Aristolochic acid mutagenesis: molecular clues to the aetiology of Balkan endemic nephropathy-associated urothelial cancer

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Balkan endemic nephropathy (BEN) is found in certain rural areas of the Balkans and affects at least 25,000 inhabitants. Of the many hypotheses on BEN, the Aristolochia hypothesis has recently gained ground substantiated by the investigations on aristolochic acid nephropathy (AAN). On both clinical and morphological grounds, AAN is very similar to BEN. That exposure to aristolochic acid (AA) of individuals living in endemic areas through consumption of bread made with flour contaminated with seeds of Aristolochia clematitis is responsible for BEN is an old hypothesis, but one which is fully consistent with the unique epidemiologic features of BEN. Here, we propose an approach to investigate AA-induced mutagenesis in BEN that can provide molecular clues to the aetiology of its associated urothelial cancer. The molecular mechanism of AA-induced carcinogenesis demonstrates a strong association between DNA adduct formation, mutagenic pattern and tumour development. A clear link between urothelial tumours, p53 mutations and AA exposure should emerge as more tumour DNA from BEN patients from different endemic areas becomes available for mutation analysis. We predict that the observed p53 mutation spectrum will be dominated by AT → TA transversion mutations as has already been demonstrated in the human p53 gene of immortalized cells after exposure to AAI and urothelial tumours from BEN patients in Croatia. Moreover, the detection of AA-specific DNA adducts in renal tissue of a number of BEN patients and individuals living in areas endemic for BEN in Croatia provides new evidence that chronic exposure to AA is a risk factor for BEN and its associated cancer.

Introduction

Balkan endemic nephropathy (BEN), a chronic renal interstitial fibrosis with slow progression to end-stage renal disease and urothelial malignancy, is found in certain rural areas of Bulgaria, Bosnia, Croatia, Romania and Serbia along the Danube river basin (1,2). At least 25,000 individuals suffer from BEN or are suspected of having the disease, whereas the total number of people at risk in these countries may exceed 100,000. Although first described 50 years ago, the aetiology of BEN remains unclear and is a matter of debate (1,2). In recent years, evidence has accumulated that BEN is an environmental disease (3). Of several hypotheses that have been proposed to explain the environmental cause of this disease, the major three are (i) the mycotoxin hypothesis, which postulates that BEN is caused by the fungal mycotoxin ochratoxin A (OTA) found in contaminated foodstuffs (4,5), (ii) the Pliocene lignite hypothesis, which proposes that BEN is caused by long-term exposure to polycyclic aromatic hydrocarbons (PAHs) and other toxic organic compounds leaching into well drinking water from low-rank coals (1) and (iii) the aristolochic acid (AA) hypothesis, which postulates that BEN is caused by chronic dietary intoxication with seeds of Aristolochia clematitis (6–10).

Chinese herbs nephropathy (CHN), first reported in a group of young patients with end-stage renal disease in Belgium in 1993, is a rapidly progressive renal interstitial fibrosis associated with a high risk of urothelial cancer (11–13). The observed nephropathy has been traced to the ingestion of herbal medicinal remedies that have included Aristolochia species containing AA and is now called aristolochic acid nephropathy (AAN) (9,14). AA is a well-known human nephrotoxin and was shown to be a strong rodent carcinogen (7), being among the most potent 2% of known carcinogens (15). Herbal remedies containing species of the genus Aristolochia were recently classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC) (16).

On both clinical and morphological grounds, AAN is very similar to BEN, which has led to the hypothesis of a common aetiological agent for both diseases (10). Already some 35 years ago, a survey conducted on the geographical distribution of the plant A. clematitis in endemic areas in Serbia suggested that AA found in flour obtained from wheat contaminated with seeds of A. clematitis in endemic areas could be the aetiologic agent of BEN (6). This hypothesis, however, did not receive widespread support at the time. However, more recently the same observation was made in endemic regions of Croatia, reviving the old hypothesis that exposure to AA of individuals living in endemic areas could occur by dietary intake of bread derived from wheat grain that was contaminated with seeds of A. clematitis (8). Indeed, AA–DNA adducts have been reported in the renal tissue of a number of BEN patients and individuals living in areas endemic for BEN in Croatia (17,18). Collectively, these results provide new evidence that AA is a risk factor for BEN and BEN-associated urothelial cancer. The intention of this article is to propose an approach to investigate AA-induced mutagenesis in BEN that can provide the molecular clue to the aetiology of BEN and its associated urothelial cancer.

