jelakovic and colleagues (26) applied liquid chromatography electrospray/multistage mass spectrometry (LC-ESI/MS/MS^3) to identify the predominant DNA adduct, 7-(deoxyadenosin-N^6-yl) AL-I (dA-AL-I), in the renal cortex. Similarly, in addition to quantify the level of DNA-AL adducts using ^{32}P-postlabeling assay, Grollman and colleagues (8) and Chen and colleagues (25) used the same method to identify dA-AL adducts. One recent study (27) compared the 2 methodologies and concluded that both ultra-performance liquid chromatography/ion-trap mass spectrometry (UPLC-ESI/MS^3) and the ^{32}P-postlabeling methods are both highly sensitive for the detection of dA-AL, but that UPLC-ESI/MS^3 is superior under certain circumstances, for example, measuring trace levels of AL-DNA adducts.

It is worth noting that Chen and colleagues (25) investigated the *TP53* mutation spectra in UUC cases and controls as a biomarker of effect in addition to the AL-

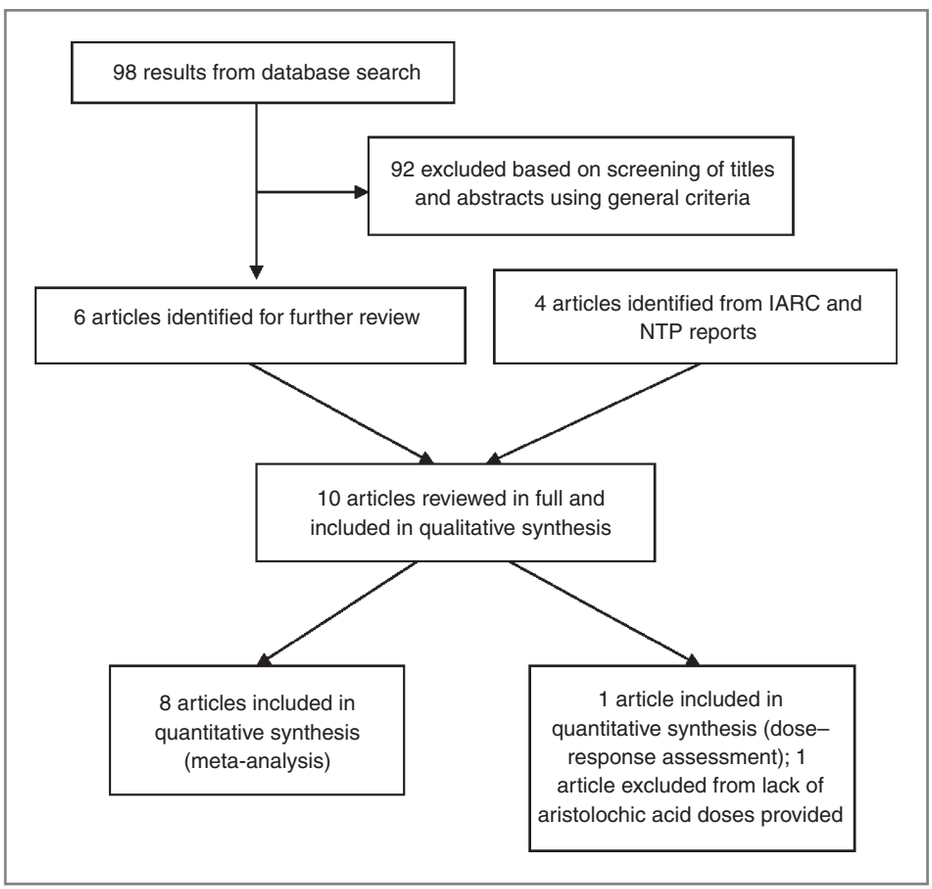

Figure 1. Selection of studies for inclusion in the systematic review for meta-analysis and dose–response assessment.

Table 1. Characteristics of the eligible studies included in the systematic review

