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The involvement of mycotoxins in the development of endemic nephropathy

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Die Rolle von Mykotoxinen bei der endemischen Nephropathie

Zusammenfassung: Die endemische Nephropathie (EN) ist eine Nierenerkrankung, die noch immer nicht wissenschaftlich erklärt werden kann. Die EN wird von einer hohen Prävalenz von urothelialen Tumoren bei der ländlichen Bevölkerung der endemischen Region begleitet. Man vermutet daher, dass eine natürlich vorkommende nephrotoxische und karzinogene Substanz in die Ätiologie involviert ist.

Am meisten wird das Mykotoxin Ochratoxin A (OTA) als schuldige Substanz verdächtigt, da dieses eine gesicherte nephrotoxische und karzinogene Wirkung hat. Die vorliegende Arbeit bringt eine Übersicht über alle relevanten Studien über OTA in der Nahrung beziehungsweise im Blut und im Harn der Bewohner der betroffenen Endemie-Gebiete. Es werden auch Daten über das gleichzeitige Vorkommen von OTA mit anderen Mykotoxinen, wie Zitronin und Fumonisin B1 (FB1) in der Nahrung vorgestellt. Leider existieren keine Studien über das gemeinsame Vorkommen von OTA und anderen Mykotoxinen beim Menschen, und es gibt nur eine Studie über die Exposition mit FB1 in endemischen Gebieten. Diese Übersicht berichtet auch über experimentelle Daten, die in Zellkulturen und Labortieren erhoben wurden, die mit OTA und anderen nephrotoxischen Mykotoxinen (da die meisten Mykotoxin-Kombinationen synergistische Wirkung zeigten) behandelt worden sind. Es wird auch das Vorkommen von OTA- und Aristolochia-Säure-bedingten DNA-Veränderungen diskutiert.

Summary. Endemic nephropathy is a human kidney disease that still escapes scientific explanation. It is accompanied by a high incidence of urothelial tumors in rural populations in endemic areas, which suggests that a natural nephrotoxic and carcinogenic compound may be involved in the etiology.

The most imputed causative agent of endemic nephropathy is the mycotoxin ochratoxin A (OTA), because of its confirmed nephrotoxic and carcinogenic action. This paper presents a review of studies of OTA in food collected in the endemic areas and in blood and urine of their residents. Data on the co-occurrence of OTA and other nephrotoxic and carcinogenic mycotoxins such as citrinin and fumonisin B_1 in food are also presented. Unfortunately, there is no study on the co-occurrence of OTA and other mycotoxins in humans and there is only one study on fumonisin B_1 exposure in endemic areas. The paper also presents experimental data on cultured cells and laboratory animals treated with combinations of OTA and other nephrotoxic mycotoxins, because most such combinations show a synergistic effect. The occurrence of OTA- and aristolochic acid-DNA adducts is also presented.

Key words: Aristolochic acid, citrinin, fumonisin B_1, nephrotoxicity, ochratoxin A.

Introduction

Endemic nephropathy (EN) is a renal disease that occurs in some rural areas of Bosnia and Herzegovina, Bulgaria, Croatia, Kosovo, Romania, and Serbia. The etiology of EN is still unknown, although a number of more or less plausible hypotheses have been put forward [1]. The main characteristics of EN are the focused and limited geographical distribution, occurrence in farming households, high mortality from uremia, and high incidence of urothelial tumors [2]. The lesions occur primarily in the proximal tubules of the kidney and can develop into end-stage renal disease. Although patients suffering from EN show some familial clustering, the endemic nature of the disease in rural populations who have lived in the endemic area for at least a decade indicates that the cause should be sought in the environment, primarily in the form of a toxic and carcinogenic substance of natural origin [3].