AA: an old herbal drug known since antiquity

AAs are found primarily in various species of the genus Aristolochia (e.g. A. clematitis, Aristolochia fangchi and Aristolochia manshurien-sis), but have also been described in certain Asarum species (16). In Europe, A. clematitis is indigenous to Mediterranean regions. The plant extract AA mainly consists of the structurally related derivatives AAI and AAI (Figure 1). Herbal drugs derived from Aristolochia species have been known since antiquity and were used in obstetrics and in the treatment of snakebites (7). 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 (19). Subsequently, in 1982 all pharmaceutical preparations containing AA were withdrawn from the market in Germany and many other countries. However, Aristolochia plants are still used in traditional medicine in some parts of the world (7).

AAN and urothelial cancer

The outbreak of so-called CHN in Belgium in 1993 was associated with the ingestion of Chinese herbal remedies prescribed by a single
Chinese herbal products (29). Instead, vent many health care practitioners from effectively verifying the in-
This form of nephropathy and associated urothelial cancers may be
AAN cases are reported worldwide, it is of great concern that
system or alleviate gastrointestinal symptoms (28). Since more and
Chinese medicines meant to effect weight loss, improve the immune
Natural health products containing AA are often sold as traditional
(25–27), highlighting the human carcinogenic potential of AA.
but it has also been diagnosed in AAN cases outside this epidemic
Aristolochia species. As for the majority of these cases, exposure to
Fangji presumably because both plants are used in Chinese
folk medicine under similar names, Fangji (20). Exposure of CHN
patients to AA was substantiated by the identification of specific AA–
DNA adducts by 32P-post-labelling in urothelial tissue of these
patients (12,21,22). In the majority of cases, progression to end-stage
renal disease occurred despite discontinuation of AA ingestion,
necessitating dialysis and subsequent renal transplantation. Within
a few years, CHN patients developed a high risk of urothelial cancer;
urothelial malignantancy of the upper urinary tract arose in almost half of
the patients (12,13). The cumulative dose of A.fangchi was a signific-
ificant risk factor; patients with an intake of 200 g of herbs (the average
herbal intake) had a 50% risk of developing cancer (12,23). More
recently, it was found that even patients who do not display the char-
acteristic histological features of CHN are also at risk of malignancy
(24). The so-called CHN has been described in patients in other
European (e.g. UK, France, Spain and Germany) and in Asian coun-
tries and USA (in total ~170 cases) (7), and had no relationship with
the Belgian cohort. As for the majority of these cases, exposure to
Aristolochia species was proven that it has been proposed to designate
this novel nephropathy in which the unequivocal role of AA has been
fully documented as AAN (9). As long as the intake of AA has not
been documented, it was recommended to classify these cases as
phytotherapy-associated interstitial nephritis. Urothelial cancer has
been documented, it was recommended to classify these cases as
fully documented as AAN (9). As long as the intake of AA has not
been investigated in this study, organ-specific AA-induced
Carcinogenic mechanism of AA in rodents. AA is a strong carcinogen
in rodents (19). In rats treated orally with 0.1, 1 and 10 mg AA/kg
body wt/day for 3 months, a high incidence of tumours was observed
(25, 85 and 100%, respectively). Main targets for tumour formation
were forestomach, kidney and urinary tract, with 72, 28 and 17% of the
animals treated with 10 mg AA/kg body wt having tumours of these
organs, respectively (31). DNA adduct formation was examined in rats
by 32P-post-labelling and the structures of the major AA–DNA adducts
were identified as 7-(deoxyadenosin-N2-yl)aristolactam I (dA-AAI),
7-(deoxyguanosin-N2-yl)aristolactam I and 7-(deoxyguanosin-N6-
yl)aristolactam II (32). AA-induced mutagenicity was investigated at
the same doses in the transgenic Big Blue rat model (31,33).
Dose-dependent increases in mutant frequency (MF) and AA–DNA
adduct formation measured by 32P-post-labelling were observed for
liver (non-target organ) and kidney (target organ) (Figure 2A and B).
MF was at least 2-fold higher in kidney compared with liver as were
AA–DNA adduct levels, suggesting higher genotoxicity in the target
organ for tumour development. Here, we show that there is a strong
correlation between DNA adduct levels and MF both in liver (r = 0.995,
P < 0.001) and kidney (r = 0.999, P < 0.001) (Figure 2C), indicat-
ing that mutagenic effects of AA are clearly associated with the for-
mation of AA–DNA adducts. Carcinogenic effects were also observed
in mice. Oral treatment with 5 mg AA/kg body wt/day for 3 weeks
resulted in tumour formation in the forestomach, lungs and kidneys
(34). In Muta 3 TmM Mouse treated with 15 mg AA/kg body wt once a
week for 4 weeks, high MFs were found in the target organs (forest-
omach and organs of the urogenital tract), whereas only small
increases in MFs were seen in non-target organs (e.g. glandular stom-
ach and liver) (Figure 3) (35). Although DNA adduct formation has not been investigated in this study, organ-specific AA-induced
type (Figure 4A and B). Similarly, in AA-treated Muta TMMouse, the AT spectrum of controls was dominated by GC and Big Blue assays was similar (Figure 4A and B) (31,33,35). In different doses of AA (adapted from ref. 31). (P<0.001)

