REVIEW ARTICLE

Drug Safety 2003; 26 (1): 33-48
0114-5916/03/0001-0033/$30.00/0

Aristolochic Acid and 'Chinese Herbs Nephropathy'

A Review of the Evidence to Date

Jean-Pierre Cosyns

Department of Pathology, Medical School, Cliniques Universitaires St-Luc, Université Catholique de Louvain, Brussels, Belgium

Contents

Abstract

Chinese herbs nephropathy (CHN) is a rapidly progressive interstitial nephropathy reported after the introduction of Chinese herbs in a slimming regimen followed by young Belgian women. It is characterised by early, severe anaemia, mild tubular proteinuria and initially normal arterial blood pressure in half of the patients. Renal histology shows unusual extensive, virtually hypocellular cortical interstitial fibrosis associated with tubular atrophy and global sclerosis of glomeruli decreasing from the outer to the inner cortex. Urothelial malignancy of the upper urinary tract develops subsequently in almost half of the patients.

Suspicion that the disease was due to the recent introduction of Chinese herbs in the slimming regimen was reinforced by identification in the slimming pills of the nephrotoxic and carcinogenic aristolochic acid (AA) extracted from species of *Aristolochia*. This hypothesis was substantiated by the identification of premutagenic AA-DNA adducts in the kidney and ureteric tissues of CHN patients. Finally, induction of the clinical features (interstitial fibrosis and upper urothelial malignancy) typical of CHN in rodents given AA alone removed any doubt on the causal role of this phytotoxin in CHN, now better called aristolochic acid nephropathy (AAN).

AAN is not restricted to the Belgian cases. Similar cases have been observed throughout the world, but AA is sometimes incriminated on the basis of the known content of AA in the herbs. The possibility remains that in some individuals in whom AA has not been demonstrated, other phytotoxins might be implicated.

Biological and morphological features of AAN are strikingly similar to those reported in another fibrosing interstitial nephropathy of still unknown aetiology, Balkan endemic nephropathy (BEN). Interestingly, AA was incriminated as the cause of BEN many years ago, a hypothesis yet to be fully explored. The intake of AA and the presence of tissular AA-DNA adducts in patients with an unequivocal diagnosis of BEN remains to be demonstrated.

The tragic phenomenon of CHN, recognised only 10 years ago, has been at the root of significant research and progress both in nephrology and oncology. It has provided a fascinating opportunity to understand the link between a fibrosing interstitial nephropathy and urothelial carcinoma. It allows the categorisation of interstitial nephritis on the basis of histological findings, of initiating toxic substances and of associated clinical features. Finally, it has led to the withdrawal in several countries of a previously unsuspected carcinogenic and nephrotoxic substance.

1. Breakthrough

During the first half of 1992, Vanherweghem et al.[1] were puzzled by the discovery in Brussels, Belgium, of several young women under the age of 50 years who developed end-stage renal disease due to interstitial nephritis. All patients had followed the same slimming regimen at the same clinic in Brussels, suggesting that it was the cause of the nephropathy. The regimen, prescribed since 1975 apparently without untoward effects, included intradermal injections of artichoke extract and euphyllin, and pills containing fenfluramine, diethylpropion, meprobamate, powders of pancreas, of laminaria and of cascara, fucus extracts, and acetazolamide (table I). From May 1990 onwards, the pancreas and laminaria powders along with the fucus extract were replaced by belladonna extract and two herbs imported from China, *Magnolia officinalis* and *Stephania tetrandra*, raising the hypothesis that these herbs were responsible for the development of the disease. The disease was initially designated as 'Chinese herbs nephropathy' (CHN).[2]

Table I. Slimming regimens (values presented in mg) [reproduced from Vanherweghem et al.,[1] with permission from Elsevier Science. *The Lancet* 1993; 341: 387-91]

	Formula 1 (1975–May 1990)	Formula 2 (May 1990–May 1992)
Injection[a]		
Artichoke extract (chophytol S)	0.2	0.2[b]
Euphyllin	0.5	0.5
Capsule A[c]		
Fenfluramine	17–25	17–25
Diethylpropion	17–25	17–25
Meprobamate	0–50	0–50
Capsule B[c]		
Pancreas powder	100	0
Laminaria powder	50	0
Fucus extract	50	0
Cascara powder	20–150	20–150
Acetazolamide	25–45	25–45
Belladonna extract	0	1–2
Stephania tetrandra	0	100–200
Magnolia officinalis	0	100–200

a Intradermal injection once a week.

b Withdrawn July 1991.

c Three times a day, orally.

2. Clinical Data

Characteristically, the disease runs a subacute course despite cessation of toxic exposure, with initially normal blood pressure in half of the patients, early severe anaemia, normoglycaemic glucosuria and mild tubular proteinuria. The urine sediment is usually normal. Aseptic leucocyturia is found in half of the cases.[3-6] The rate of progression of renal failure is significantly related to the duration of the slimming regimen[4] and to the cumulative dose of *S. tetrandra*,[7] and inversely related to the interval between withdrawal of Chinese herbs and diagnosis.[4] Corticosteroid therapy has been claimed to slow the progression of renal failure.[8]

3. Pathology

The interstitial fibrosis initially reported on the basis of a few renal biopsies[1] has been subsequently integrated into a unique pathological picture[2,9] (figure 1): extensive hypocellular interstitial sclerosis, tubular atrophy and global glomerular sclerosis decreasing from the outer to the inner cortex, including the columns of Bertin; mild to severe hyalinisation, fibromucoid or fibrous intimal thickening mainly of interlobular arteries, and normal or collapsed residual glomeruli. The possibility of the subsequent development of urothelial malignancy was suggested by the constant presence of mild to moderate atypia of the epithelial lining of the collecting ducts and of the pelviureteric urothelium discovered in nephroureterectomy specimens.[2,10,11] Indeed, foci of papillary and *in situ* transitional cell carcinoma were observed in two of these patients in both pelves and ureters, and in one of the pelves, respectively.[12,13] These findings were confirmed in several patients' native kidneys and ureters at the time of renal transplantation or shortly thereafter: urothelial malignancy was indeed observed in 40–46% of patients with CHN mainly in the upper urinary tract.[10,11] Clearly, the ingested Chinese herbs also had a carcinogenic effect on the urothelium.

4. Differential Diagnosis

The association of interstitial fibrosis with urothelial atypia and malignancy is reminiscent of two other nephropathies, analgesic nephropathy (AN) and Balkan endemic nephropathy (BEN).

A history of long-term analgesic consumption distinguishes AN from the other nephropathies. Like CHN, classic AN is characterised by marked early anaemia and slowly progressive renal impairment.[15] Blood pressure level is influenced by the degree of renal failure. Overall it is elevated, usually mildly, in 40–50% of the patients. In contrast to CHN, sterile pyuria is present on urinalysis[16] and renal colic, with occasionally reversible acute renal failure and macro- or microscopic haematuria, is occasionally reported. The latter results from sloughing and elimination of necrotic papillae.

On morphological grounds, the hallmark of classic AN is diffuse renal papillary necrosis, a feature that is absent in CHN. Papillary necrosis starts in the mid-papilla and may subsequently become calcified and associated with secondary patchy cortical areas of interstitial fibrosis and tubular atrophy leading to cortical retractions. In contrast to the situation in CHN, these areas contain inflammatory cells, are not predominantly subcapsular and spare the columns of Bertin.[17] On renal imaging, both kidneys are atrophic, with bumpy contours and papillary calcifications. Together with a history of analgesic abuse, these features are taken to be diagnostic of AN.[18] In CHN, renal atrophy is homogeneous with very few mild cortical retractions and no calcifications.[2] Urothelial cancer of the upper urinary tract associated with urothelial atypia is frequently found in AN,[19] but with a lower incidence (5–24%) than in CHN.[20,21]

In contrast to AN, the analogy between CHN and BEN is striking on clinical and on histological grounds. Blood pressure is elevated in around 40% of patients with advanced renal failure.[22] In both conditions, anaemia develops early and may be severe,[23] and mild tubular proteinuria, glucosuria and aseptic leucocyturia are found.[5,6,24] Upper

