Modernization of Traditional Medicine

Nephrotoxicity and carcinogenesis of aristolochic acids and their derivates

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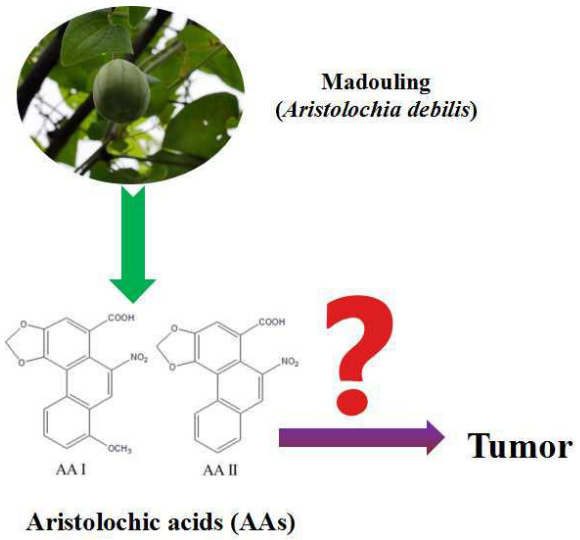
Highlights

This review summarized the toxicity and carcinogenesis of aristolochic acids and the underlying mechanisms.

Editor's Summary

The mutational signature of aristolochic acids is related to the occurrence of HCC. However, the frequency of administration and dose, exposure time to aristolochic acids, and infectious situations of hepatitis B virus should also be further identified.



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Abstract

Aristolochic acids (AAs), a natural mixture of 8-methoxy-6-nitro-phenanthro-(3,4-d)-1,3-dioxolo-5-carboxylic acid (AAI) and 6-nitro-phenanthro-(3,4-d)-1,3-dioxolo-5-carboxylic acid (AAII), derived from aristolochiaceae species, has been reported to cause AAS-induced nephropathy and upper urothelial cancer. In this review, we summarize the information on the nephrotoxicity and carcinogenesis of AAs and their derivatives. AAs nephrotoxicity can lead to apoptosis and oxidative stress of renal tubular cells, and inhibition of the expression of aquaporins. AAs can also reduce the capability for renal tubular epithelial cell repair after acute injury and further produce renal fibrosis by activating TGF-β-Smad signaling and promoting the migration of macrophages. Moreover, AAs-induced carcinogenesis may be due to the formation of covalent adducts with DNA which can lead to the mutation in certain tumor suppressor genes or proto-oncogenes and the different catalyzing capacity of the microsomal cytochrome P450 of individuals in AAI metabolism.

Keywords: Aristolochic acids, Aristolochic acids nephropathy, Nephrotoxicity, Carcinogenesis

摘要

马兜铃酸(Aristolochic acids, AAs)，主要是由来源于马兜铃科属植物的 8-methoxy-6-nitro-phenanthro-(3,4-d)-1,3-dioxolo-5-carboxylic acid (Aristolochic acids I, AAI), 6-nitro- phenanthro-(3,4-d)-1,3 -dioxolo-5-carboxylic acid (Aristolochic acids II, AAII)组成的天然混合物，能够引起马兜铃酸肾病和上尿路上皮癌。本文概述了 AAs 及其衍生物的肾毒性及致癌性。AAs 肾毒性能够诱导肾小管上皮细胞凋亡及氧化应激的发生，并能抑制水通道蛋白的表达。AAs 还可以降低肾小管上皮细胞急性损伤后的修复能力，并通过 TGF-β-Smad 信号和促进巨噬细胞的迁移进一步促进肾脏纤维化。另外，AAs 的致癌性可能是因为导致某些肿瘤抑癌基因或原癌基因突变的 DNA 加合物的形成，和不同个体的细胞色素 P450 在 AAI 代谢中的不同催化能力导致的。

关键词：马兜铃酸；马兜铃酸肾病；肾毒性；致癌性

Abbreviations: AAs, Aristolochic acids; AAN, Aristolochic acids nephropathy; UUC, Upper urothelial carcinoma; AQPs, Aquaporins; AL, Aristolactams; TSP1, Thrombospondin-1; CYP, Cytochrome P450.