The mycotoxin theory of EN origin is based on the analogy between pig kidney lesions caused by ochratoxin A (OTA) in Scandinavian countries and human kidney lesions in patients with EN [4]. Most studies on the involvement of mycotoxins in the etiology of EN were intended to prove that residents of endemic areas were more exposed to OTA than residents of other regions. However, low concentrations of OTA are often found in food and

blood of residents of non-endemic areas and in the blood of persons with chronic kidney diseases all round the world [5, 6]. Nevertheless, this does not discard the theory that mycotoxins are involved in the development of EN, because people in endemic areas are exposed to variable amounts of OTA that often exceed the exposure in non-endemic areas. In addition, food is never contaminated with a single mould strain, and other nephrotoxic mycotoxins are produced by the moulds that produce OTA. It is also known that natural mycotoxicoses are often caused by exposure to a combination of mycotoxins, and of course we normally ingest different foodstuffs at the same time. The climate in endemic areas favors mould production and the most common nephrotoxic mycotoxins that contaminate food in mild climates are OTA, citrinin, and fumonisin B_1 (FB_1). The International Agency for Research on Cancer has classified OTA and FB_1 as possibly carcinogenic to humans (Group 2B); citrinin is not classifiable with regard to its carcinogenicity to humans (Group 3) [7–9]. Exposure to OTA and other mycotoxins may therefore be involved in the etiology of EN and urothelial tumors.

This review presents research data on OTA and other nephrotoxic mycotoxins in food and blood of residents of endemic areas, as well as experimental data on the effects of OTA given in combination with other nephrotoxic mycotoxins. We also briefly present the theory of aristolochic acid (AA) in the development of EN.

Human exposure to OTA and other nephrotoxic mycotoxins in endemic areas

Most of Bulgarian and Croatian research has been limited to finding out whether OTA exposure is higher in residents of the endemic areas than in other populations. After the first finding of OTA in food and feed in the endemic area in Croatia [10], a number of other studies found either higher OTA concentrations or higher frequency of OTA-positive samples in endemic than in non-endemic areas [11–16]. In two studies using a duplicate diet method the weekly intake of OTA varied significantly and in two subjects it was near the provisional tolerable weekly intake established by the Joint FAO/WHO Expert Committee on Food Additives (100 ng/kg b.w.) [17, 18]. Studies of co-occurrence of nephrotoxic mycotoxins in maize and beans in endemic regions of Bulgaria and Croatia revealed significant co-occurrence of OTA with either citrinin or FB_1 (Table 1).

OTA persists in the human organism for a very long time and its calculated half-life in plasma after a single oral dose is 35 days [19]. It is not surprising that OTA is found in low concentrations in humans all over the world, even in countries where no EN has ever been identified [5]. There are speculations that exposure to OTA and other mycotoxins may be the cause of interstitial nephropathies all round the world [20]. In some countries in North Africa (Tunisia and Egypt) OTA has been detected in high concentrations in the blood and food of patients with kidney impairment of unknown etiology [21–23]. The appearance of OTA in kidney disease or impairment other than EN has been reviewed earlier [6].

In humans, OTA was first detected in the blood of residents in the endemic area of Croatia [24]. In a two-year study (1979–1980) the frequency of OTA-positive blood samples from residents of a non-endemic village did not differ significantly in the first and second years (7.6% and 7.8%, respectively). However, in residents of an endemic village the frequency dropped considerably (from 16.6% to 6.0%). In a ten-year follow-up of the same endemic village the highest frequency of OTA-positive blood samples was 4.5% (range 2–50 ng/ml) and in a control village 2.4% (range 1–10 ng/ml) [25]. Similar results were obtained in the endemic area of Vratza in Bulgaria [26], where OTA was detected more frequently and at higher levels in the blood samples of persons with urinary tract tumors and/or EN than in samples from unaffected people in the endemic or a non-endemic area. The same was true for urine samples [27].

No published study has yet measured OTA and another mycotoxin at the same time in residents of endemic areas.