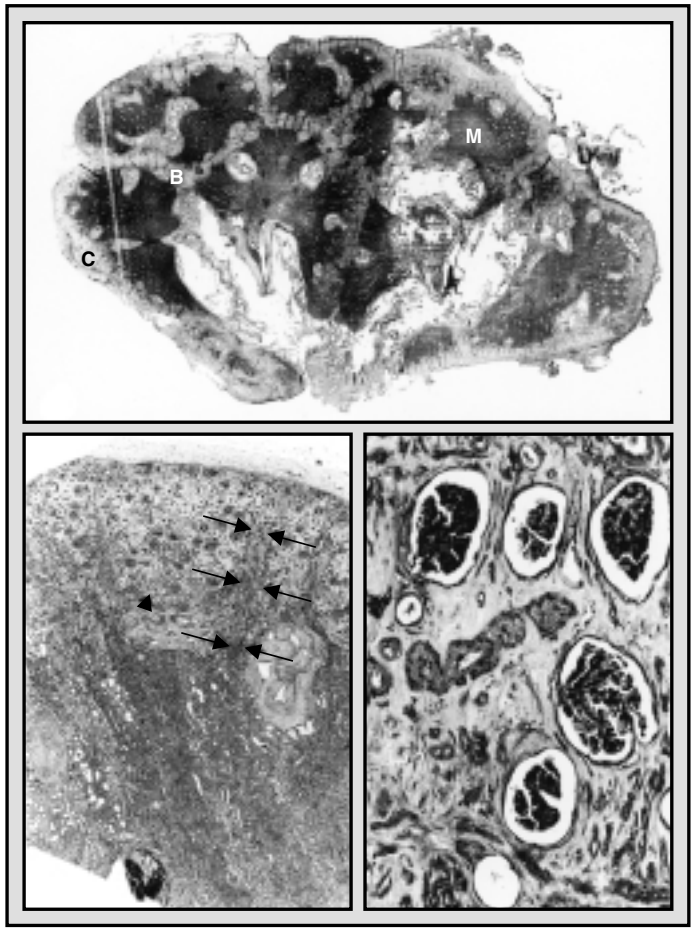

Fig. 1. Kidney section of patient with end-stage Chinese herbs nephropathy. (**Top**) Diffuse atrophy of cortex (C) including columns of Bertin (B) down to the renal sinus. Slight involvement of the pyramids (M). (Haematoxylin and eosin stain, × 4). (**Bottom left**): Hypocellular interstitial fibrosis and tubular atrophy are more marked in medullary ray (arrows) and the outer cortical labyrinth. Tubules are still visible in the inner cortical labyrinth (arrowhead). [Periodic acid-Schiff stain, × 30]. (**Bottom right**): Severe fibrous intimal thickening of interlobular artery. Acellular interstitial sclerosis with few atrophic tubules. (Hematoxylin and eosin stain, × 280) [reproduced from Cosyns et al.,[2,14] with permission from Blackwell Publishing. *Kidney Int* 1994; 45: 1680-1688; *Kidney Int* 2001; 59: 2164-2173].

urothelial cancer develops with the same frequency (40%)[25] and is associated with atypia, a constant finding in CHN. The pathological lesions of CHN are amazingly close to those of BEN: both kidneys are symmetrically small and shrunken, with a smooth outline. On histological examination there is widespread remarkably acellular or hypocellular interstitial fibrosis and tubular atrophy associated with only focal mononuclear cellular infiltration and either normal or globally sclerosed glomeruli. Fibrotic changes are more conspicuous in the cortex than in the medulla and progress from the subcapsular area to the deep cortex.[26-31] Interestingly, interlobular fibroelastosis, which is absent to moderate in early stages of CHN[9] and found in only 35% of pre-uraemic BEN kidney samples,[26]

is a symptom that becomes severe in later stages of both diseases,[2,32] suggesting that it is secondary to the progressive kidney destruction.

Several differences between the two diseases should be pointed out. BEN is characterised by a familial[25,33] and an environmental clustering.[25] The sex ratio in BEN is approximately 1 : 1, in contrast to the female preponderance in CHN. The latter probably reflects the almost exclusive female attendance at the slimming clinic. Progression towards end-stage renal failure is more rapid in CHN (a few months) than in BEN (decades).[25] Finally, the development of urothelial malignancy requires a much longer exposure in BEN: induction times of 20 and 27 years, respectively, have been calculated for renal pelvic and urinary bladder carcinomas associated with BEN.[34] In contrast, in CHN, exposure to the toxic slimming regimen averaged 20 months with a delay between the end of the regimen and the discovery of urothelial malignancy ranging from 2 to 6 years.[10,11] The single pathological difference between the two diseases lies in the more extensive and constant involvement of the columns of Bertin in CHN than in BEN.[30,35]

The similarities between CHN and BEN suggest a common aetiological agent. As discussed in section 5, aristolochic acid (AA), the mutagenic and nephrotoxic alkaloid extracted from the plant *Aristolochia*,[36-41] is the main cause of CHN. It has also been incriminated many years ago in the genesis of BEN,[42] a hypothesis yet to be fully explored.[2,43,44]

5. Aetiology

The aetiopathogenesis of CHN has been disputed. Serotonin, a vasoconstrictive agent endowed with renal interstitial fibrotic properties,[45] claimed to be injected at the time of mesotherapy in CHN patients,[46,47] as well as the concomitant consumption of analgesics or of ochratoxin A (OTA) [both fibrogenic and carcinogenic substances] have been incriminated but not confirmed. Renal failure with or without urothelial carcinoma has been observed in some CHN patients despite the absence of mesotherapy or of regular analgesic or tobacco consumption.[11] OTA was not identified in the pills used in the slimming cure.[1] Only a small number of renal tissue samples contained weakly positive OTA-related DNA adducts.[11,48]

The possibility of confusion between Chinese herbs by the Chinese export company was initially entertained but, at first, neither OTA nor AA was found in the capsules taken by the patients.[1] However, the Chinese Medicinal Material Research Center at the Chinese University of Hong Kong quickly recognised *Aristolochia fangchi*, a non-prescribed AA-containing herb, in a sample of Chinese herbs imported into Belgium under the name of *S. tetrandra* (fangji).[49] Tetrandrine, the alkaloid present in the latter herbs, was not found in the slimming pills,[1] whereas variable amounts of AA were subsequently identified in 10 of 12 batches of so-called *S. tetrandra* herbs imported in Belgium;[50] the absence of AA in the original pill samples was attributed to analytic difficulties. The maximal daily dose of AA has been estimated at 0.025 mg/kg bodyweight for an average of 13 months[11,51] These observations supported the hypothesis that AA had inadvertently been included in the slimming pills perhaps as a result of confusion between 'fangji' and 'fangchi' and that this nephrotoxic and carcinogenic alkaloid was the cause of CHN.[1] The role of AA remained to be further evaluated.

5.1 Aristolochic Acid

AA is the active principle extracted from *Aristolochia*.[52] The roots or rhizomes provide 0.24–0.40% essential oil, tannin, resin, β-sitosterin, allantoin and a yellow, bitter and acidic substance, designated as AA. AA is a mixture of structurally related nitrophenanthrene carboxylic acids, 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), differing from each other only by one