The AA-induced mutation pattern in the kidney in the Muta™TMMouse and Big Blue assays was similar (Figure 4A and B) (31,33,35). In addition, in Big Blue rats the overall pattern of mutations induced by AA in liver was similar to that in kidney (31,33). While the mutation spectrum of controls was dominated by GC → AT transitions, AT → TA transversions were the predominant AA-induced mutation type (Figure 4A and B). Similarly, in AA-treated Muta™TMMouse, AT → TA mutations were also predominant in target organ forestomach and bladder (35). Translesional bypass of adenine adducts of AA (dA-AAI and 7-(deoxyadenosin-^-N6-yl)aristolactam II) points to a mutagenic potential resulting from dAMP incorporation opposite the adduct by DNA polymerase (36), suggesting that AT → TA transversion mutations would be the mutagenic consequence. AT → TA transversions are typical mutations observed in H-ras in tumours of rodents treated with AA and correspond with DNA adduct formation at adenine residues (37–39). This mutation occurs exclusively at the first adenine of codon 61 (CAA) in all forestomach and ear duct tumours of rats treated with AAI (37). This selectivity for AAI for mutations at adenine residues is consistent with the extensive formation of dA-AAI adducts in the target organ and their long-term persistence in forestomach DNA (22,40). Collectively, these data may indicate the probable molecular mechanism whereby AA induces tumours in rodents.

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 (41,42). This may not only be of primary importance to explain the strong nephrotoxicity of AA but also indicates a potential mechanism on the seemingly tissue specificity of AA-induced oncogenesis. Changes in gene expression were examined in liver and kidney of rats treated with 10 mg AA/kg body wt five times per week for 3 months (43,44). More many genes had altered expression levels in the target kidney than in the non-target liver following AA treatment. Significant alterations of biological processes related to defense response, apoptosis and immune response, as well as organic acid metabolism were found in kidney but not in liver (43).

Carcinogenic mechanism of AA in humans. Human cytosolic enzymes [e.g. NAD(P)H:quinone oxidoreductase (NQO1)] and microsomal enzymes (e.g. CYP1A1 and CYP1A2) activate AA by simple nitroreduction leading to DNA-binding species (45,46). Among the activating metabolizing enzymes is also prostaglandin H synthase that is highly expressed in urothelial tissue. Interestingly, it was reported that to date only 3–5% of the patients treated with the slimming regimen in Belgium have suffered from nephropathy (9,13,47). One possible explanation for the differential responses of patients may be individual differences in the activities of the enzymes catalysing bio-transformation (activation and/or detoxification) of AA. The most abundant DNA adduct detected by 32P-post-labelling in urothelial tissue is da-AAI (12,21,22,24–27). However, AA–DNA adducts are also found in various tissues outside the urinary tract (24,26,48), indicating that additional factors may be critical for the high incidence of urothelial tumours. The long-term persistence of the da-AAI adduct in various organs (including kidney) in rats is in line with its detection in Belgian AAN patients almost 10 years after the patients stopped taking the herbal slimming regimen (12), thus demonstrating that AA–DNA adducts are not only suitable biomarkers of exposure to AA but also markers of cancer risk. In Belgian AAN patients, the risk to develop urothelial tumours was related to the cumulated intake of Aristolochia fangchi (12,23).