Competing interests: The authors declare that there is no conflict of interests regarding the publication of this paper.

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Background

Chinese medicine and Chinese materia medica preparations are known to contain aristolochic acids (AAs), a type of nitrophenolic carboxylic acids, such as Guanmutong (*Aristolochia manshuriensis*), Guangfangji (*Aristolochia fangchi*), Madouling (*Aristolochia debilis*), Xixin (*Asarum sieboldii*) and Qingmuxiang (*Aristolochia debilis*), the dry root of Madouling (*Aristolochia debilis*) (Tables 1 and 2). According to the records in *Shengjizonglu* published in 1117 A.D (Northern Song Dynasty of China) and *Bencaohuiyan* in 1624 A.D.(Ming Dynasty of China), Madouling (*Aristolochia debilis*) was capable of clearing heat from the lung and descending Qi, relieving cough and asthma, as well as clearing the intestine and eliminating hemorrhoids. Xixin (*Asarum sieboldii*) was also recorded in *Shennongbencaojing* in Qin and Han Dynasty (221B.C.-25A.D.) as being warm in nature and toxicity, with the function of expelling wind and cold, warming the lungs, and inducing dieresis.

However, with time, these herbs were found to contain AAs and their derivatives including nitro-group, which is the most important toxic group. AAs nephropathy was first reported following the substitution of Hanfangji (*Stephania tetrandra*) with Guangfangji (*Aristolochia fangchi*), which contains AAs, in slimming pills in Belgium [1]. Administration of the pills containing AAs caused kidney disease that required dialysis or kidney transplantation during that time. Then, *Aristolochiaceae* species were banned worldwide because of this incidence.

AAs derived from *Aristolochiaceae* species mainly consists of a natural mixture of 8-methoxy-6-nitro-phenanthro-(3,4-d)-1,3-dioxolo-5-carboxylic acid (AAI) and 6-nitro-phenanthro-(3,4-d)- 1,3-dioxolo-5- carboxylic acid (AAII). The chemical structures are shown in Figure 1. It can be used as a snake bite antidote and was found to lead to AAs nephropathy (AAN) [2]. AAN, once called Chinese herbal nephropathy, is the disease caused by the ingestion of botanicals containing AAs, which can progress to acute kidney injury, chronic kidney disease, or interstitial renal fibrosis depending on the doses, frequency, and exposure time [3]. Exposure to AAs can also lead to a chronic renal disease called Balkan endemic nephropathy, which can often progress to upper urothelial carcinoma (UUC) [4]. It is believed that dietary contamination with the seed of *Aristolochia* species might be the source of exposure.

AAs are not only toxic to the kidneys but are also carcinogenic. A recent study reported that the mutational signature of AAs was detected in patients with hepatocellular carcinomas (HCC) in Taiwan and Asia. In this study, the author indicated AAs and their derivatives were widely implicated in the occurrence of HCC [5]. However, the conclusion still needs further studies. Firstly, are HCC-associated mutations caused by AAs themselves? When these patients began the treatment with AAs-containing drugs and the amount of the administered dose were not reported in the study. Secondly, the occurrence of HCC can be caused by many factors except