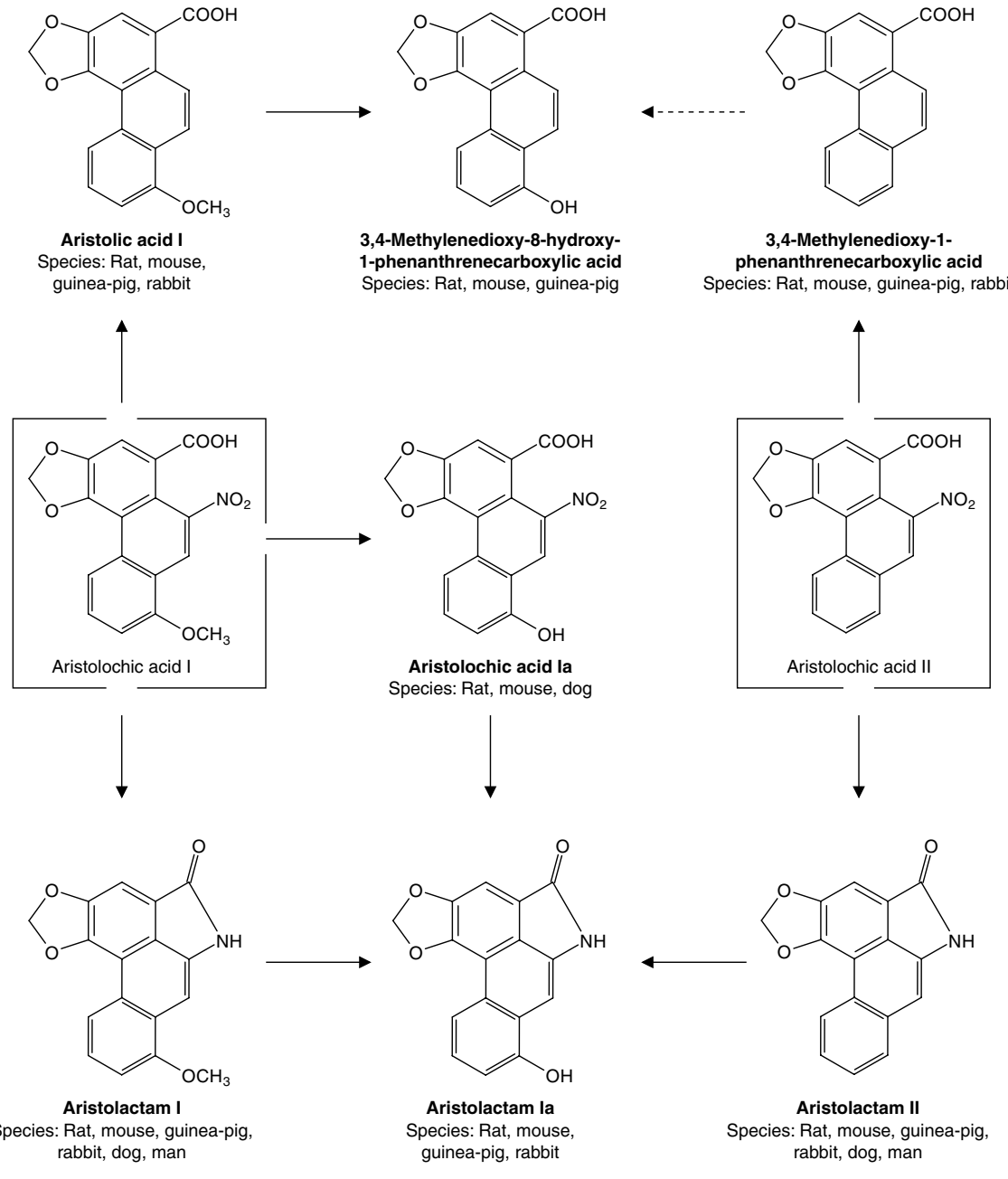

Fig. 2. Metabolic transformation of aristolochic acid (AA)I and AAII (reproduced from Krumbiegel et al.,[59] with permission from Taylor and Francis Group. *Xenobiotica* 1987; 17: 981-91; www.tandf.co.uk/journals). Dotted arrow indicates a postulated reaction.

methoxy group (figure 2). Besides these major components, extracts of species of *Aristolochia* also contain several derivatives such as aristolactams predominantly[53] and AAIII, AAIIIa, AAIV and AAIVa in minor quantities.[54-56] Most importantly, analysis by high pressure liquid chromatography of the so-called *S. tetrandra* herbal powder used to prepare the slimming pills showed that AA was actually present within the natural mixture, as both AAI and AAII were found in two of the three tested samples.[57,58]

AA has been used in Europe, South America and the Far East as herbal medicine in obstetrics, in the treatment of tumours, and especially in folk medicine as snake venom antidotes.[60-62] During the last century, AA was used for its antiviral, antibacterial and antineoplastic properties.[63-65]

There is little information in the literature regarding the metabolism of AA in mammals.[66] After oral administration of AAI and AAII to several species, Krumbiegel et al.[59] showed that most AA metabolites in urine and faeces were reduction products (figure 2).

Under aerobic conditions, AAI is extensively demethylated into AAIa and then reduced to aristolactam Ia, a stable metabolic end-product. Under anaerobic conditions a minor amount of AAI is reduced to aristolactam I and eventually to aristolactam Ia. In contrast, AAII is not metabolised aerobically. Under anaerobic conditions, it undergoes a rapid reduction of the nitro group to the amino derivative and subsequent ring closure to aristolactam II. Only a minor amount of aristolactam II is subsequently transformed: after a single dose of AAII, only trace amounts of aristolactam Ia can be found in urine and faeces.[59]

Reductive replacement of the nitro group of AAI or AAII produces aristolic acid I and 3,4-methylenedioxy-1-phenanthrenecarboxylic acid, respectively. Demethylation of aristolic acid produces 3,4-methylenedioxy-8-hydroxy-1-phenanthrenecarboxylic acid. Whether the latter compound can also evolve from AAII awaits further elucidation.

Metabolic transformations of AA differ among species. Rats and mice show identical metabolite patterns. In contrast, urine samples of guinea-pigs, rabbits, and to an even greater extent dogs and humans, do not yield all the metabolites identified in rats. Metabolites produced by reductive replacement of the nitro group of AAI and AAII are found in rats, mice, guinea-pigs and rabbits, but not in dogs and humans.[59]

5.2 Evidence for the Aetiological Role of Aristolochic Acid in Chinese Herbs Nephropathy

AA is a known nephrotoxic agent inducing acute renal failure and tubular lesions in several species; acute tubular damage has been reported after a single intravenous injection of 1 mg /kg[67] in rabbits and after less than 1 mg/kg/day for 3 days or more in humans.[68] A similar effect in rats and mice requires administration of much higher amounts: 20 and 30 mg/kg, respectively.[69] Dogs, cats, frogs and porpoises do not develop acute tubular lesions after AA administration.[67]

AA is also a potent carcinogen in rodents. Its mutagenicity has been established in several short-term tests.[38-41] Time- and dose-dependent multisystemic tumours have been observed in rats and mice after long-term oral intake of AA.[36,37] All rats given a daily oral intake of 10 mg/kg develop tumours within 3 months, mainly in the forestomach and bladder.

Mutagenic and carcinogenic effects of AA are associated with the formation of AA-DNA adducts.[70] Nitroreduction of AA is essential to produce the DNA-binding species.[70] Using the ^{32}P-postlabelling assay,[71] Pfau et al.[72] demonstrated that on metabolic activation both *in vitro* and *in vivo* in various rat organs, AA binds covalently to the exocyclic amino group of DNA purine nucleotides, leading eventually to the formation of DNA adducts. *In vitro*, DNA adducts are produced by the reaction of enzymatically activated AA with 3′-purine phosphonucleosides.[70] Enzymatic activation of AA may be achieved by butter-

milk xanthine oxidase, rat DT-diaphorase, nicotinamide adenine dinucleotide phosphate: cytochrome P450 (CYP) reductase, CYP1A1 and CYP1A2, peroxidases and ram seminal vesicle prostaglandin-H-synthase.[66] These adducts obtained *in vitro* were assigned to the 7-(deoxyadenosin-N^6-yl)-aristolactam I (dA-AAI), the 7-(deoxyadenosin-N^6-yl)-aristolactam II (dA-AAII), the 7-(deoxyguanosin-N^2-yl)-aristolactam I (dG-AAI) and the 7-(deoxyguanosin-N^2-yl)- aristolactam II (dG-AAII) and have been used as reference compounds for identification and quantification of DNA adducts in the target organ (forestomach) of male Sprague-Dawley rats treated orally with AA.[71] The pattern produced included dA-AAI, dA-AAII, dG-AAI and dG-AAII.[72] According to the structure of the formed AA-DNA adducts, an intermediate cyclic nitrenium ion with a delocalised positive charge is postulated as the ultimate electrophilic species binding to the exocyclic amino groups of purine nucleotides.[66,73] It is noteworthy that the lifelong persistence of AA-DNA adducts in AA-treated animals makes their detection in tissues a valid biological marker even years after cessation of AA exposure.[11,57]