In AAN, urothelial atypia were associated with the over-expression of the p53 protein (13), suggesting that p53 is mutated in AAN-associated cancer (49). More than 50% of all human tumours contain a mutation in p53 (50). Interestingly, in one AAN patient from the UK available for analysis, a characteristic AT → TA transversion mutation was found in p53 (exon 5; codon 139 AAG) in urothelial tumour cells (48). It is noteworthy that the mutated base adenine has the same neighbouring bases in codon 138/139 (GCC AAG) of p53 as in codon 61 (CAA) of H-ras, suggesting a sequence-specific mechanism during mutation induction. Other mutations in p53 were also found in a papillary transitional cell carcinoma resected from the bladder of one Belgian AAN patient (see below) (9). To examine AA-induced mutation spectra in the human p53 gene in laboratory animals, a human p53 knock-in (Hupki) mouse has been constructed (51,52). When the Hupki p53 of immortalized cells derived from primary Hupki embryonic fibroblasts exposed to AAI were sequenced, specific AT → TA
transversion mutations in p53 were observed (Figure 4C) (53,54). Interestingly, in one cell line a characteristic AT → TA transversion was found at the first adenine of codon 139 (AAG) identical to the mutation found in the urothelial tumour cells of the UK AAN patient (48,54). These data may indicate a probable molecular mechanism whereby AA causes urothelial cancer.

Mutational specificity of a carcinogen often serves as indirect evidence for tumour initiation caused by interaction of the carcinogen with these specific DNA sequences (55–57). In the same AAN patient from the UK, a GC → AT transition mutation (exon 7; codon 245) was also found in p53 in a breast tumour and a liver metastasis, indicating that the mutation most probably arose before metastasis (48). However, given that GC → AT transitions are not the typical mutations induced by AA (usually AT → TA transversions), it was concluded that it is not likely that the p53 mutation in the breast and liver tumours was induced by AA.

**Genetic variation of AA-metabolizing enzymes as a risk factor in AAN and/or BEN?**

Variations in AA-activating enzymes such as NQO1 and CYP1A1 and regulatory proteins controlling expression of these enzymes may play a role in cancer susceptibility to AA. The role of genetic polymorphism in several genes relevant for detoxification has already been investigated in BEN patients (2,58,59). BEN patients homozygous for a polymorphism in several genes relevant for detoxification has already been extensively studied, those participating in its detoxification pathway (M.Stiborová, unpublished data). A large-scale investigation in BEN patients on the role of genetic polymorphism in genes of some phase I detoxification enzymes, such as CYP2D6, −3A4 and −3A5 as well as in those of the conjugation enzymes NAT1, NAT2, GSTT1 and GSTM1, revealed a higher risk for BEN (odds ratio = 2.41) in individuals carrying CYP1A1*1 allele G6989 (58). However, whether or not this cytochrome P450 isoenzyme is involved in AA detoxification remains to be determined.

**AA mutagenesis: a clue to BEN-associated urothelial cancer?** The p53 tumour suppressor protein plays an important role in DNA damage responses, cell cycle control, apoptosis and activation of certain DNA repair systems (60). Disabling p53 function by mutations in the p53 coding sequence leads to the development of tumours. More than 50% of all human tumours contain a mutation in p53 (50). Over 20,000 human tumour mutations in p53 have been registered in the IARC TP53 database (61). In principle, this information can be used to generate hypotheses regarding disease risk factors in a defined population. Three often-cited observations that draw a link between a particular mutation profile and specific environmental risk factor are (i) the high prevalence of tandem CC → TT mutations in squamous and basal cell carcinoma of skin and exposure to sunlight, (ii) the nearly exclusive occurrence of a codon 249 AGG to AGT hotspot mutation in hepatocellular carcinoma from high-incidence areas where aflatoxin exposure and chronic hepatitis B infection are common and (iii) the high prevalence of p53 GC → TA transversion mutations in lung cancers of tobacco smokers (50).

