Commentary

Traditional herbal medicines containing plant species of the genus *Aristolochia* are carcinogenic to humans

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Aristolochic acids (AAs) are primarily found in different species of the genus *Aristolochia* (e.g. *Aristolochia clematitis*, *Aristolochia fangchi* and *Aristolochia manshuriensis*), but have also been described in certain *Asarum* species [1]. In Europe *Aristolochia clematitis* is indigenous to Mediterranean regions (see Figure). The plant extract of *Aristolochia* species (AA) mainly consists of the structurally related derivatives aristolochic acid I (AAI) and aristolochic acid II (AAII) [1]. Herbal drugs derived from *Aristolochia* species have been known since antiquity and were used in obstetrics and in the treatment of snake bites [2]. Contemporary medicine has used *Aristolochia* plant extracts for therapy of arthritis, gout, rheumatism and festering wounds. In the 1970s the anti-inflammatory properties of AA encouraged the development of pharmaceutical preparations in Germany until AA was shown to be a strong rodent carcinogen [2]. AA is among the most potent 2% of known carcinogens [3]. Subsequently, in 1982 all pharmaceutical preparations containing AA were withdrawn from the market in Germany and many other countries [2]. However, *Aristolochia* plants are still used in traditional medicine in some parts of the world [1,2].

So-called Chinese herbs nephropathy (CHN) associated with the ingestion of Chinese herbal remedies was first reported in 1993 in a series of kidney failure cases all treated for weight-loss in the same clinic in Belgium [4]. Over 100 CHN patients have been identified so far in Belgium, mostly women [2,5]. In 1990 this clinic in Brussels began prescribing Chinese herbal slimming pills intended to contain, in part, *Stephania tetrandra* (for its purported diuretic effects). However, it was shown that *Stephania tetrandra* was inadvertently replaced by *Aristolochia fangchi* containing nephrotoxic AA because both plants are used in Chinese folk medicine under the same name, *Fangji* [2,5]. Exposure of CHN patients to AA was
substantiated by the identification of specific AA-DNA adducts in urothelial tissue of these patients [6,7]. In the majority of cases, progression to end-stage renal failure occurs despite discontinuation of AA ingestion, necessitating dialysis and subsequent renal transplantation [5]. Follow-up investigations published in 2000 showed that only years later CHN patients developed a high risk of urothelial cancer. Out of 39 CHN patients with end-stage renal disease that underwent prophylactic removal of the kidneys and ureters, urothelial tumours in 18 of them were diagnosed (a prevalence of 46%) [6]. The cumulative dose of *Aristolochia* was a significant risk factor; patients with an intake of 200g of herbs (the average herbal intake) had a 50% risk of developing cancer. Recently, it was found that even patients who do not display the characteristic histological features of CHN are also at risk of malignancy [8].

So-called CHN has been described in patients in other European (e.g. UK, France, Spain and Germany) and in Asian countries and the USA, who were exposed to *Aristolochia* species containing AA and had no relationship with the Belgian cohort [2,9-12]. Therefore, it has been proposed to designate this novel nephropathy in which the unequivocal role of AA has been fully documented as aristolochic acid nephropathy (AAN) [2,5]. In many AAN patients urothelial tumours have been diagnosed, highlighting the carcinogenic potential of AA in human beings [9,11,12]. Natural health products containing AA are often sold as traditional Chinese medicines meant to effect weight loss, improve the immune system or alleviate gastrointestinal symptoms [3,13]. However, as with most herbal products, there is little and often no evidence from clinical trials of their efficacy for any of these benefits [13]. Herbal remedies containing species of the genus *Aristolochia* were recently classified as carcinogenic to humans (group 1 carcinogen) by the International Agency for Research on Cancer (IARC) [1]. Therefore, products containing AA have been banned in many countries world-wide and consumers were advised to immediately discontinue use of any botanical
products containing AA. However, despite warnings, there still appear to be a number of herbal products containing *Aristolochia* species offered for sale through the internet [3].

There is clear evidence that AA is genotoxic forming covalent DNA adducts [2]. Specific AA-DNA adducts were detected in various tissues of AA-treated rats as well as in urothelial tissue of AAN patients [6,7]. The most abundant DNA adduct detected by $^{32}$P-postlabelling is the adenosine adduct of AAI, 7-(deoxyadenosin-N$^6$-yl)aristolactam I (dA-AAI) [6,7,9,11,12,14]. However, AA-DNA adducts are also found in various tissues outside the urinary tract [8,9,15] indicating that factors other than DNA adduct formation by AA may be critical for the high incidence of urothelial tumours. The life-long persistence of the dA-AAI adduct in various organs (including kidney) in rats is in line with its detection in Belgian CHN patients almost 10 years after the patients stopped taking the herbal slimming regimen [2,6], thus demonstrating that AA-DNA adducts are not only suitable biomarkers of exposure to AA but also markers of cancer risk. Both human cytosolic enzymes (e.g. NQO1) and microsomal enzymes (e.g. CYP1A1 and CYP1A2) activate AA by simple nitroreduction leading to DNA binding species [16]. Among the metabolic enzymes is also prostaglandin H synthase which is highly expressed in urothelial tissue [16]. Interestingly, to date only 5% of the patients treated with the slimming regimen in Belgium have suffered from nephropathy. One possible explanation for the different responses of patients may be individual differences in the activities of the enzymes catalysing biotransformation (activation and/or detoxification) of AA.