5.3 Carcinogenic Potential of Aristolochic Acid

The suspected aetiological role of AA found in the slimming pills was substantiated by the ^{32}P-postlabelling method and cochromatographic analyses with authentic markers: AA-DNA adducts were identified in the kidney tissue of patients with CHN. One major DNA adduct was identified as dA-AAI and two minor DNA adducts as dG-AAI and dA-AAII, respectively, in all of eight kidney samples and in one ureter removed from six patients a mean of 20 months [range 9–44] after the end of the slimming regimen.[57] These DNA adducts were absent in six control end-stage kidneys from patients with various renal diseases different from CHN. These specific adducts are similar to those reported in the DNA of AA-treated rats, the dA-AAI being the major adduct.[71] These results have been subsequently confirmed in a second, larger series of CHN patients with a longer interval between the end of AA exposure and the nephroureterectomy.[11] As a result, the levels of AA-DNA adducts were lower (levels of predominant adduct, dA-AAI, equal to 70–530 and 1.2–165 per 10^9 nucleotides in the first and second series, respectively). The detection of specific DNA adducts up to 89 months after toxic exposure suggests non-reparable genomic lesions. This is in line with the apparently life-long persistence of AA-DNA adducts in the kidneys of rats given AA.[57,74] Altogether these findings conclusively demonstrate that CHN is associated with the intake of AA and that the amount of AA is sufficient to alter cellular DNA. The absence of OTA in the slimming pills used in the slimming cure[1] and the finding of only a small number of renal tissue samples weakly positive for OTA-related DNA adducts in tissue samples taken late as well as shortly after renal transplantation indicate that OTA does not play a key role in CHN.[11,48]

Specific DNA adducts within the genomic DNA have an important biological significance as premutagenic lesions providing a pathophysiological clue for the associated urothelial atypia and cancer.[10,11] This has been verified for the principal AA-DNA adduct found in the tissues of CHN patients, the dA-AAI adduct.[11,57,75] C-Ha-*ras* proto-oncogenes are activated with high frequency by an A → T transversion mutation in codon 61 from CAA to CTA in AAI-induced forestomach carcinomas in rats.[76] '*In vitro*' primer extension studies with site-specifically adducted oligonucleotides containing AA-DNA adducts suggest a higher mutagenic potential of adenine adducts than of guanine adducts.[77] The p53 tumour suppressor gene is overexpressed in malignant urothelial cells of 4/4 CiS and/or papillary transitional cell carcinoma developed in CHN patients,[10] a finding that suggests this gene is mutated in AA-associated malignancy. Sequencing analysis of the papillary transitional cell carcinoma resected from the bladder in one CHN patient showed the existence of an

A → C transversion and of a G → A transition mutation in exon 7 of p53; in codon 230, ACC (Thr) is mutated to CCC (Pro), and in codon 248, CGG (Arg) is mutated to CAG (Gln) [C. Lurquin, personal communication]. This result has to be further investigated by the analysis of p53 mutations in a large number of urothelial tumours from CHN patients. Of note, codons 248 and 230 for exon 7 of human p53 were found to be, respectively, a strong and weak AA-DNA binding site *in vitro*.[78] It is of interest to note that codon 248 of p53 is a mutational hotspot in many tumours, whereas mutation in codon 230 has been reported only once so far.[79,80]

The mechanism of the carcinogenic activity of AA remains to be determined.[66] Site-specifically synthetic adducted oligonucleotides obtained *in vitro* after modification with AAI and AAII have been used as templates in primed DNA replication reactions with prokaryotic (sequenase)[77] and eukaryotic (human pol α)[81] DNA polymerases. Analysis of nucleotide incorporation directly across from all AA purine adducts showed a preferential incorporation of dCMP opposite the deoxyguanosine adducts. In contrast, no preferential incorporation of dAMP and dTMP was found opposite the deoxyadenosine adducts. These findings suggest that the mutagenic potential of AA results from dAMP incorporation by DNA polymerase opposite the adenine adducts. This is in line with the frequent A → T transversion mutation observed in the c-Ha-*ras* gene of AA-induced tumoral cells in rodents.

5.4 Nephrotoxic Potential of Aristolochic Acid

In contrast to the well known acute nephrotoxic and carcinogenic effects of AA, little is known about its chronic nephrotoxicity. NZW rabbits given AA intraperitoneally 0.10 mg/kg/day for 17–21 months develop not only urothelial atypia (at 17 and 21 months) and malignancies (at 21 months) but also impaired renal function (at 16 months) and extensive interstitial fibrosis (at 17 months) which is hypocellular and presents a conspicuous decreasing corticomedullary gradient of intensity, two characteristic features of CHN.[14] The intensity of the renal lesions varied between animals and allowed a more refined pathological analysis. The mildest lesions concerned the straight part (S3) of the proximal tubule of superficial nephrons; more severe lesions further involved the convoluted parts (S1, S2) of the proximal tubule, also of superficial nephrons. The most severe lesions also affected the proximal tubules of the deep nephrons. These data are compatible with a toxic effect of AA on tubular cells, first of the S3 and then of the more proximal S1 and S2 segments of proximal tubules with an attendant interstitial fibrosis. The preferential involvement of superficial nephrons might account for the corticomedullary gradient of interstitial fibrosis. In contrast, normal rats given a higher dosage of AA orally (10 mg/kg/day) for 3 months developed urothelial atypia and malignancies 3 months later but no renal failure or interstitial fibrosis.[58] In rats given an intraperitoneal AA dose of 5 mg/kg/day for 4 months, renal dysfunction and hypocellular interstitial sclerosis developed at 4 and 6 months, respectively. Neither a corticomedullary gradient for fibrosis nor urothelial atypia and tumours are reported.[82] In previously dehydrated rats, AA 10 mg/kg/day given subcutaneously during 35 days increased serum creatinine (at day 10) and produced interstitial fibrosis (at day 35) with marked lymphocytic infiltration and without documented evidence of a characteristic corticomedullary gradient. Urothelial atypia and malignancies were reported at days 10, 35 and 105 and malignancies at 3 months.[51] The variation in interstitial fibrosis induced by AA in rats might result at least in part from different routes of administration.

The cellular mechanisms of AA toxicity have been recently delineated[83] in an established model of proximal tubule cells, the OK cell line. A transient exposure to AA significantly decreases expression of megalin, forms specific DNA ad-

ducts similar to those found in kidneys from CHN patients, and permanently inhibits tubular protein reabsorption.[83] Whether a DNA mutation is also responsible for the kidney-destructive fibrotic process awaits further elucidation. It is of interest to note that p53 mRNA expression is increased in both kidneys of rats with a unilateral ureteral obstruction, suggesting that p53 is also involved in renal fibrosis.[84]

The relevance of these rodent models of chronic AA intoxication to the understanding of CHN should now be examined. The presence of extensive flattened proximal tubular cells in CHN patients[2,9] associated in the two last animal models with interstitial oedema is reminiscent of acute toxicity studies.[67-69] Repeated proximal tubule epithelial cell damage might lead to inflammation and fibrosis. The early development of tubular proteinuria and glucosuria observed in CHN patients and in these AA-treated animals together with the reported adult onset of Fanconi syndrome in patients after the consumption of Chinese herbs containing AA[85-90] strongly suggest that proximal tubule cells play a key role in the pathogenesis of CHN lesions. Several other findings are common to CHN patients and AA-treated rodents: arterial blood pressure remains normal;[14] serum creatinine increases;[14,51,82] mild tubular proteinuria develops;[14,51,82] normoglycaemic glucosuria[14,51] and anaemia[14] are noted; and upper urinary tract cancer and urothelial atypia develop.[14,51] Interestingly, arterial and glomerular lesions are absent in rodents,[14,51] whereas arteries are normal in the early stages of CHN.[9] Fibroelastosis of the interlobular arteries is present in all patients with end-stage CHN.[2] These data are compatible with the hypothesis that the renal vasculature is not the primary target of AA toxicity but that vascular lesions are secondary to the progressive kidney destruction.

Some differences between CHN patients and AA-treated rodents should be pointed out. CHN patients progress to end-stage renal failure within a few months or years, whereas serum creatinine levels rise slowly in AA-treated rodents[14,82] unless they are dehydrated, which accelerates renal functional impairment.[51] The daily dosage of AA used is highest in rats, 200–400 times that calculated in CHN patients,[11,51] and lower in rabbits (4 times that calculated in CHN patients). This could reflect interspecies differences in the susceptibility to AA[67] due to differences in the metabolic transformation of AA between humans and rodents.[59] Alternatively, the toxicity of AA might have been potentiated in humans by the other compounds contained in the herbal preparation. Urothelial malignancies develop earlier in humans (2–6 years)[10,11] than in rodents when their respective life expectancies are taken into account.

Taken together, the experimental data and the clinical observations are compatible with the following hypothetical scheme. AA induces tubular cell lesions in the S3 and subsequently in the S1 and S2 segments of proximal tubules, initially in the superficial and later in deep nephrons, with an attendant interstitial hypocellular fibrosis with a striking corticomedullary gradient. These tubular lesions account for the various tubular disorders encountered in CHN. AA does not initially affect the vasculature but progressive renal destruction is associated with secondary arterial lesions. The mutagenic potential of AA is responsible for the development of urothelial malignancies. Whether it is also involved in the tubular lesions remains to be explored.

