

ARISTOLOCHIC ACID- AND OCHRATOXIN A-DNA ADDUCTS: POSSIBLE MARKERS OF BALKAN ENDEMIC NEPHROPATHY AND ASSOCIATED UROTHELIAL TUMORS?

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Summary. *The etiology of Balkan endemic nephropathy (BN) is still debated. The similar prevalence of the disease in immigrants and natives in endemic areas points to environmental etiological factors. Viral agents, selenium deficiency, cadmium, lead and long-term exposure to polycyclic aromatic hydrocarbons and amines have received little support. By contrast, BN is amazingly reminiscent of the findings characterizing aristolochic acid (AA) and ochratoxin A (OTA) chronic intoxications. Both drugs have nephrotoxic and carcinogenic activities providing a possible pathophysiological clue for the development of the renal fibrosis and the urothelial malignancies characterizing BN. Preliminary studies indicate that both toxic substances may be food contaminants in endemic areas. Actual exposure of inhabitants has recently been supported by the identification of AA- and OTA-related DNA adducts in their renal tissue. Identification of these specific DNA adducts in a large number of patients with BN and in control subjects remains to be assessed. Molecular hallmarks like an A → T transversion mutation in the human p53 tumor suppressor gene characterizing AA-induced malignancy may become additional disease markers which are presently missing in BN.*

Key words: *Balkan nephropathy, aristolochic acid, ochratoxin A, interstitial nephritis, urothelial cancer*

So-called Balkan endemic nephropathy (BN) is a chronic fibrosing tubulointerstitial renal disease with insidious onset and slow progression to end-stage renal failure associated with urothelial malignancies. It is characterized by the development of early tubular proteinuria, anemia and normal blood pressure, and of progressive diffuse hypocellular interstitial fibrosis with tubular atrophy predominating in the outer cortex. It affects families of rural populations in a mosaic-like pattern in several regions of Serbia, Bulgaria, Rumania, Bosnia-Herzegovina, and Croatia. It is a major health problem in endemic regions, with up to 10% of the population affected.

Although the recognition of the disease dates back some 50 years, the etiology of BN is still debated. Despite familial clustering suggesting involvement of hereditary factors, the similar prevalence of BN in immigrants and natives in endemic areas points to environmental etiological factors. Viral agents, selenium deficiency and long-term exposure to polycyclic aromatic hydrocarbons and amines have been considered but received little support (1-6).

Biological and pathological features of BN are to some extent reminiscent of those observed in cadmium and lead nephropathy (7-8). Cadmium exposure is characterized by the adult-onset of low molecular weight proteinuria and glucosuria or by the develop-

ment of a full Fanconi syndrome. Once the renal manifestations become evident, there is a trend towards progressive renal damage even if exposure is discontinued (9). Osteoporosis with osteomalacia may ensue. As illustrated in the so-called Itai-Itai or Ouch Ouch disease (7), the kidneys have macroscopically a sand-paper-like appearance and weigh 50 to 90 g. Hypocellular interstitial fibrosis associated with flattened tubular epithelia predominates in the outer cortex and is less marked in the columns of Bertin or in the medullae. Varying degrees of glomerulosclerosis without evidence of primary glomerulonephritis are found. Moderate to severe atherosclerosis is noted. The pelvis and calyces have a normal appearance. Cadmium is first detoxified in the liver cells by binding to metallothionein. It is subsequently filtered by the glomerulus and reabsorbed by the proximal tubular cells where lysosomes release the metal causing cell damage.