No	Source	Location/period	Age, y	Relevant doses/ exposure	Health effect measured	No. of total participants	No. of UUC events	Measure of exposure	Results (95% CI)	Adjustment for covariates
1	Wu and colleagues, 2004 (ref. 19; survival analysis, multivariate Cox proportional model)	Taiwan 1983–2003		Chinese herbal medicines	UUC	730	30	Medical records/ questionnaire	HR = 5.2	Age, sex, compound analgesic usage, underground water intake
2	Li and colleagues, 2005 (ref. 20; hospital-based case-control study, 283 patients)	Beijing, China 2004	22–88	Chinese herbs containing aristolochic acid	UUC	264	24	Questionnaire	OR = 49 (11.1–215.8)	n/a
3	Chang and colleagues, 2007 (ref. 21; survival analysis, univariate Cox proportional model)	Taiwan 1993–2002	46–87	Chinese herbal medicines	UUC	1537	26	Medical records/ questionnaire	HR = 6.21	n/a
4	Li and colleagues, 2008 (ref. 22; hospital-based cohort study, 1735 patients)	Beijing, China 1996–2005	8–79	Chinese herbs containing aristolochic acid	UUC	1429	27	Medical records	RR = 5.85 (2.4–11.1)	n/a
5	Lai and colleagues, 2009 (ref. 23; population-based case-control study, 179,295 subjects in total)	Taiwan 1997–2002		Aristolochic acid >500 mg	UUC	577	36	Prescription history	OR = 2.0 (1.4–2.9)	Age, sex, chronic arsenic exposure, history of chronic urinary tract infection
6	Xiao and colleagues, 2011 (ref. 24; hospital-based case-control study, 3790 renal transplant recipients)	Beijing 1974–2011	27–71	Aristolochic acid	Urothelial Carcinoma	3790	100	Clinical records	OR = 11.6 (7.62–17.66)	n/a
7	Chen and colleagues, 2012 (ref. 25; case-control study)	Taiwan		Aristolochic acid	UUC	148	89	AL-DNA adducts	OR = 1.01 (0.39–2.61)	n/a
						151	47	*TP53* A→T transversion	OR = 23.18 (1.38–388.15)	
						148	38	AL-DNA adducts plus *TP53* A→T transversion	OR = 17.77 (1.06–297.80)	
8	Jelakovic and colleagues, 2012 (ref. 26; case-control study)	Croatia, Bosnia, and Serbia	43–89	Aristolochic acid	UUC	72	47	AL-DNA adducts	OR = 48.66 (2.72–870.27)	n/a
						63	16	*TP53* A→T transversion	OR = 3.82 (0.2–72.92)	

DNA adduct. The *TP53* mutational signature is dominated by rare A:T to T:A transversion in patients with UUC. AL-DNA adducts were present in the renal cortex of 83% of patients with A:T to T:A mutations in *TP53*, *FGFR3*, or *HRAS* oncogenes. With the data provided in the article, we estimated several ORs for aristolochic acid-related UUC. Using the signature *TP53* A→T transversion as a biomarker of exposure, and applying a continuity correction $\varepsilon = 0.5$ (because the number of exposed controls was zero), the OR of aristolochic acid-related UUC is 23.18 (1.38–388.15). ORs were also estimated using, as measures of exposure, the presence of AL-DNA adducts, and the presence of both *TP53* A→T transversions and AL-DNA adducts. The corresponding ORs are 1.01 (0.39–2.61) and 17.77 (1.06–297.80), respectively.

Benchmark dose modeling

Two studies (28, 29) provided data on disease prevalence corresponding to at least 4 different cumulative doses of aristolochic acid-containing herbal medicines. The Lai and colleagues (29) study was used for the purposes of BMD modeling, as this study provided a conversion factor from doses of the medicine MuTong into aristolochic acid, the substance of interest. Muniz Martinez and colleagues (28) measured disease progression as a function of doses of *Aristolochia fangchi*, but this was not translated to doses of aristolochic acid.

Lai and colleagues (29) conducted a case–control study in Taiwan to investigate the relationship between kidney failure and cumulative consumption of herbal medicines MuTong or Fangchi. The authors drew ESRD cases and random samples from the national health insurance reimbursement database from 1997–2002. Adjusted ORs obtained for ESRD associated with MuTong were 1.47 (1.01–2.14) for 61 to 100 g cumulative dose, and 5.82 (3.89–8.71) for more than 200 g cumulative dose; and for ESRD associated with Fangchi were 1.60 (1.20–2.14) for 61 to 100 g cumulative dose and 1.94 (1.29–2.92) for more than 200 g cumulative dose. Hence, total consumption of more than 60 g MuTong or Fangchi from herbal supplements was associated with a statistically significant increased risk of ESRD. However, when we fitted statistical BMD models on the more extensive MuTong and Fangchi dose ranges, all the *P* values were zero for the Fangchi data, which meant that no statistical models in the software fit the data well.