6. Time to Abandon the Term Chinese Herbs Nephropathy

Compelling evidence now demonstrates that CHN is not restricted to the young women given the suspected slimming phytotherapy. The worldwide use of AA since antiquity indicates that the pathological picture of CHN has not been recognised in the past and that the Belgian epidemic is only 'the tip of the iceberg'.[3,91,92] This assumption has been confirmed by two recent observations made in China and in the UK. A Chinese woman presented with clinical features of subacute

interstitial nephritis after consumption of pills bought in Shanghai (Pan Long Yun Hai Pharmaceutical Co. Ltd, Chuxiong City, China).[93] The components mentioned on the package insert were root of *Panax quinquefolius* (Xiyangshen), root of *Cynanchum otophyllum* (Qingyangshen), root of *Rubia yunnanensis* (Xiaohongshen), rhizome of *Rheum officinale* (Dahuang), leaf of *Nelumbo nucifera* (Heye) and others, without mention of *Aristolochia*. Renal biopsy disclosed hypocellular interstitial fibrosis with severe tubular atrophy. Glomeruli were either globally sclerosed or normal by light microscopy. AA was identified in the pills and the major AA-DNA adduct, dA-AAI, has been demonstrated in a fragment of the frozen kidney biopsy. A second woman, living in the UK, developed rapidly progressive interstitial nephritis after 2 years' treatment of eczema with a herbal preparation Mu Tong (*Aristolochia manshuriensis*) containing AAs.[94] A preterminal renal biopsy sample showed a virtually hypocellular cortical interstitial fibrosis typical of CHN. Three years after renal transplantation she developed multifocal urothelial malignancies of both native pelves and ureters. dA-AAI was demonstrated in native ureteric and renal tissue samples.[95] None of the other substances contained in the Belgian slimming cure were ingested by these two patients; they are thus not required to induce CHN lesions. Thus, the morphological and biological features of CHN observed in rodents given AA alone, together with these two last observations, remove any doubt concerning the aetiological role of AA in the genesis of CHN. Therefore, the interstitial nephropathies in which the unequivocal role of AA has been fully demonstrated should better be called AA nephropathy, or AAN. This term is preferred to the term CHN not only for scientific reasons,[93] as it points more precisely to the culprit, but for addressing the possible prejudicial character of the latter term, which may imply to readers that Chinese herbs in general cause renal impairment.[96-98] However, use of the term AAN requires demonstration of AA intake and, whenever possible, of a typical renal morphological picture including hypocellular interstitial fibrosis with a decreasing corticomedullary gradient together with urothelial atypia and occasional malignancy along with AA-DNA adducts in the urinary tract tissue.

6.1 Patients with Aristolochic Acid Nephropathy

Although an accurate number is difficult to establish because of duplicate publications, more than 100 patients have developed AAN caused by the Belgian slimming regimen.[99] Renal histology, available in about 50 patients,[1,2,9-13,48,100] showed the typical hypocellular interstitial fibrosis with a decreasing corticomedullary gradient of intensity in all the cases. Urothelial atypia and malignancy were found, respectively, in virtually all[2,10-13,48,100] and in nearly half[10-13,100] of the cases. AA-DNA adducts have been identified in the kidney tissue in more than 40 patients tested.[11,48,57,75]

It is interesting to note that only a small fraction, about 3%[10] to 5%,[101] of the patients who followed the slimming regimen, developed the disease. This may result from differences in the content of AA in the batches of so-called *S. tetrandra*,[11,50] in the patient's compliance to the regimen and in the susceptibility to toxic and/or carcinogenic substances, which is partially determined by the genetic polymorphism of the cytochrome P450-dependent mono-oxygenases involved in the metabolic activation of toxic compounds.[102] The effect of the genetic polymorphism on the enzymatic activation of AA by patients with AAN remains to be evaluated.[66]

Outside the Belgian epidemic, at least 65 cases of CHN have been reported worldwide and most of these cases could be classified as AAN patients. The ingested herbs correspond to the following: Mu Tong (*A. manshuriensis*);[94,95,103] Kan Mokutsu or *A. manshuriensis* ('Tenshin-toki-shigyaku-ka-gosyuyu-syokyo-to'[85,88] and 'toki-shigyaku-ka-gosyuyu-syokyo-to'[104]); *Aristolochia pistolochia*;[105] species of *Stephania*;[106-108] a Chinese herbal remedy called Akebia 14;[90] a combination

of various herbs without mention of the *Aristolochia* species (content mentioned in section 6)[93] and including,[89] among others, alismatis rhizoma [*Alisma orientale*], ginseng [*Panax ginseng*], Japanese angelica root [*Angelica autiloba*], *Poria sclerotium* [Poria], atractylodis lanceae rhizoma [*Atractylodes lancea*], *Atractylodes* rhizome [*Atractylodes japonica*] and astragali radix [*Astragalus membranaceus*]); and a Chinese herb mixture named 'Kidney-Protection'.[87] In several cases, the names of the consumed herbs are not reported[86,88,109] or are available in Chinese or Japanese characters only.[110-112] AA has been identified in all but three[88,103,105] of the herbal preparations used by these patients. In these cases, we rely on the identification of the herb and its reputed AA content. AA intake by the patient reported by Pena et al.[105] is likely, since the ingested herb belongs to the genus *Aristolochia*. In the cases reported by Li et al.,[103] the evidence of AA intake is limited to the possibility that *A. manshuriensis* was included in the Chinese herbal remedy Mu Tong, whose name may derive from *A. manshuriensis* and whose content has proved to be AA in cases of AAN.[94,95] Finally, seven of eight patients reported from the literature in Japan by Tanaka et al.[88] as CHN patients may be probably classified as AAN patients, as four of them took 'Tenshin-toki-shigyaku-ka-gosyuyu-syokyo-to' (Kan Mokutsu), which was found to contain AA in cases of AAN, and three of them consumed 'Boui-ougi-to' and 'Ryutan-shakan-to', which may derive from species of *Aristolochia*.

Renal histology available in all of these patients[85-89,93-95,103-106,109-111] revealed interstitial fibrosis which was hypocellular in all the cases. Because of the small size of biopsy material in the majority of these cases, the topography of the interstitial fibrosis is not reported and urothelial atypia are mentioned only twice.[93,106] Of note, glomerular mesangial matrix increase and double contour appearance of capillary walls was seen in one case.[89] Upper urinary tract malignancy developed in two cases.[86,95] The kidney tissue tested for the presence of AA-DNA adducts in two patients was found to be positive.[93,95] Of note, Fanconi syndrome is reported in few of these patients.[85-90]

It is also interesting to note that in China, despite the use of AA for centuries, adverse effects have been only occasionally reported. They may have been overlooked. Alternatively, they may be less frequent because AA is given without admixture of Western drugs such as fenfluramine and acetazolamide, which may have potentiated the toxicity of AA in the Belgian epidemic. An epidemiological study collecting anamnestic, clinical, pathological and molecular data (identification of AA-DNA adducts in the kidney and urinary tract tissues) is mandatory before the secular use of AA is proved innocent.