Lead nephropathy is characterized by the development of hypertension, tubular glucosuria and hyperuricemia with gout (10,11). Whether the disease progresses unabated despite the cessation of lead exposure remains to be determined. The kidneys have macroscopically a granular surface and are homogeneously and symmetrically shrunken weighing 30 to 110 g (12,13). Hypocellular interstitial fibrosis is irregularly distributed throughout the cortex without reported predominance in

any kidney compartments. Its severity varies greatly from case to case. It is associated with flattened tubular epithelia of atrophic tubules and dilation of preserved tubules. The medullae are well preserved except in some cases showing microtophi in the medullary interstitial tissue. Several irregularly distributed obsolescent glomeruli are found. Interlobular and larger arteries usually show severe arteriosclerosis and arterioles display prominent hyalinosis. Nonspecific immune deposits may be found by immunofluorescent microscopy. Lead binds to proteins and accumulates in proximal tubular cells where it leads to the formation of eosinophilic inclusions surrounded by a clear halo, which are the hallmark of lead nephropathy by light microscopy (14).

In addition to discrepancies like the presence of osteoporosis and osteomalacia in cadmium intoxication, of hypertension with hyperuricemia, gout and renal microtophi without predominance of interstitial fibrosis in the outer cortex, no evidence points at present to an etiological role of cadmium or lead in the Balkan area. By contrast, BN is amazingly reminiscent of the findings characterizing aristolochic acid (AA) and ochratoxin A (OTA) chronic intoxications (15-19). The hypothesis that AA, the alkaloid found in the plant *Aristolochia*, is a common etiological factor for the development of BN and the new interstitial fibrosing nephropathy associated with a high incidence of urothelial cancer due to the consumption of Chinese herbs containing AA, AA nephropathy (AAN), is highly suggested by several similarities between both diseases (17,20,21). On clinical grounds, normal blood pressure, early and severe anemia, mild tubular proteinuria and glucosuria characterize both diseases. On morphological grounds, the association of hypocellular interstitial fibrosis decreasing in severity from the outer to the inner cortex with a 40% incidence of urothelial malignancy is a unique feature found in both illnesses (17). Moreover, the etiological role of AA has been incriminated many years ago in BN (16), a hypothesis still to be fully explored. The main difference between both diseases is the progression towards end-stage renal failure which lasts decades versus months in BN and AAN, respectively.

On the other hand, OTA, a mycotoxin produced by several species of *Aspergillus* and *Penicillium*, may also play a role in the genesis of BN. Similarly to AAN, low molecular weight proteinuria, glucosuria, flattened proximal tubular epithelial cells and interstitial fibrosis with tubular atrophy characterize OTA nephropathy (22). In endemic areas, samples of staple-foods are contaminated with a higher frequency than in nonendemic regions (23). Blood levels of OTA in people living in endemic areas are often higher in those who develop BN and/or urinary tract tumors (24).

Nephrotoxicity and carcinogenicity of both OTA and AA provide a possible pathophysiological clue for the development of the interstitial fibrosing nephropathy and urothelial cancer characterizing BN. Acute tubular

necrosis has been described after exposure of animals and man to high doses of both drugs (25). Both toxics react with cellular DNA and form pre-mutagenic DNA adducts in mice and man (26-29). OTA-related DNA adducts have been identified in DNA extracted from urinary tract tumors found in Bulgarian subjects living in endemic areas (28). In contrast, they were virtually absent in DNA isolated from kidney tissue in patients with AAN (30). AA-DNA adducts have been extensively documented in tissues from AAN patients whereas their identification in BN patients remains to be determined. It is of interest to note that both OTA-related and AA-DNA adducts were found in DNA extracted from the kidneys of 2 out of 3 adult females who lived and had a farming activity in an endemic region. Interestingly, one of both exposed patients had developed pelvic urothelial malignancy (31).

So-called Chinese herbs nephropathy described in the Belgian epidemic was renamed AAN as soon as AA was identified as the culprit. Moreover, it turned out that AAN was not restricted to the attendants of the Belgian slimming clinic. An increasing number of similar cases due to the consumption of herbs in which AA has been identified or was reputedly contained have been subsequently reported throughout the world (32,33). As expected (34), similar patients with OTA-associated interstitial fibrosing nephropathy have recently been described outside the Balkan area. Patients with high blood levels of OTA and interstitial fibrosing nephropathy have been reported in France (22) and Tunisia (35). The source of OTA exposure was respectively unknown and probable contaminated food. Like several other diseases initially designated by the geographical area where they were described for the first time, BN should be renamed once the etiology is known. As suggested by the hitherto extensive reported research mentioned above, the etiological factor is likely not to be specific to the Balkan area. Whether, in contrast to AAN, BN results from the combined toxicity of AA and OTA remains to be elucidated. This should be evaluated by the identification and quantification of specific DNA adduct assays in kidney and urinary tract tissue from patients having the highest epidemiological, clinical and morphological likelihood of BN and from control subjects.