For MuTong-related ESRD, 6 dose ranges and their associated numbers of ESRD cases and controls were provided. These data were entered into the BMDS, and the corresponding dose–response model is shown in Fig. 2. For the MuTong dose–response data, the Weibull distribution was the most appropriate fit, resulting in a BMDL value of 162.8 g cumulative exposure to MuTong (equivalent to 0.42 g aristolochic acid, according to the conversion provided in the article) resulting in a 10% increased risk to the population of ESRD. This specific conversion factor of amount of aristolochic acid in MuTong was derived by the authors of the study themselves for this particular study (29), and is not necessarily the same conversion factor that should be used to estimate the amount of aristolochic acid in a particular Chinese herbal medicine. Indeed, Lai and colleagues (29) noted that their conversion factor was slightly different from those derived in other Dutch and Belgian studies.

Meta-analysis

A forest plot with estimates from a random effects model and the contribution of each epidemiologic study to the meta-analysis is shown in Fig. 3. The inverse-variance (I-V) method pooled OR denotes fixed-effects estimates, and the DerSimonian and Laird (D+L) method pooled OR denotes random-effects estimates. Heterogeneity in the study pool was significant (*P* value from Cochran test <0.001; $I^2 = 88.7\%$). Therefore, in this study,

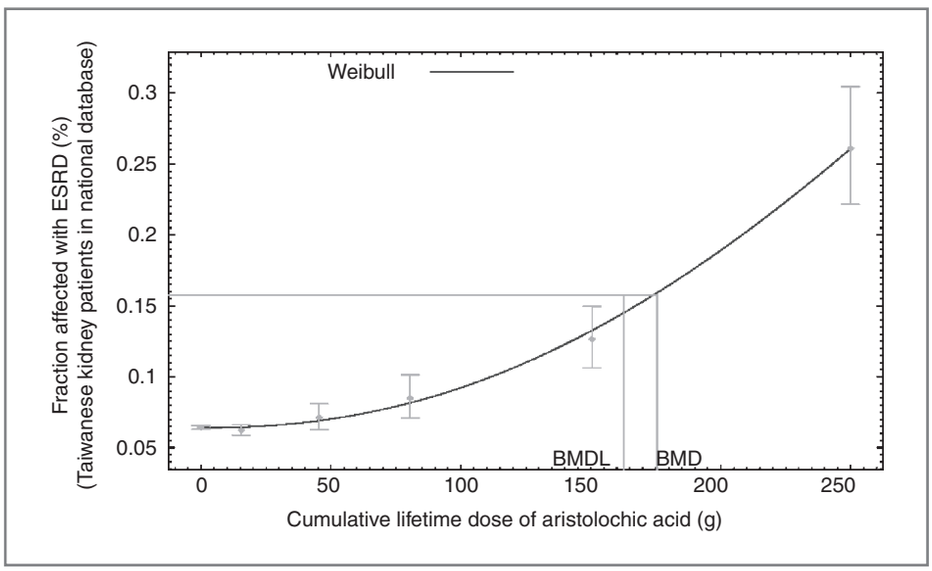

Figure 2. BMD model for Lai and colleagues (29) on ESRD prevalence as a function of cumulative MuTong dose in Taiwan.

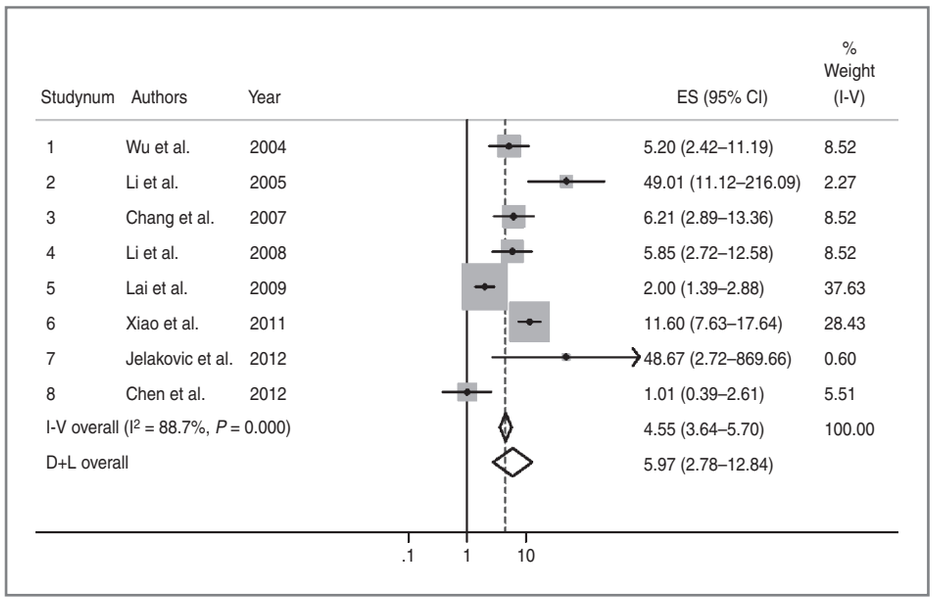

Figure 3. Forest plot of risk ratios (ES) of aristolochic acid-related urothelial cancer with the contribution of each study to the meta-analysis. I-V pooled OR denotes the fixed-effects estimates, and the D+L pooled OR denotes the random-effects estimates.

the random-effects estimates were reported as the primary analysis, whereas fixed-effects estimates were provided for comparison. Aristolochic acid exposure is significantly associated with UUC, with a pooled OR of 5.97 (2.78–12.84). The Egger test of asymmetry suggests no presence of bias (intercept = 1.84; $P = 0.446$).