6.2 Patients with Phytotherapy-Associated Interstitial Nephritis

After considerable discussion on the *Am J Kidney Dis* Discussion Forum (27 January 2001) and on the NEPHROL e-mail discussion group concerning whether the term CHN is prejudicial and should be abandoned,[96] it has been recommended that the term 'phytotherapy-associated interstitial nephritis' (PAIN)[97,98] be used to designate patients in whom phytotherapy has been incriminated without the causative agent being identified. More than 30 such patients, at least, have been reported.[88,113-115] One report concerned a 36-year-old female patient who presented after 6 months of malaise and progressive weight loss with normal blood pressure (146/90mm Hg), anaemia (haemoglobin 8.3 g/dl), increased serum creatinine (203 μmol/L), mild proteinuria (0.78 g/24h) and normal urinary sediment.[113] Both kidneys had a reduced size. Renal biopsy showed mainly extensive hypocellular interstitial fibrosis with tubular atrophy. No medical data or history could account for this progressive renal failure except the consumption of Jia Wey Guo Sao pills (six pills, 3–4 times/day) during the last 7 years until admission. The pills contained *Angelica sinensis*

radix 90mg, *Rethmania* radix et rhizoma 90mg, ligustici rhizoma 50mg, *Paeonia lactiflore* radix 50mg, ginseng radix 35mg, *Eucommia* cortex 20mg and mel (honey) 165mg. Contamination of the pills by *Aristolochia* is unlikely, as the pills were made from the Chinese plants directly rather than from the plants reduced to powders, which makes them all look alike. Renal failure requiring renal replacement therapy has recently been reported in ten Chinese and 20 Taiwanese patients after consumption of various unidentified herbs for various purposes.[114,115] Finally, one of the patients reported from the Japanese literature as a CHN patient had ingested unidentified herbs included in 'health food' without mention of AA identification.[88] Whether phytotoxins other than AA may cause pathological changes similar to those of AAN awaits further evaluation. These observations suggest that PAIN is more common than previously thought. They demonstrate the necessity of investigating the use of herbal remedies and their possible toxicity in cases of renal failure of unknown origin.[115]

7. Conclusion

The dramatic history in Belgium of AA-induced nephropathy has led to the withdrawal of a formerly unsuspected carcinogenic and nephrotoxic substance. Several countries, including Canada, Australia, Germany and the UK, banned the use of herbs containing AA.[116] It has provided a fascinating opportunity for understanding the association between fibrosing interstitial nephropathy and urothelial carcinoma. It has further allowed detection of a worldwide problem, the extent of which is now under investigation. Finally, it allows categorisation of interstitial nephritis on the basis of histological findings, of initiating toxic substances and of associated clinical features.

The tragic phenomenon of CHN recognised only 10 years ago has thus been at the root of significant progress both in nephrology and oncology.[3]

Acknowledgements

The author thanks Professor C. van Ypersele de Strihou for helpful comments, Dr C. de Burbure for the revision of the manuscript, Mrs R.M. Goebbels for technical assistance and Mr S. Lagasse for preparing the photographs.

This research has not been supported by public or private grants. The author has no potential conflict of interest that is directly relevant to the content of this manuscript.

References

1. Vanherweghem JL, Depierreux M, Tielemans C, et al. Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. Lancet 1993; 341: 387-91
2. Cosyns JP, Jadoul M, Squifflet JP, et al. Chinese herbs nephropathy: a clue to Balkan endemic nephropathy? Kidney Int 1994; 45: 1680-8
3. van Ypersele de Strihou C, Vanherweghem JL. The tragic paradigm of Chinese herbs nephropathy. Nephrol Dial Transplant 1995; 10: 157-60
4. Reginster F, Jadoul M, van Ypersele de Strihou C. Chinese herbs nephropathy presentation, natural history, and fate after transplantation. Nephrol Dial Transplant 1997; 12: 81-6
5. Kabanda A, Jadoul M, Lauwerys R, et al. Low molecular weight proteinuria in Chinese herbs nephropathy. Kidney Int 1995; 48: 1571-6
6. Nortier JL, Deschodt-Lanckman MM, Simon S, et al. Proximal tubular injury in Chinese herbs nephropathy: monitoring by neutral endopeptidase enzymuria. Kidney Int 1997; 51: 288-93
7. Martinez MC, Nortier J, Vereerstraeten P, et al. Progression rate of Chinese herb nephropathy: impact of *Aristolochia fangchi* ingested dose. Nephrol Dial Transplant 2002; 17: 408-12
8. Vanherweghem JL, Abramowicz D, Tielemans C, et al. Effects of steroids on the progression of renal failure in chronic interstitial renal fibrosis: a pilot study in Chinese herbs nephropathy. Am J Kidney Dis 1996; 27: 209-15
9. Depierreux M, Van Damme B, Vanden Houte K, et al. Pathologic aspects of a newly described nephropathy related to the prolonged use of Chinese herbs. Am J Kidney Dis 1994; 24: 172-80
10. Cosyns JP, Jadoul M, Squifflet JP, et al. Urothelial lesions in Chinese-herb nephropathy. Am J Kidney Dis 1999; 33: 1011-7
11. Nortier JL, Muniz Martinez MC, Schmeiser HH, et al. Urothelial carcinoma associated with the use of a Chinese herb (*Aristolochia fangchi*). N Engl J Med 2000; 342: 1686-92
12. Cosyns JP, Jadoul M, Squifflet JP, et al. Urothelial malignancy in nephropathy due to Chinese herbs [letter]. Lancet 1994; 344: 188
13. Vanherweghem JL, Tielemans C, Simon J, et al. Chinese herbs nephropathy and renal pelvic carcinoma. Nephrol Dial Transplant 1995; 10: 270-3
14. Cosyns JP, Dehoux JP, Guiot Y, et al. Chronic aristolochic acid toxicity in rabbits: a model of Chinese herbs nephropathy? Kidney Int 2001; 59: 2164-73
15. De Broe ME, Elseviers MM. Analgesic nephropathy. N Engl J Med 1998; 338: 446-52
16. Duggin GG. Combination analgesic-induced kidney disease: the Australian experience. Am J Kidney Dis 1996; 28 (1 Suppl.): 39S-47S