Carcinogen-DNA adducts have an important biological significance. AA-DNA adducts in AA-induced forestomach epidermoid carcinomas in the rat are frequently associated with the activation of *Ha-ras* protooncogenes by an A → T transversion mutation in codon 61 from CAA to CTA (36). Site-specifically synthetic adducted oligonucleotides obtained *in vitro* after modification with AA used as templates in primed DNA replication reactions with prokaryotic (Sequenase) and eukaryotic (Human pol α) DNA polymerases suggest a higher mutagenic potential of adenine adducts than of guanine adducts (37,38). dCMP was preferentially matched at the level of the deoxyguanosine adducts. In contrast, no preferential match of dAMP and

dTMP was found on the complementary strand of the nucleotide facing the deoxyadenosine adducts. These findings suggest that dAMP incorporation by DNA polymerase at the site of adenine adducts is the molecular mechanism of AA mutagenicity (39). This is in line with sequencing analysis of an urothelial carcinoma resected from the bladder in an AAN patient from the Belgian epidemic showing the existence of mutations at purine residues in exon 7 of the p53 tumor suppressor gene (32), which was expected to be mutated in AAN associated urothelial malignancies (19). Moreover, the first adenine of codon 139 (AAG) of the p53 gene in urothelial neoplastic cells from an AAN patient outside

the Belgian epidemic has recently been found to be the site of an A → T transversion mutation (40). Finally, similar transversion mutations associated with the identification of AA-DNA adducts were found in the human p53 gene in primary embryonic cells from an AA-exposed human p53 knock-in (Hupki) mouse strain (41). Further characterization of mutations in the p53 tumor suppressor gene as well as possibly in oncogenes of a large number of AA-induced malignancies may provide molecular hallmarks susceptible to become additional disease markers, which are presently missing in BN and its associated urothelial tumors.

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ARISTOLOHIČNA KISELINA I OHRATOKSIN A DNK ADUKTI: MOGUĆI MARKERI ENDEMSKE NEFROPATIJE I TUMORA UROTELIJUMA

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Kratka sadržaj: Etiologija endemske nefropatije (EN) još uvek nije poznata. Slična prevalencija bolesti kod imigranata i starosedelaca endemskih naselja upućuje na etiološku ulogu faktora sredine. Smatra se da virusi, deficit selena, kadmijum, olovo i dugotrajno izlaganje policikličnim aromatičnim ugljovodnicima i aminima nema etiološki značaj. Nasuprot tome, pažnju zaslužuje hronična intoksikacija aristolohičnom kiselinom (AA) i ohratoksinom A (OTA). Oba ova agensa imaju nefrotoksično i kancerogeno dejstvo sa patofiziološkom osnovom za razvoj fibroze bubrega i karcinoma urotelijuma koji karakterišu EN. Prethodna ispitivanja ukazuju da ove toksične supstance mogu biti prisutne u hrani iz endemskih područja. Izloženost stanovništva bila je skoro potvrđena prisustvom AA i OTA DNK-adukta u bubrežnom tkivu. Ostaje da se na velikom broju obolelih ispita ovaj nalaz. Ključni molekularni nalaz A-T transverzionalne mutacije humanog p53 tumorskog supresornog gena, koji karakteriše AA-izazvane malignitete, može da bude dodatni marker bolesti, kakav danas ne postoji u EN.

Ključne reči: Endemska nefropatija, aristolohična kiselina, ohratoksin A, intersticijski nefritis, karcinom urotelijuma