Table 1 Chinese medicine known or suspected to contain aristolochic acids

Chinese medicine name	Latin name
Dayeqingmuxiang	*Aristolochia austrozechuanica*
Dabaijie	*Aristolochia chuii*
Zhushalian	*Aristolochia cinnabarina*
	Aristolochia tuberosa
Jiuyuesheng (Zhushalian)	*Aristolochia Tuberosa* C. F. Liang et S.M
Tianxianteng	*Aristolochia contorta*
	Aristolochia debilis
Madouling	*Aristolochia contorta*
	Aristolochia debilis
Fangji	*Aristolochia heterophylla*
	Aristolochia austrozechuanica
	Aristolochia moupinensis
Hanfangji	*Aristolochia heterophylla*
Huaitong	*Aristolochia moupinensis*
Mufangji (Shuichengmufangji)	*Aristolochia moupinensis*
	Aristolochia ovatifatia
Muxiangmadouling	*Aristolochia moupinensis*
	Aristolochia griffithii Yhoms ex Duchartre
Daqingmuxiang	*Aristolochia kwangsiensis* Chun *et* How
Mianningfangji	*Aristolochia moupinensis* Franch
Xungufeng	*Aristolochia mollissima*
Tiaoyexixin	*Asarum caudigerellum* C.Y.Cheng
	Asarum caudigerum Hance
	Asarumsplendens (Maekawa) C.Y.Cheng et C.S.Yang
	Asarum caulescens Maxim.
Wujinqi	*Asarum caulescens* Maxim.
Duheng	*Asarum forbesii* Maxim.
	Asarum ichangense C.Y.Cheng et C.S.Yang
Xiangxixin	*Asarum forbesii* Maxim.
	Asarum ichangense C.Y.Cheng et *C.S.Yang*
	Asarum wulingense C.F.Liang
Xixin	*Asarum heterotropoides* Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag.
	Asarum sieboldii Miq.*var.seoulense* Nakai
	Asarum sieboldii Miq
Gansuxixin	*Asarumhimalaicum* Hook. f. et Thoms. ex Klotzsch
Nanpingxixin	Asarumhimalaicum Hook. f. et Thoms. ex Klotzsch
Maoxixin	*Asarumhimalaicum* Hook. f. et Thoms. ex Klotzsch
Jinerhuan	*Asarum insigne* Diels
Shancigu	*Asarum sagittarioides* C. F. Liang

Source: CFDA http://www.sda.gov.cn/WS01/CL1991/215894.html

for gene mutation, such as hepatitis B virus, hepatitis C virus and aflatoxin. Therefore, this reminds us of the need to review the literature on the toxicity and carcinogenesis of AAs and their derivatives as well as the underlying mechanisms (Table 3).

Nephrotoxicity

The kidneys are susceptible to toxicity, which includes that induced by chemicals, medications, and natural nephrotoxins. The types of kidney dysfunction include nephrotoxicity, necrosis, urinary tract carcinoma, acute kidney injury, and chronic kidney disease. Among them, nephrotoxicity could result in a direct tubular effect or acute interstitial nephritis [6].

Histopathological analysis of the kidneys of AAs-treated mice revealed that AAI caused acute tubular necrosis and interstitial fibrosis, whereas AAII showed little acute necrosis and interstitial fibrosis [7, 8]. Reactive metabolites or intermediates formed through the activation of AAs, are also associated with herbal toxicity, mutagenicity, and carcinogenicity. A study has also suggested that human hepatocyte metabolism of AAI increased the cytotoxicity to human kidney epithelial cells by the formation of AL adducts [9].

Apoptosis

Apoptosis is the major cell death pathway associated with AAs treatment [10]. Increased intracellular Ca^{2+} concentration has been linked with AAs-induced cytotoxicity by inducing apoptosis of renal tubular cells [11]. Furthermore, the mitochondria of the proximal tubular epithelial cells were fragmented into suborganelles in AAN rats [12]. In addition, AAI causes toxicity to the testis by inhibiting the Akt and ERK1/2 pathway [13, 14]. In addition, the activation of *P53* induced apoptosis and promoted injury in mice that ingested AAs [15]. AAs can also induce apoptosis by a caspase 3-dependent pathway in the human proximal tubular epithelial cell line [16].