17. Olsen S. Histopathology of drug and toxin induced kidney lesions. Zentralbl Allg Pathol 1986; 132: 365-83
18. Elseviers MM, De Broe ME. Combination analgesic involvement in the pathogenesis of analgesic nephropathy: the European perspective. Am J Kidney Dis 1996; 28 (1 Suppl.): 48S-55S
19. Mccredie M, Stewart JH, Carter JJ, et al. Phenacetin and papillary necrosis: independent risk factors for renal pelvic cancer. Kidney Int 1986; 30: 81-4
20. Blohme I, Johansson S. Renal pelvic neoplasms and atypical urothelium in patients with end-stage analgesic nephropathy. Kidney Int 1981; 20: 671-5
21. Vogt P, Frei U, Repp H, et al. Malignant tumours in renal transplant recipients receiving cyclosporin: survey of 598 first kidney transplantations. Nephrol Dial Transplant 1990; 5: 282-8
22. Polenakovic MH, Stefanovic V. Balkan nephropathy. In: Cameron JS, Davison AM, Grünfeld JP, et al., editors. Oxford textbook of clinical nephrology. 2nd ed. New York (NY): Oxford University Press, 1998: 1203-10
23. Hrabar A, Ceovic S, Aleraj B, et al. Relationship of anemia to Balkan endemic nephropathy. Kidney Int 1991; 40 (34 Suppl.): 44S-5S
24. Raicevic S, Trnacevic S, Hranisavljevic J, et al. Renal function, protein excretion and pathology of Balkan endemic nephropathy: II. Protein excretion. Kidney Int 1991; 40 (34 Suppl.): 52S-6S
25. Stefanovic V. Balkan endemic nephropathy: a need for novel etiological approaches. Q J Med 1998; 91: 457-63
26. Ferluga D, Hvala A, Vizjak A, et al. Renal function, protein excretion, and pathology of Balkan endemic nephropathy: III. Light and electron microscopic studies. Kidney Int 1991; 40 (34 Suppl.): 57S-67S
27. Petrinska-Venkovska S. Morphologic aspect of endemic nephropathy in Bulgaria. In: Puchlev A, Popov N, Astrug A, et al., editors. International Symposium on Endemic Nephropathy. Sofia: Bulgarian Academy of Sciences Press, 1965: 95-104
28. Hall III PW, Dammin GJ, Griggs RC, et al. Investigation of chronic endemic nephropathy in Yugoslavia: II. Renal pathology. Am J Med 1965; 39: 210-7
29. Hall III PW, Dammin GJ. Balkan nephropathy. Nephron 1978; 22: 281-300
30. Sindjic M. Rezultati autopsijskih i biopsijskih istrazivanjá bubreznih promena u obolelih od endemske nephropatijè. [Doktorska teza]. Beograd: Medicinskom Fakultetu Univerze u Beogradu, 1981
31. Hall III PW, Dammin GJ. Balkan nephropathy. In: Tisher CG, Brenner BM, editors. Renal pathology with clinical and functional correlations. Philadelphia: JB Lippincott, 1989: 913-24
32. Ferluga D, Hvala A, Vizjak A, et al. Hantaan nephropathy (HN) and Balkan endemic nephropathy (BEN): tubulointerstitial or vascular renal disease? [abstract]. Pathol Res Pract 1991; 187: 686
33. Danilovic V, Djurisic M, Mokranjac M, et al. Néphrites chroniques provoquées par l'intoxication au plomb par voie digestive (farine). Presse Med 1957; 65: 2039-40
34. Cukuranovic R, Ignjatovic M, Stefanovic V. Urinary tract tumours and Balkan nephropathy in the south Morava river basin. Kidney Int 1991; 40 (34 Suppl.): 80S-4S
35. Sindjic M, Calic-Perisic N, Velimirovic D, Anojcic B. Renal vascular changes and their possible role in the pathogenesis and morphogenesis of endemic Balkan nephropathy. In: Strahinjic S, Stefanovic V, editors. Endemic (Balkan) nephropathy. Proceedings of the 4th Symposium on Endemic (Balkan) Nephropathy, Nis: Univ Nis, 1979: 113-22
36. Mengs U, Stotzem CD. Renal toxicity of aristolochic acid in rats as an example of nephrotoxicity testing in routine toxicology. Arch Toxicol 1993; 67: 307-11
37. Mengs U. Tumour induction in mice following exposure to aristolochic acid. Arch Toxicol 1988; 61: 504-5
38. Robisch G, Schimmer O, Göggelmann W. Aristolochic acid is a direct mutagen in *Salmonella typhimurium*. Mutat Res 1982; 105: 201-4
39. Maier P, Schawalder HP, Weibel B, et al. Aristolochic acid induces 6-thioguanine-resistant mutants in an extrahepatic tissue in rats after oral application. Mutat Res 1985; 143: 143-8
40. Frei H, Würgler FE, Juon H, et al. Aristolochic acid is mutagenic and recombinogenic in Drosophila Genotoxicity Test. Arch Toxicol 1985; 56: 158-66
41. Schmeiser HH, Pool BL, Wiessler M. Mutagenicity of the two main components of commercially available carcinogenic aristolochic acid in *Salmonella typhimurium*. Cancer Lett 1984; 23: 97-101
42. Ivic M. The problem of etiology of endemic nephropathy. Acta Fac Med Naiss 1970; 1: 29-37
43. Stefanovic V, Polenakovic MH. Balkan nephropathy: kidney disease beyond the Balkans? Am J Nephrol 1991; 11: 1-11
44. Arlt VM, Ferluga D, Stiborova M, et al. Is aristolochic acid a risk factor for Balkan endemic nephropathy-associated urothelial cancer? Int J Cancer 2002; 101: 500-2
45. Colson CR, De Greef KE, Duymelinck C, et al. Role of serotonin in the development of Chinese herbs nephropathy [abstract]. Nephrol Dial Transplant 1999; 14 (4 Suppl.): 16S
46. Mcintyre M. Chinese herbs: risk, side effects and poisoning: the case for objective reporting and analysis reveals serious misinterpretation. J Altern Complement Med 1998; 4: 15-6
47. Malak J. Chinese herb nephropathy is not a (dex)fenfluramine nephropathy but a serotonin nephropathy. J Altern Complement Med 1998; 4: 131-2
48. Arlt VM, Pfohl-Leszkowicz A, Cosyns JP, et al. Analyses of DNA adducts formed by ochratoxin A and aristolochic acid in patients with Chinese herbs nephropathy. Mutat Res 2001; 494: 143-50
49. But PPH. Need for correct identification of herbs in herbal poisoning [letter]. Lancet 1993; 341: 637
50. Van Haelen M, Vanhaelen-Fastre R, But P, et al. Identification of aristolochic acid in Chinese herbs [letter]. Lancet 1994; 343: 174
51. Debelle FD, Nortier JL, De Prez EG, et al. Aristolochic acids induce chronic renal failure with interstitial fibrosis in salt-depleted rats. J Am Soc Nephrol 2002; 13: 431-6
52. De Smet PAGM. Aristolochia species. In: De Smet PAGM, Keller K, Hänsel R, et al, editors. Adverse effects of herbal drugs. Berlin: Springer-Verlag, 1992: 79-89
53. Stiborova M, Frei E, Breuer A, et al. Aristolactam Ia metabolite of aristolochic acid I upon activation forms an adduct found in DNA of patients with Chinese herbs nephropathy. Exp Toxicol Pathol 1999; 51: 421-7
54. Pailer M, Belohlav L, Simonitsch E. Zur Konstitution der Aristolochiasäuren. Monatsh Chem 1955; 86: 676-80
55. Pailer M, Bergthaller P, Schaden G. Über die Isolierung und Charakterisierung von vier neuen Aristolochiasäuren (aus Aristolochia clematitis L.). Monatsh Chem 1965; 96: 863-83

56. Pailer M, Bergthaller P. Die Konstitution der Aristolochiasäuren III und IIIa. Monatsh Chem 1966; 97: 484-93
57. Bieler CA, Stiborova M, Wiessler M, et al. 32P-post-labelling analysis of DNA adducts formed by aristolochic acid in tissues from patients with Chinese herbs nephropathy. Carcinogenesis 1997; 18: 1063-7
58. Cosyns JP, Goebbels RM, Liberton V, et al. Chinese herbs nephropathy-associated slimming regimen induces tumours in the forestomach but no interstitial nephropathy in rats. Arch Toxicol 1998; 72: 738-43
59. Krumbiegel G, Hallensleben J, Mennicke WH, et al. Studies on the metabolism of aristolochic acids I and II. Xenobiotica 1987; 17: 981-91
60. Rücker G, Chung BS. Aristolochic acids from Aristolochia manshuriensis. Planta Med 1975; 27: 68-71
61. Rosenmund H, Reichstein T. Zur Kenntnis der Aristolochiasäure. Pharm Acta Helv 1943; 18: 243-61
62. Pailer M, Belohlav L, Simonitsch E. Pflanzliche Naturstoffe mit einer Nitrogruppe. I. Die Konstitution der Aristolochiasäure. Monatsh Chem 1956; 87: 249-68
63. Kupchan SM, Doskotch RW. Tumor inhibitors: I. Aristolochic acid, the active principle of Aristolochia indica. J Med Pharm Chem 1962; 5: 657-9
64. Möse JR, Porta J. Weitere Studien über Aristolochiasäure. Drug Res 1974; 24: 52-4
65. Kluthe R, Vogt A, Batsford S. Doppelblindstudie zur Beeinflussung der Phagozytosefähigkeit von Granulozyten durch Aristolochiasäure. Drug Res 1982; 32: 443-5
66. Arlt VM, Stiborova M, Schmeiser HH. Aristolochic acid as a probable human cancer hazard in herbal remedies: a review. Mutagenesis 2002; 17: 265-77
67. Mehes J, Decsi L, Varga F, et al. Selektive chemische Ausschaltung der Harnkanälchen. I. Ordnung bei Kaninchen. Naunyn-Schmiedeberg's Arch Exp Path u Pharmak 1958; 234: 548-65
68. Jackson L, Kofman S, Weiss A, et al. Aristolochic acid (NSC-50413): phase I clinical study. Cancer Chemother Rep 1964; 42: 35-7
69. Mengs U. Acute toxicity of aristolochic acid in rodents. Arch Toxicol 1987; 59: 328-31
70. Arlt VM, Wiessler M, Schmeiser HH. Using polymerase arrest to detect DNA binding specificity of aristolochic acid in the mouse H-ras gene. Carcinogenesis 2000; 21: 235-42
71. Stiborova M, Fernando RC, Schmeiser HH, et al. Characterization of DNA adducts formed by aristolochic acids in the target organ (forestomach) of rats by 32P-postlabelling analysis using different chromatographic procedures. Carcinogenesis 1994; 15: 1187-92
72. Pfau W, Schmeiser HH, Wiessler M. Aristolochic acid binds covalently to the exocyclic amino group of purine nucleotides in DNA. Carcinogenesis 1990; 11: 313-9
73. Schmeiser HH, Frei E, Wiessler M, et al. Comparison of DNA adduct formation by aristolochic acids in various *in vitro* activation systems by 32P-post-labelling: evidence for reductive activation by peroxidases. Carcinogenesis 1997; 18: 1055-62
74. Fernando RC, Schmeiser HH, Scherf HR, et al. Formation and persistence of specific purine DNA adducts by 32P-postlabelling in target and non-target organs of rats treated with aristolochic acid I. IARC Sci Publ 1993; 124: 167-71
75. Schmeiser HH, Bieler CA, Wiessler M, et al. Detection of DNA adducts formed by aristolochic acid in renal tissue from patients with Chinese herbs nephropathy. Cancer Res 1996; 56: 2025-8
76. Schmeiser HH, Janssen JW, Lyons J, et al. Aristolochic acid activates ras genes in rat tumors at deoxyadenosine residues. Cancer Res 1990; 50: 5464-9
77. Broschard TH, Wiessler M, Von Der Lieth CW, et al. Translesional synthesis on DNA templates containing site-specifically placed deoxyadenosine and deoxyguanosine adducts formed by the plant carcinogen aristolochic acid. Carcinogenesis 1994; 15: 2331-40
78. Arlt VM, Schmeiser HH, Pfeifer GP. Sequence-specific detection of aristolochic acid-DNA adducts in the human p53 gene by terminal transferase-dependent PCR. Carcinogenesis 2001; 22: 133-40
79. Olivier M, Eeles R, Hollstein M, et al. The IARC TP53 Database (R6, January 2002): new online mutation analysis and recommendations to users. Hum Mutat 2002; 19: 607-14
80. Taniere P, Martel-Planche G, Saurin JC, et al. TP53 mutations, amplification of P63 and expression of cell cycle proteins in squamous cell carcinoma of the oesophagus from a low incidence area in Western Europe. Br J Cancer 2001; 85: 721-6
81. Broschard TH, Wiessler M, Schmeiser HH. Effect of site-specifically located aristolochic acid DNA adducts on i*n vitro* DNA syntheses by human DNA polymerase α. Cancer Lett 1995; 98: 47-56
82. Zheng F, Zhang X, Huang Q. Establishment of model of aristolochic acid-induced chronic renal interstitial fibrosis in rats. Zhonghua Yi Xue Za Zhi 2001; 81: 1095-100
83. Lebeau C, Arlt VM, Schmeiser HH, et al. Aristolochic acid impedes endocytosis and induces DNA adducts in proximal tubule cells. Kidney Int 2001; 60: 1332-42
84. Morrissey JJ, Ishidoya S, McCracken R, et al. The effect of ACE inhibitors on the expression of matrix genes and the role of p53 and p21 (WAF1) in experimental renal fibrosis. Kidney Int 1996; 49 (54 Suppl.): 83S-7S
85. Tanaka A, Nishida R, Maeda K, et al. Chinese herb nephropathy in Japan presents adult-onset Fanconi syndrome: could different components of aristolochic acids cause a different type of Chinese herb nephropathy? Clin Nephrol 2000; 53: 301-6
86. Yang SS, Chu P, Lin MS, et al. Two clinical variants of Chinese-herb nephropathy: case reports and review of the literature. J Med Sci 2001; 21: 217-24
87. Yang SS, Chu P, Lin YF, et al. Aristolochic acid-induced Fanconi's syndrome and nephropathy presenting as hypokalemic paralysis. Am J Kidney Dis 2002; 39: E14
88. Tanaka A, Nishida R, Yoshida T, et al. Outbreak of Chinese herb nephropathy in Japan: are there any differences from Belgium? Intern Med 2001; 40: 296-300
89. Ubara Y, Hara S, Kobayasi M, et al. A case of Chinese herbs-induced renal interstitial fibrosis associated with fibrosis of salivary glands. Am J Kidney Dis 1999; 33: E6
90. Krumme B, Endmeir R, Vanhaelen M, et al. Reversible Fanconi syndrome after ingestion of a Chinese herbal 'remedy' containing aristolochic acid. Nephrol Dial Transplant 2001; 16: 400-2
91. van Ypersele de Strihou C. Médecine douce, médecine innocente? Nephrologie 1998; 19: 5-6
92. Vanherweghem JL. Nephropathy and herbal medicine. Am J Kidney Dis 2000; 35: 330-2