The only study of mycotoxin exposure other than OTA measured in the serum and urine of residents in an endemic area was performed in Croatia [28], where expo-

Table 1. Co-occurrence of mycotoxins in beans and maize collected in endemic and non-endemic areas

			EN region		Control		
Commodity	Year	Mycotoxins	% positive	Range (ng/g)	% positive	Range (ng/g)	Ref.
Beans	1989	OTA	36.6	25–240	8.0	25–200	13
		citrinin	40.0	50–800	12.0	50–120	
	1990	OTA	40.0	85–260	5	10–220	
		citrinin	36.0	30–800	10.0	20–200	
Maize	1989	OTA	43.7	25–900	8.0	10–230	
		citrinin	43.7	50–1100	12.0	150–380	
	1990	OTA	44.0	25–890	5.0	20–235	
		citrinin	40.0	50–1000	10.0	50–140	
	1996	OTA	9.0	0.36–1.1	10.0	0.36–223.6	14
		FB_1	95.6	12–11661	98.3	18–11278	
	1997	OTA	50.0	0.29–613.7	20.0	0.26–216.6	
		FB_1	96.0	12–2524	90.7	12–970	

sure to fumonisins was investigated by measuring sphinganine (Sa) and sphingosine (So) concentration in the serum and urine of persons from the endemic and a control area. Results indicated an impairment of sphingolipid metabolism in residents of the endemic areas, which may have been induced by fumonisins or fumonisin-like mycotoxins [28].

Experimental data on combined exposure to OTA and other nephrotoxic mycotoxins

The effects of various combinations of mycotoxins have been studied on cultured cells and in experimental animals.

The most frequently tested combination of mycotoxins is OTA and citrinin, whose combined effects have been reviewed by Speijers and Speijers [29]. In more than a dozen studies reported in this review, OTA and citrinin showed a synergistic or additive effect in most animal species and in all cell cultures. This combination of mycotoxins was recently confirmed as having a synergistic cytotoxic effect on porcine renal cells (LLC PK1) [30].

The synergistic toxic effect of OTA and FB_1 was suspected to be the cause of the sudden death of Yorkshire piglets in Venezuela [31]. The finding of OTA (20–39 ppm) and FB_1 (10–40 ppm) in feed was assumed to be related to the kidney, liver and lung lesions.

The few studies on the combined effects of OTA and FB_1 on cell cultures or experimental animals all confirm synergistic or additive effects (Table 2). This combination synergistically decreased the viability of C6 glioma cells, Caco-2 cells and Vero cells after 48 hours of exposure [32]. A combination of low-dose OTA and FB_1 for 24 and 48 hours decreased the viability of porcine kidney cells (PK 15) and glutathione concentration, and increased malondialdehyde concentration in an additive or less than additive way [33, 34]. In turkey poults exposed to feed containing OTA (3 mg/kg) and FB_1 (300 mg/kg), serum parameters (cholesterol, AST, LDH) and the relative weight of organs (kidney, liver, pancreas, spleen, gizzard, and heart) changed, showing additive or less than additive toxicity but not toxic synergy [35]. In contrast, we have observed a synergistic increase in DNA damage in kidney homogenates of rats treated with an oral combination of OTA and FB_1 [36]. This effect, measured with the comet assay, was noticed even at extremely low doses (5 ng OTA/kg b.w. + 200 ng FB_1/kg b.w.) that correspond to the daily human exposure in Europe.

Ochratoxin A- and aristolochic acid-DNA adducts in patients with EN

It is well known that chemical carcinogenesis is initiated by the formation of covalently bound chemical carcinogens or their reactive metabolites to the DNA molecule, thus forming DNA adduct. The presence of DNA adducts of a certain compound or its metabolites is not proof of the compound's involvement in the etiology of the disease, but rather of exposure.