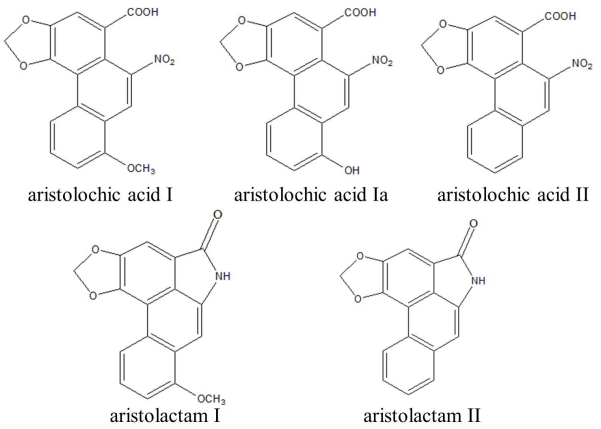

Figure 1 Chemical structures of aristolochic acids and aristolactams

Table 2 Chinese materia medica preparation containing aristolochic acids

Names	Corresponding herbs containing aristolochic acids
Longdanxiegan pills	Mutong (*Caulis Clematis Armanoii*)
Fukefenqing pills	Mutong (*Caulis Clematis Armanoii*)
Ganluxiaodu pills	Mutong (*Caulis Clematis Armanoii*)
Fufangshedan chuanbei powder	Madouling (*Aristolochia debilis*)
Jiming pills	Madouling (*Aristolochia debilis*)
Qingguozhike pills	Madouling (*Aristolochia debilis*)
Chuanxiling capsules	Madouling (*Aristolochia debilis*)
Fei'an pills	Madouling (*Aristolochia debilis*)
Qishiweisongshi pills	Madouling (*Aristolochia debilis*)
Weifu granule	Madouling (*Aristolochia debilis*)
Xiaoerzhike oral liquid	Madouling (*Aristolochia debilis*)
Zhikehuatan pills	Madouling (*Aristolochia debilis*)
Xiaokepingchuan oral liquid	Madouling (*Aristolochia debilis*)
Zhikehuatan capsules	Muxiangmadouling (*Aristolochia ovatifatia*)
Fengshisailong capsules	Muxiangmadouling (*Aristolochia ovatifatia*)
Fengshizhitong pills	Muxiangmadouling (*Aristolochia ovatifatia*)
Shuganliqi pills	Qingmuxiang (*Aristolochia debilis*)
Tianxianteng powder	Tianxianteng (*Aristolochia contorta*)
Heweijiangni capsules	Tianxianteng (*Aristolochia contorta*)
Dangguisini decoration	Duheng (*Asarum forbesii* Maxim.)
Baowei capsules	Zhushalian (*Aristolochia cinnabarina*)
Fufangweitong capsules	Zhushalian (*Aristolochia cinnabarina*)
Zhushalian capsules	Zhushalian (*Aristolochia cinnabarina*)
Fufangquancan tablets	Xungufeng (*Aristolochia mollissima*)
Shennong liquor	Xungufeng (*Aristolochia mollissima*)
Duzhongzhuanggu pills	Xungufeng (*Aristolochia mollissima*)
Qufengchushi medicinal liquor	Xungufeng (*Aristolochia mollissima*)
Sanshe medicinal liquor	Xungufeng (*Aristolochia mollissima*)

Oxidative Stress

Ingestion of AAs can increase oxidative stress in proximal tubular epithelial cells through the generation of reactive oxygen and nitrogen species [17, 18]. Furthermore, a low-dose of AAs can induce cell cycle arrest in the G2/M phase by DNA damage, which is triggered by the production of reactive oxygen species [14]. Vitamin C and E can decrease hydrogen peroxide levels to attenuate AAs-induced cell cytotoxicity [17, 19].

Aquaporins

Polyuria and nocturia are the main symptoms of patients with acute AAN. Aquaporins (AQPs) transmembrane proteins are critical in regulating the kidney function. AQPs distribution in the kidney allows the transport of water across the permeable epithelia and plays a crucial part in the urinary concentrating and diluting processes [20, 21]. AQP1 is mainly expressed in the apical and basolateral membrane of epithelial cells in the proximal tubule and the descending thin limb of long looped nephrons and plays an important role in maintaining the body-water balance [22, 23]. AQP2 is a water-channel protein and regulates body-water homeostasis and urine concentration. Dysfunction of AQP2 can lead to conditions such as an acute or chronic renal failure or electrolyte disturbance [24]. AQP4 is located in the basilar membrane of the principal cells and provides an exit passage for reabsorbed water [22]. Furthermore, AAI and aristolactams (AL) can inhibit the expression of AQP 1, AQP2, and AQP4 [25].