Figure 5A shows the distribution of mutations along p53 in tumours of the urinary tract (kidney, renal pelvis, ureter and other urinary organs excluding bladder) as found in the IARC TP53 database (http://www-p53.iarc.fr). In the current database (R11 release, October 2006), 137 mutations (with 121 mutations in the coding region) are recorded for these tissues. The mutation spectrum is characterized by

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**Fig. 3. MF in the eII gene from various organs of Muta™Mouse treated with AA (adapted from ref. 35).**

**Fig. 4. p53 mutation pattern.** (A) Mutation pattern in the kidney eII gene of Muta™Mouse treated with AA (adapted from ref. 35). (B) Mutation pattern in the kidney eII of Big Blue rat treated with AA (adapted from ref. 33). (C) Immortalized Hupki murine embryonic fibroblasts (MEFs). Cell lines are derived from primary cells treated with AAI (N = 12) or not treated (N = 12) (adapted from refs 51–53). (D) Left: mutation pattern of human urinary tract tumours (kidney, renal pelvis, ureter and other urinary organs excluding bladder) as recorded in the IARC TP53 database (R11 release, October 2006), 137 mutations. Morphology inclusion criteria: carcinoma not otherwise specified, papillary carcinoma not otherwise specified, squamous cell carcinoma not otherwise specified, transitional cell carcinoma not otherwise specified, papillary transitional cell carcinoma, carcinoma in situ not otherwise specified and dysplasia not otherwise specified. (D) Right: mutation pattern of human bladder tumours as recorded in the IARC TP53 database (R11 release, October 2006), 898 mutations. Morphology inclusion criteria: carcinoma not otherwise specified, papillary carcinoma not otherwise specified, squamous cell carcinoma not otherwise specified, transitional cell carcinoma not otherwise specified, transitional cell carcinoma in situ, papillary transitional cell carcinoma, carcinoma in situ not otherwise specified, urothelial papilloma not otherwise specified and dysplasia not otherwise specified. (E) Mutation pattern in transitional cell carcinoma from BEN patients in Croatia (N = 11) (adapted from ref. 18).
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mutation hotspots in codons 65, 175, 214, 248, 249, 258, 280 and 285. Some of these codons (175, 248 and 282) contain 5-methylcytosine within a CpG sequence and represent mutation hotspots in many other tumours (50,62). Mutations at these codons may be formed by a methylation–deamination mechanism but enhanced adduct formation at guanines in CpG sites by bulky carcinogens, such as PAHs, may also contribute to mutations at these mutation hotspots (57). Codons 65, 214, 258 and 280 seem more specific for the mutation spectrum of urinary tract tumours. Some of the mutations recorded for these codons are associated with adenine, mutations in codon 65 are even related to AT→TA transversion mutations. However, overall the mutation pattern is dominated by G→A (39%; G→A at CpG 19%) and A→G (20%), followed by G→T (12%), whereas A→T transversion mutations account for only 6% of mutations (Figure 4D). Although it is unclear whether these mutations are formed by endogenous or exogenous sources, some of the tumours have been attributed to smoking (exposure to aromatic amines) or to treatment with analgesic drugs (treatment with phenacetin). In fact, the database also lists the AT→TA transversion mutation found in codon 139 of the AAN patient (48). On the basis of patient data, however, it is likely that all except one of these mutations (as noted above) are not associated with AA exposure. However, in a recent report examining p53 mutations in urothelial tumours of BEN patients in Croatia (N=11), mutations at A:T pairs accounted for 89% (17/19) of all mutations, with the majority of these (15/17) being AT→TA transversions, representing 78% of all base substitutions detected in the p53 gene (Figure 4E) (18). Interestingly, the mutations in p53 found in two of the cases (AT→TA transversions at codons 209 and 280) were also induced in immortalized cells derived from primary Hupki embryonic fibroblasts exposed to AAI (53,54). In general, we suggest that a clear link between urothelial tumours, p53 mutations and exposure to AA will emerge as more tumour DNA from afflicted persons becomes available for mutation analysis. We predict that this mutation spectrum will be dominated by AT→TA transversion mutations as has already been demonstrated in immortalized cells derived from primary Hupki embryonic fibroblasts after exposure to AAI and in
urothelial tumours from patients with BEN in Croatia (Figure 4C and E) (18,53,54). With respect to AA as a risk factor for BEN-associated urothelial tumours observed outside Croatia, we would also predict that many of these tumours carry characteristic AT → TA transversion mutations in p53. Those studies in combination with the detection of AA-DNA adducts in BEN patients (18) can provide the molecular clues demonstrating that chronic exposure to AA is a risk factor in areas endemic for BEN and its associated cancer.