A theory of AA involvement in EN was put forward in the early 1990s [37]. AA-nephropathy (AAN) is a rapidly progressive kidney fibrosis with a high frequency of urothelial malignancies and was reported in young women first in Belgium and afterwards in other European countries [38]. AA from *Aristolochia fangchi*, which is known to have been mixed with other herbs by mistake and used for slimming therapies, is a known nephrotoxic and carcinogenic compound that causes cortical fibrosis without primary glomerular abnormalities and also malignancies of the urothelial system soon after exposure.

The differences between EN and AAN have recently been reviewed [39]. In EN the first clinical signs appear after a longer period of exposure to toxicant, initial anemia is milder, the length of progression from the first signs to end-stage kidney disease is much longer, and, in contrast to AAN, progression of the disease cannot be slowed by steroid treatment [38, 40]. It should be stressed that no analysis of food has yet demonstrated the presence of AA in the food of residents of endemic areas. The only paper dealing with this problem reported that residents with verified EN in the Croatian endemic area remembered more frequently than healthy individuals that their fields were severely infested with birthwort (*Aristolochia clematitis*), which contains AA in the seeds [41].

Using the ^{32}P-postlabelling method, OTA- and AA-related DNA adducts have been found in the liver and kidney of experimental animals after OTA and AA treatment [42–44]. Some authors have expressed doubt about the origin of OTA-related DNA adducts, suggesting that OTA and its metabolites cannot form covalent bonds with

Table 2. Effects of combined treatment of OTA and FB_1 on cell cultures and in experimental animals

Cell cultures/animals	Parameter	Effects	Ref.
C6 glioma CaCo-2 Vero cells	Cell viability	Synergistic	32
PK 15	Cell viability Malondialdehyde Glutathione	Additive Additive Additive	33
PK 15	Cytotoxicity Apoptosis	Additive Additive	34
Turkey poults	Relative weight of organs Serum parameters	Additive Additive or less than additive	35
Rats	DNA damage in kidney cells	Synergistic	36

DNA, and that adducts seen in the tissues of OTA-treated animals are due to products derived from OTA-mediated cytotoxicity [45, 46].

These adducts have been detected both simultaneously and separately in human kidney and in tumor tissues of the urinary tract in several studies. In the first study on kidney tissue from three EN patients living in Croatia, OTA- and AA-related DNA adducts were found simultaneously in samples from two of the patients, but in the third sample there were no adducts [47]. OTA-related DNA adducts were also detected in eight samples of tumor kidney and bladder tissues of Bulgarian patients who lived in a high-risk area for EN [48]. Such adducts were not found in tumor kidney tissues of French patients with AAN, analyzed as controls. These samples, and all tissues from surgically removed kidneys, urothelial or tumor tissues of patients with AAN in Belgium, contained only AA-related DNA adducts [49]. In a recent paper, OTA- and citrinin-related DNA adducts were detected in tumor kidney tissues of patients with EN from Croatia, Bulgaria and Serbia, but AA-related DNA adducts were not found [50]. In contrast, the presence of AA-related DNA adducts in four samples of renal cortex of patients with EN and in three upper urinary tract tumors in patients from the endemic area of Croatia have been reported [51]. These samples were not tested for the presence of OTA-related DNA adducts.

Conclusion

Most available studies of mycotoxin exposure of residents in endemic areas of Bulgaria and Croatia have focused on OTA contamination of food and feed, and on the presence of OTA in human blood and urine. The fact that OTA has been found in very low concentrations in humans in non-endemic areas does not negate the mycotoxin theory of the development of EN. The endemic area of Croatia has a humid climate which favors the growth of moulds and production of mycotoxins. Studies on experimental animals and cell cultures have shown that most combinations of other mycotoxins and OTA, particularly OTA and citrinin, have a synergistic effect. Further investigation into possible exposure to combinations of nephrotoxic and carcinogenic mycotoxins in the endemic areas is needed. Some features of AAN are similar to those of EN, but the endemic occurrence of nephropathy points to a particular regional exposure, and field contamination with *Aristolochia clematitis* cannot be regarded as such.

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