Renal fibrosis

Acute kidney injury can lead to the regeneration of most tubules, but some of the regeneration processes can induce local inflammation. Then interstitial fibroblasts can proliferate and convert to myofibroblasts with matrix deposition, which can induce the chronic development of tubulointerstitial fibrosis. Numerous factors can cause tubular injury such as endothelial toxins and inflammation. AAs can diminish the reparative ability of renal tubular epithelial cells after acute injury and, then, result in the occurrence of renal fibrosis [26].

Renal fibrosis can be induced by TGF-β–Smad signaling and macrophage migration. AAs-induced acute tubular necrosis has a tendency to develop into fibrosis, and an increased expression of TGF-β1 can be found in patients with AAN, which might be activated by thrombospondin-1 (TSP1) in the injured kidney [27-29]. The blockage of TSP1 can decrease the activity of TGF-β [30]. TSP1 may play a vital role in the prognosis of AAs-induced renal interstitial fibrosis. Furthermore, the epithelial-mesenchymal transition of tubular epithelial cells is crucial for the induction of kidney fibrosis, which can also be regulated by TGF-β1-Smad2/3 and hedgehog signaling [31, 32]. In addition, peritubular capillary loss and the upregulation of hypoxia-inducible factor-1α can also increase the tubulointerstitial fibrosis area [33]. A previous study indicated that bone morphogenetic protein-7 could inhibit AAs-induced epithelial-mesenchymal transition in a dose-dependent manner [34]. In addition, bortezomib was also reported to have an anti-fibrotic effect by reducing the expression of renal fibrosis-related protein and kidney injury markers in an AAs-induced mouse model of fibrosis by suppressing TGF-β1/Smad3 signaling [35].

Macrophages may be involved in the progression of AAN and renal fibrotic diseases because macrophage accumulation occurs as an early feature of AAN. Treatment with fms-I, a selective inhibitor of the macrophage colony-stimulating factor receptor, suppressed the disease progression in a model of chronic AAN [36]. In addition, macrophage migration inhibitory factor was found to be associated with inflammation and matrix deposition after kidney tissue injury, which could inhibit fibrotic kidney diseases [37].

Table 3 The mechanisms of nephrotoxicity and carcinogenesis of aristolochic acids

Items	Mechanisms	
Nephrotoxicity	Apoptosis	Increased intracellular Ca^{2+} concentration [11]
		Mitochondria injury [12]
		Inhibiting Akt and ERK1/2 [13, 14]
		Activating of *P53* [15]
		Inducing a caspase 3-dependent pathway [16]
	Oxidative stress	Generation of reactive oxygen and nitrogen species [17, 18]
	Aquaporins	Inhibiting the expression of aquaporins [25]
	Renal fibrosis	Activating TGF-β-Smad signal [27, 35]
		Promoting Macrophage migration [36, 37]
Carcinogenic	Formation of AL adducts with DNA [44]	
	Gene mutation including *lacZ, cll ,TP53* and *H-RAS* [50, 52]	
	Different activity of microsomal cytochrome P450 [57]	
	Increase the level of C-myc and Lin28B as well as G protein-coupled receptor 87 [58, 59]	

Carcinogenic

Current, compelling evidence suggests that exposure to AAs can lead to UUC [38, 39]. Recent studies have indicated that AAs could also induce renal cell carcinoma and premalignant liver alterations [40,41]. Rats treated with AAs 0.1, 1.0, and 10.0 mg/kg body weight/day for 3 months developed tumor incidences with frequencies of 25%, 85%, 100%, respectively. In addition, 72%, 28%, and 17% of the rats treated with 10.0 mg/kg had tumors of the forestomach, kidney, and urinary tract, respectively [42]. Moreover, AAs has been classified as a carcinogen by the International Agency for Research on Cancer [43].