Exposure to aristolochic acid worldwide

There are at least 2 major routes of human exposure to aristolochic acid. The more well-characterized exposure, potentially affecting millions worldwide (25), is consumption of *Aristolochia* plants in certain herbal medicines. The less-understood exposure route concerns the inadvertent presence of some part of *Aristolochia* plants commingled into the diets of farming populations in the Balkans (and possibly other parts of the world). It is estimated that more than 100,000 individuals may be at risk for aristolochic acid-related BEN in the Balkans alone (30).

At national levels, regulatory efforts are being made to reduce risk of human exposure to herbal medicines containing aristolochic acid. All *Aristolochia*-based medicines were prohibited for supply, sale, or use in therapeutic goods in Australia (17, 31). In 2001, the United Kingdom Committee on Safety of Medicines issued a statutory instrument to prohibit the sale, supply, and importation of any medicinal products containing *Aristolochia* (17, 32). Herbal products containing aristolochic acid were prohibited in 2003 in Taiwan (29). In addition, advisories concerning the use and marketing of botanical products that may contain aristolochic acid have been issued by the European Agency for the Evaluation of Medicinal Products (33), Health Canada, and the U.S. Food and Drug Administration (17). In these nations, it is likely that aristolochic acid exposure has decreased over the last decade and will continue to decrease, as less herbal medicines available in the market will contain aristolochic acid. However, unreported sales of these herbal medicines may continue for some time. Moreover, many who have already been exposed to aristolochic acid in the past may develop aristolochic acid-related cancer or other aristolochic acid-related diseases in the years to come, despite recent regulatory efforts to curb exposure. Given the BMDs estimated above, probably many individuals who have regularly consumed these herbal medicines for years have reached cumulative doses that could result in disease.

Inadvertent exposures to aristolochic acid are more difficult to characterize and control. It has been speculated that, because *Aristolochia* weeds have been seen to grow in cereal fields, *Aristolochia* seeds may commingle with cereal grains in the field and during harvest. AL-DNA adducts have been found in the kidneys of patients with BEN (8), which indicates aristolochic acid exposure in these individuals. Moreover, aristolochic acid was detected in the seeds of *Aristolochia clematitis* growing in the endemic regions (9). Although it is highly likely that aristolochic acid has caused BEN in this population, the toxin has not actually been detected in the food consumed in this region; in part, because of lack of efforts in the past to detect it, and the difficulty of chemical analysis whether aristolochic acid binds to cereal components. It may not be possible at this point to find such food samples as relevant exposures may have occurred from food consumed decades ago. However, it is known that *Aristolochia clematitis* has grown continuously in the cultivated fields in Balkan endemic regions for at least 5 decades (34).

Discussion

The review of the epidemiologic literature indicates that aristolochic acid exposure is causally related to urothelial carcinoma and ESRD in humans, in a dose-dependent manner. Thus, the more herbal medicines containing

aristolochic acid are consumed over the course of a lifetime, or the longer that inadvertent exposures to aristolochic acid continue, the higher the risk that one or both of these diseases will develop.

From a risk assessment standpoint, it is useful to understand the cumulative dose of aristolochic acid that is associated with an increased risk of human disease. The BMD has been used in recent governmental risk assessments as a point of departure to establish tolerable human intakes of certain substances. In this study, we found a BMD of cumulative aristolochic acid exposure of 0.42 g, associated with increased risk of ESRD in humans, based on a Taiwanese study that analyzed extensive data on Chinese herbal medicine intake and disease incidence (29). This value may be useful in providing risk analysts with information about establishing a cumulative dose of aristolochic acid exposure, below which there may not be increased risk of ESRD in humans.