More recently, an increased incidence of bladder tumours has been observed in AAN patients (63). Bladder tumours have also been reported in BEN patients. Figure 5B shows the mutation spectrum of bladder tumours as recorded in the IARC TP53 database. In the current database, 822 single-base substitutions (898 mutations in total) are recorded for bladder. The mutation spectrum is characterized by hotspots in codons 175, 220, 241, 245, 248, 271, 273, 280 and 285. There is some overlap with the codon distribution of mutations as found in tumours of the urinary tract (Figure 5A), and again, the mutation pattern is dominated by G → A (51%; G → A at CpG 19%), followed by G → C (13%), G → T (10%) and A → G (10%), whereas A → T transversion mutations account for only 5% (Figure 4D). Mutations associated with adenine are found in codons 220, 271, 280 and 285, whereas in codon 220 A → G transition is the exclusive mutation type (N = 17), mutations associated with adenine are rare at the other mutation hotspots, respectively. As mentioned above, it is noteworthy that a papillary transitional cell carcinoma from the bladder in one Belgian AAN patient showed an AT → CG transversion and a GC → AT transition mutation in exon 7, codon 230 (ACC) and codon 248 (CGG), respectively (9). However, we predict that a characteristic mutation spectrum (dominated by AT → TA transversions) in relation to AA exposure will emerge in bladder as more tumours from afflicted persons become available for mutation analysis.

A significant amount of research into the aetiology of BEN over the past decades has focused on OTA. Evidence both in favour (5,64–67) and against (68–71) the genotoxicity of OTA in humans and rodents has been published. The literature on short-term and against (68–71) the genotoxicity of OTA in humans and rodents over the past decades has focused on OTA. Evidence both in favour (5,64–67) and against (68–71) the genotoxicity of OTA in humans and rodents has been published. The literature on short-term and against (68–71) the genotoxicity of OTA in humans and rodents is still lacking, or is disputed by other studies (67,71). Two DNA adduct standards obtained by photooxidation have been identified, indicating that OTA can react with dG to yield carbon (C)-bonded (C-C8-dG OTA) and oxygen (O)-bonded (O-C8-dG OTA) adducts (74). Further studies suggest that a spot that comigrated with C-C8-phenyl-dG adduct have been shown to be weakly miscoding, generating GC → TA and GC → CG transversion mutations (Figure 6) (65). Oxidative DNA lesions such as 8-oxo-dG are also capable of inducing such mutations (52), indicating that it may be difficult to distinguish mutations induced directly by OTA or caused indirectly by OTA through the formation of oxidative damage to DNA (Figure 6). However, in both cases a mutation pattern induced by OTA would be quite different from that induced by AA, providing a new approach to find a link between urothelial tumours and environmental exposure in endemic areas. It is noteworthy that the Pliocene lignite hypothesis has linked BEN to exposure to coal-derived organic compounds present in the water supplies of villages endemic for BEN. Such compounds include genotoxic aromatic amines and PAHs (1). However, although aromatic amines and PAHs have been linked with cancer in urogenital tissues, the currently identified organic compounds in the well water from the endemic areas do not account sufficiently for the aetiology and pathology of BEN and its associated tumours. Moreover, not all the described endemic areas are topographically connected to the known Pliocene coal beds (2). Mutagenic PAHs like benzo(a)pyrene induce mainly GC → TA transversions (52,57), which would result in a different mutation pattern to that induced by AA. Collectively, the mutation pattern would provide the molecular clue to the aetiology of BEN-associated urothelial cancer.

Conclusions

Epidemiological evidence gathered during the last decades strongly suggests that BEN is an environmentally induced disease. Of the many hypotheses put forward to disclose the causative agents of BEN, the Aristolochia hypothesis has recently gained ground. That dietary intake of AA may be responsible for BEN and its associated urothelial cancer is a theory that was first proposed in 1969 by Ivic and is fully consistent with the unique epidemiologic features of BEN. Experimental evidence such as the detection of AA–DNA adducts in BEN patients and the identification of AA-specific mutation spectra in tumours of BEN patients would establish a molecular link between AAN and BEN. However, the role of other factors in the pathogenesis of BEN cannot be ruled out.

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References


43. Chen, T. et al. (2006) Gene expression profiles distinguish the carcinogenic effects of aristolochic acid in target (kidney) and non-target (liver) tissues in rats. BMC Bioinformatics, 7 (suppl. 2), S20.


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