Forming covalent adducts with DNA

DNA adducts were analyzed in the AAs-treated liver and kidney using ^{32}P-post-labeling, and three major AA-DNA adducts were detected including dA-AAI, dG-AAI, and dA-AAII [42]. Moreover, AL-DNA adducts were identified in the renal cortex of patients as a biomarker of AAs exposure [44]. The study of AAs metabolism demonstrated that ALI and ALII were the major metabolites found in the urine and feces [45, 46]. They both undergo reduction of nitro groups to form reactive cyclic acylnitrenium ions that can bind to the exocyclic amino groups of adenine and guanine, forming covalent adducts with DNA [42, 47, 48].

Gene Mutation

AAs have been shown to have mutagenic effects *in vivo*. First, AAs can induce a subcutaneous granuloma of rats caused by gene mutation determined using the granuloma pouch assay [49]. Moreover, the mutant frequency of gene *lacZ* and *cll* in the forestomach, kidney, and bladder were higher than those of control mice after intragastric administration of AAs [50]. In addition, AAs can lead to malformed kidney phenotypes in zebrafish embryos [51]. Mutation in certain tumor suppressor genes (*TP53*) or proto-oncogenes (*H-RAS*) can be detected in patients who have AAN-associated carcinoma [52]. This includes A:T to T:A transversion mutation (mutational signature), which can be used as an indicator of AAs exposure [53,54]. The mutation in AAs-induced urothelial tumors corresponds to a high prevalence of dA-AAI adducts in the tissue of patients with AAN. The adduct persists in the renal tissue and can be detected in patients with AAN after exposure for decades [55], which may be a reason for oncogenesis.

Differential activity of microsomal cytochrome P450

The differential activity of the enzymes catalyzing the biotransformation of AAs may lead to carcinogenesis. Various cytosolic and microsomal enzymes can activate AAs to reactive forms such as microsomal cytochrome P450 (CYP) 1A1, CYP1A2, and NADPH: CYP reductase [56]. Among them, CYP1A1 and 1A2 prevail in AAI metabolism *in vivo*. Furthermore, CYP1A1/2 can increase the detoxification of AAI to its metabolite AAIa and, thereby, suppress AAI-DNA adduct levels *in vivo* [57]. Hence, when the activity of the enzymes catalyzing biotransformation of AAs differs in the population, only certain people develop cancer due to the formation of high AAI-DNA adducts level.

Other proteins related to carcinogenesis

In addition, a study showed that 10 days of oral administration of AAI (3 mg/kg/day) could increase the level of C-myc and Lin28B, which are an oncoprotein and oncofetal RNA-binding protein, respectively [58]. In addition, a proteomics analysis of altered proteins in the kidney of mice with AAN revealed that G protein-coupled receptor 87, a tumorigenesis-related protein involved in signal transmission, was increased after administration of AAs [59].

These data may indicate possible molecular mechanisms by which AAs causes UUC.

Prevention and treatment

In 2002, Food and Drug Administration prohibited the use of AAs-containing herbs in America. In 2004, the usage of Guangfangji (*Aristolochia fangchi*) and Qingmuxiang (*Aristolochia debilis*) was canceled by China Food and Drug Administration.

Besides, some studies have indicated the possibility of preventing AAN. Firstly, the female hormone 17β-estradiol was reported to have protective effects on the renal ischemia-reperfusion injury of acute AAN by attenuating AA-induced cell apoptosis and downregulating the expression of *P53*, and phosphor-*P53* [60]. Secondly, L-Arg supplementation in AAs-treated mice significantly increased nitric oxide bioavailability, which could also restore renal function and reduce renal injury [61]. In addition, low-dose darbepoetin alpha treatment prevents acute tubular necrosis and interstitial fibrosis in human AAN [62]. Moreover, β-naphthoflavone, a non-carcinogen CYPA1 inducer, facilitated the disposal of AAI in the liver by inducing the expression of CYP1A1 in the liver [63], which could reduce the kidney toxicity.

Conclusion

In conclusion, AAs has been involved in the development of AAN and is associated with UUC. If the formation of AAs-DNA adducts could be used as a marker for assessing AAs exposure and evaluating the risk of HCC, then additional effects of AAs and their derivatives warrant further research. Furthermore, the frequency of administration and the amount of dose, exposure time to AAs, and infectious situations involving the hepatitis virus should also be identified.

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