Our meta-analysis found a pooled OR of aristolochic acid-related UUC of 5.97 (2.78–12.84). As UUC is a relatively rare condition, the ORs can be used to estimate RR. Hence, the risk of developing UUC is about 6 times higher for those exposed to aristolochic acid than for those who have not been exposed to aristolochic acid. One limitation of our approach is the differences in how exposure to aristolochic acid was measured amongst the studies in the meta-analysis. Several studies were based upon medical or prescription records, with or without an accompanying questionnaire; others were based on detection of the AL-DNA adduct. In particular, one weakness of questionnaire-based studies is the possibility of recall bias. Although in the case of these studies, it is likely that the participants would have remembered whether they had consumed a particular Chinese herbal medicine, the amount consumed would be more difficult to remember or estimate.

In addition, there are differences in how much aristolochic acid is present in different preparations of the *Aristolochia*-containing Chinese herbal medicines, as well as differences in methods for quantifying the aristolochic acid in these medicines. Several classic analytic methods, including thin-layer chromatography, liquid chromatography UV-vis, and liquid chromatography mass spectroscopy (LC/MS), were used to determine aristolochic acids in Chinese herbs and dietary supplements (35, 36). An enhanced method of LC/MS, liquid chromatography/tandem mass spectrometry with superior sensitivity, selectivity, and specificity, was also developed (35). Simple and rapid techniques, such as capillary zone electrophoresis and capillary electrophoresis with laser-induced fluorescence detection (37, 38), were also proposed as alternatives for analyzing aristolochic acids.

Finally, there are limitations associated with extrapolating from the results of the BMD modeling linking aristolochic acid to ESRD. Even if aristolochic acid exposure was fairly well characterized in (29) because the conversion factor from the MuTong to aristolochic acid was directly provided by the authors for their study, this conversion factor cannot be used for other herbal medicine exposure studies worldwide, because there is a wide variability in the amount of aristolochic acid in different medicinal preparations. Thus, in future studies of this nature, new conversion factors must be found for the relevant herbal medicine preparations, which themselves may not be consistent over the lifetime of an individual's use of these medicines. In the future, measuring aristolochic acid exposure through the AL-DNA biomarker may be a much more accurate way to assess individuals' cumulative exposures.

Two unique routes of human exposure to aristolochic acid exist. The route for which there is the greatest evidence for causality of adverse health effects is through consumption of traditional Chinese herbal medicines labeled Fangchi or MuTong. Even within populations that consume these herbal medicines, however, some may take the medicine continually, whereas others may take it for several days or weeks in a lifetime (e.g., in response to a bout of illness). Hence, lifetime exposures to aristolochic acid can vary substantially among these herbal medicine-consuming populations. Another, less defined, route of human exposure concerns inadvertent presence in foods due to presence of *Aristolochia* plants growing alongside food crops, thus incorporated unintentionally in human diets. This route of exposure has been postulated in certain Balkan populations, in which aristolochic acid exposure has been associated with BEN. Although aristolochic acid has not been detected in food, it has been detected in the seeds of plants growing in those regions, and aristolochic acid-specific DNA adducts have been found in the kidneys of those with the disease.

Possible genetic factors associated with chronic kidney diseases and UUC specifically, were investigated in several studies. In a recent meta-analysis focusing on genome-wide linkage scans for renal function traits (39), no chromosomal region reached genome-wide statistical significance, and subgroup analyses by status of chronic kidney diseases did not yield additional information. On the other hand, 3 other very recent studies (40, 41) identified a particular genotype—a polymorphism located at the T allele of rs9642880 on chromosome 8q24 that seems to confer susceptibility to urothelial carcinomas of the upper urinary tract. Thus, the genetic link to the diseases associated with aristolochic acid should be studied further, particularly, in identifying vulnerable subpopulations in terms of both genetics and aristolochic acid exposure.

Because the association between aristolochic acid exposure and severe renal disease is strong, efforts must be made to prevent exposures in at-risk human populations. Both herbal medicine producers and the populations that consume these medicines must be informed of these risks, and where possible, regulations should be enforced to prevent the presence of *Aristolochia* plants in medicines. Fortunately, many regulatory bodies worldwide have in the last decade taken measures to reduce the sales of herbal medicines containing *Aristolochia*. In

communities in which inadvertent exposure to aristolochic acid is suspected through commingling of *Aristolochia* seeds with cereal grains, particularly in the Balkans, farmers should be alerted to the possible presence of harmful weeds in their fields and encouraged to adopt primary prevention methods: removing the weeds or carefully sorting weed plants or seeds from the grains following harvest. Primary prevention is key to preventing aristolochic acid-related diseases in human populations in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Authors' Contributions

Conception and design: F. Wu
Development of methodology: F. Wu
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F. Wu, T. Wang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): F. Wu, T. Wang
Writing, review, and/or revision of the manuscript: F. Wu, T. Wang
Study supervision: F. Wu

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