In AAN patients urothelial atypia were associated with the overexpression of the P53 protein [17], suggesting that *p53* is mutated in AAN-associated cancer [18]. More than 50% of all human tumours contain a mutation in the p53 gene. Interestingly, in one AAN patient available for analysis a characteristic A to T transversion mutation was found in the p53 gene in urothelial tumour cells [15]. Translesional bypass of adenine adducts of AA (dA-AAI and
dA-AAII) indicates a mutagenic potential resulting from dAMP incorporation by DNA polymerase, suggesting that an A to T transversion mutation would be the mutagenic consequence [2]. A to T transversions are typical mutations observed in the H-ras oncogene of tumours in rodents treated with AA and correspond with DNA adduct formation at adenine residues [19]. To examine AA-induced p53 mutation spectra in the human p53 gene in laboratory animals a human p53 knock-in (Hupki) mouse has been constructed [20]. When the Hupki p53 gene of immortalized cell lines derived from primary Hupki embryonic fibroblasts (HUFs) exposed to AA were sequenced specific A to T transversion mutations in the p53 gene were observed [20,21]. Interestingly, in one cell line a characteristic A to T transversion was found at the first adenine of codon 139 (AAG) identical to the mutation found in the urothelial tumour cells of the AAN patient yet available for mutation analysis [15,21]. These data may indicate the probable molecular mechanism whereby AA causes urothelial cancer. On the other hand, specific DNA damage due to AA in urothelial cells and cell-specific alterations at the transcription level of proteins might impair physiological processes [22,23]. This may not only be of primary importance in explaining the rapidly progressive nature of AAN but also indicates a potential mechanism on the seeming tissue specificity of the AAN-associated oncogenesis compared to the widespread occurrence of AA-DNA adducts.

On both clinical and morphological grounds, AAN is very similar to the Balkan endemic nephropathy (BEN) [24], which was first recognized in the 1950s in certain rural areas of Rumania, Croatia, Serbia and Bulgaria along the Danube river basin [25,26]. At least 25,000 individuals may suffer from BEN or are suspected of having the disease, while the total number of people at risk in these countries may exceed 100,000 [24]. For many years evidence has accumulated that BEN is an environmentally induced disease strongly associated with the intake of foodstuffs contaminated with the fungal mycotoxin ochratoxin A,
which is nephrotoxic and carcinogenic in experimental animals [25,26]. However, similarities between AAN and BEN have led to the hypothesis of a common aetiological agent for both diseases – AA [14,24]. Already some 35 years ago, a survey conducted on the geographical distribution of the plant *Aristolochia clematitis* in the endemic areas suggested that AA found in flour obtained from wheat contaminated with seeds of *Aristolochia clematitis* in endemic areas could be the aetiologica l agent of BEN [26]. This hypothesis, however, has not yet received widespread support. Nevertheless, it was demonstrated recently that flour used to bake bread, a dietary staple in the endemic region of Croatia, was derived from wheat grain which was contaminated with seeds of *Aristolochia clematitis* [27]. This suggests that chronic exposure to AA of individuals living in endemic areas has occurred. Indeed, the dA-AAI adduct was found in randomly collected kidney tissue from a small number of farmers (2 out of 3 cases) coming from endemic areas for BEN with end-stage renal disease and upper urinary tract malignancy, although it was not possible to classify these patients as clearly suffering from BEN [14]. Moreover, preliminary data presented by colleagues in the U.S. clearly show that AA-DNA adducts are detectable in urothelial tissue of patients with unequivocal diagnosis of BEN and that some urothelial tumours samples available to them for analysis carried characteristic A to T transversion mutations in the p53 gene [28]. Collectively, these results provide new evidence that AA is a clear risk factor for BEN and BEN-associated urothelial cancer. The specific role of AA in the development of BEN now awaits further investigation.

There is clear evidence that the plant extract AA derived from *Aristolochia* species plays a causal role not only in AAN but also and even more importantly in the development of AAN-associated urothelial cancer. AA-DNA adducts are a suitable biomarker of exposure to AA and a marker of cancer risk. Individuals exposed to AA who have not developed AAN may also at risk of urothelial malignancy. The multi-systemic formation and persistence of
AA-DNA adducts in AAN patients strongly suggests the possibility of the future development of multi-systemic tumours in AAN cases similarly to what has previously been observed in AA-exposed rodents [2]. Since more and more AAN cases besides those reported in Belgium have been described world-wide and all are related to exposure to AA, it is of great concern that this form of nephropathy and associated urothelial cancers may occur more commonly in the future due to the widespread availability of herbal medicines containing AA. Language barriers may prevent many health care practitioners from effectively verifying the ingredients listed on many Chinese herbal products [29]. Instead, purchasers and prescribers of Chinese herbal products should be warned to ensure that the products they are using are not listed on the safety alerts published by the U.S. Food and Drug Administration (FDA) [30,31] or other regulatory agencies. Any patient with suspected exposure to AA should be monitored for signs of nephropathy and urothelial cancer. The increasing reports on AAN also point once more to the urgent need to submit the so-called “natural” harmless drugs of herbal medicine to a critical evaluation of benefits and side effects prior to their release for medical use just as it is mandatory for drugs in the Western world [32]. Owing to the fact that AA is both a powerful nephrotoxic and carcinogenic substance all products containing botanicals known to or suspected of containing AA should be banned from the market world-wide.

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