93. Gillerot G, Jadoul M, Arlt VM, et al. Aristolochic acid nephropathy in a Chinese patient: time to abandon the term 'Chinese herbs nephropathy'? Am J Kidney Dis 2001; 38: E26
94. Lord GM, Tagore R, Cook T, et al. Nephropathy caused by Chinese herbs in the UK. Lancet 1999; 354: 481-2
95. Lord GM, Cook T, Arlt VM, et al. Urothelial malignant disease and Chinese herbal nephropathy. Lancet 2001; 358: 1515-6
96. Chen HY, Ma B-Y, Grant A, et al. Time to abandon the term 'Chinese herbs nephropathy' Kidney Int 2001; 60: 2039-40
97. Solez K, Daugirdas J, Gregory MC, et al. Is 'Chinese herbs nephropathy' a prejudicial term [letter]. Kidney Int 2001; 38: 1141-2
98. Cosyns JP. When is 'aristolochic acid nephropathy' more accurate than 'Chinese herbs nephropathy'? Kidney Int 2002; 61: 1178-81
99. van Ypersele de Strihou C. Chinese herbs nephropathy or the evils of nature. Am J Kidney Dis 1998; 32: 1-1ii
100. Kanaan N, Cosyns JP, Jadoul M, et al. The importance of a histology-based diagnosis of interstitial nephropathy in two patients with renal insufficiency. Nephrol Dial Transplant. In press.
101. Vanherweghem JL. Misuse of herbal remedies: the case of an outbreak of terminal renal failure in Belgium (Chinese herbs nephropathy). J Altern Complement Med 1998; 4: 9-13
102. Wolf CR. Individuality in cytochrome P450 expression and its association with the nephrotoxic and carcinogenic effects of chemicals. IARC Sci Publ 1991; 115: 281-7
103. Li X, Yang L, Yu Y. An analysis of the clinical and pathological characteristics of Mu-Tong (a Chinese herb) induced tubulointerstitial nephropathy. Chin J Intern Med 2001; 40: 681-7
104. Nishimagi E, Kawaguchi Y, Terai C, et al. Progressive interstitial renal fibrosis due to Chinese herbs in a patient with calcinosis Raynaud esophageal sclerodactyly telangiectasia (CREST) syndrome. Intern Med 2001; 40: 1059-63
105. Pena JM, Borras M, Ramos J, et al. Rapidly progressive interstitial renal fibrosis due to a chronic intake of a herb (Aristolochia pistolochia) infusion. Nephrol Dial Transplant 1996; 11: 1359-60
106. Vanherweghem JL, Cuykens JJ, Vandenbergh Ph, et al. Valvular heart disease and Chinese-herb nephropathy [letter]. Lancet 1998; 351: 991
107. Pourrat J, Montastruc JL, Lacombe JL, et al. Néphropathie associée à des herbes chinoises 2 cas [abstract]. Presse Med 1994; 23: 1669
108. Stengel B, Jones E. Insuffisance rénale terminale associée à la consommation d'herbes chinoises en France. Néphrologie 1998; 19: 15-20
109. Cronin AJ, Maidment G, Cook T, et al. Aristolochic acid as a causative factor in a case of Chinese herbal nephropathy. Nephrol Dial Transplant 2002; 17: 524-5
110. Chen W, Chen Y, Li A. The clinical and pathological manifestations of aristolochic acid nephropathy: the report of 58 cases. Zhonghua Yi Xue Za Zhi 2001; 81: 1101-5
111. Tanaka A, Nishida R, Sawai K, et al. Traditional remedy-induced Chinese herbs nephropathy showing rapid deterioration of renal function. Jpn J Nephrol 1997; 39: 794-7
112. Tanaka A, Shinkai S, Kasuno K, et al. Chinese herbs nephropathy in the Kansai area: a warning report. Jpn J Nephrol 1997; 39: 438-40
113. Ng YY, Yu S, Chen TW, et al. Interstitial renal fibrosis in a young woman: association with a Chinese preparation given for irregular menses. Nephrol Dial Transplant 1998; 13: 2115-7
114. Yang CS, Lin CH, Chang SH, et al. Rapidly progressive fibrosing interstitial nephritis associated with Chinese herbal drugs. Am J Kidney Dis 2000; 35: 313-8
115. Chang CH, Wang YM, Yang AH, et al. Rapidly progressive interstitial renal fibrosis associated with Chinese herbal medications. Am J Nephrol 2001; 21: 441-8
116. Kessler DA. Cancer and herbs. N Engl J Med 2000; 342: 1742-3

Correspondence and offprints: Dr *Jean-Pierre Cosyns*, Department of Pathology (ANPS 1712), Cliniques Universitaires St-Luc, 10 Avenue Hippocrate, 1200 Bruxelles, Belgium.
E-mail: cosyns@anps.ucl.ac.be