Biomarkers, 2012; 17(7): 577–589
© 2012 Informa UK, Ltd.
ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.3109/1354750X.2012.692392

informa
healthcare

COMMENTARY

Ochratoxin A exposure biomarkers in the Czech Republic and comparison with foreign countries

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Abstract
Among ochratoxins, ochratoxin A (OTA) occupies a dominant place and represents significant risk for human and animal health which also implies economic losses around the world. OTA is nephrotoxic, hepatotoxic, teratogenic and immunotoxic mycotoxin. OTA exposure may lead to formation of DNA adducts resulting to genotoxicity and carcinogenicity (human carcinogen of 2B group). Now it seems that OTA could be "a complete carcinogen" which obliges to monitor its presence in biological materials, especially using the suitable biomarkers. In this article, OTA findings in urine, blood, serum, plasma and human kidneys (target dose) in the Czech Republic and comparison with foreign countries are presented.

Keywords: Monitoring, ochratoxin A, OTA, exposure, biomarkers

Introduction

The attention of public health authorities, toxicologists, specialists in food safety, and clinicians have been focused on systematic control of the presence mycotoxins including ochratoxins which might endanger the health of the population.

Ochratoxins are one of the important groups of mycotoxins. In this group, many forms of ochratoxins and their metabolites have been described including phenylalanine-based ochratoxin A, B, and C (Wu et al., 2011). Among them, ochratoxin A (OTA) occupies a dominant place and represents significant risk in terms of impacts on human (and animal) health and economic losses. In terms of risks to human health, OTA is no doubt important. It is also non-negligible food contaminant. Humans are exposed to ochratoxin A – like to others mycotoxins – through several routes, with dietary intake being the most prominent one. Dermal contact or inhalation exposures are of minor importance for general population (Jørgensen, 2005; Degen et al., 2007).

OTA, 7-carboxyl-5-chloro-8-hydroxyl-3,4-dihydro-3-R-methylisocoumarin-7-L-β-phenylalanine, see Figure 1.

OTA is produced as a secondary metabolite by the fungal species of *Aspergillus* (Frisvad et al., 2004; Samson et al., 2004; Perrone et al., 2006a,b) and *Penicillium* (Larsen et al., 2001; Bogs et al., 2006; Battilani et al., 2007). This mycotoxin is a common contaminant in food such as cereal products, beer, coffee, cacao, spices, legumes, green tea, figs, pistachios, raisins, grape juice, wine and animal origin e.g. pork meat, pork blood products, kidney, liver of poultry (Malir and Ostry, 2003; EFSA, 2006). In the recent years, several "new" sources of OTA exposure (e.g. wine, raisins, dried red pepper, liquorice and curcuma) have been identified (EFSA, 2006; Report on carcinogens, 2011).

OTA has been shown to exhibit number of toxic effects to animals (Wu et al., 2011). Of these, the most prominent ones include nephrotoxicity (Krogh, 1992), carcinogenicity in the kidney and liver in mice (Kanizawa and Suzuki, 1978), rats (Bendele et al., 1985) and chicks

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(Received 25 March 2012; revised 02 May 2012; accepted 07 May 2012)

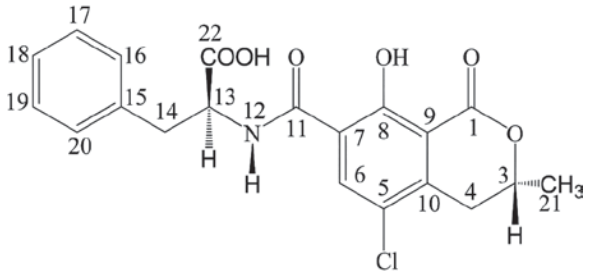

Figure 1. Ochratoxin A.

(Stoev, 2010) in addition to numerus specific toxic effects, such as hepatotoxicity (Chopra et al., 2010), teratogenicity (Mayura et al., 1976), and immunosuppressivity (Haubeck et al., 1981).

The International Agency for Research on Cancer (IARC) has evaluated the experimental evidence for carcinogenicity of OTA as sufficient, and has, thus, classified OTA as "possibly carcinogenic to humans", group 2B (IARC, 1993). At present, OTA is thought to be complete carcinogen (Pfohl-Leszkowicz and Manderville, 2012).

Apart from carcinogenic effects, OTA is suspected to have immunosuppressive, genotoxic, nephrotoxic, hepatotoxic or teratogenic effects on humans (Baudrimont, 1995; Pfohl-Leszkowicz and Manderville, 2007; Wu et al., 2011; Pfohl-Leszkowicz and Manderville, 2012).

In terms of human pathologies, OTA is suspected of being the main etiological agent responsible for Balkan Endemic Nephropathy (BEN) and associated urinary tract tumors in humans (Vukelic et al., 1992) and Chronic Interstitial Nephropathy (CIN) in North Africa (Wafa et al., 1998, Grosso et al., 2003), a chronic kidney disease in humans.

The toxicity of OTA originates from chemical structure of the molecule. This molecule consists of isocoumaric and phenylalanine parts connected by peptidic bond through amine group of phenylalanine (van der Merwe et al., 1965). OTA inhibits protein synthesis by competing with phenylalanine in the phenylalanyl-tRNA synthase-catalyzed reaction (Konrad and Röschenthaler, 1972; Bunge et al., 1978; Creppy et al., 1983). Chronic exposure to OTA may result in formation of DNA adducts and damage of genetic information. The exposure to OTA may lead to formation of DNA adducts accounting for its genotoxicity, cancerogenicity (Manderville, 2005; Manderville and Pfohl-Leszkowicz, 2008). Also, chronic exposure to OTA has influence on metabolism of calcium, saccharides and it increases lipid peroxidation (Rahimtula and Chong, 1991; Krogh et al., 1988; Halliwell, 1991; Baudrimont, 1995).

It is known that the toxicokinetic profile of OTA varies greatly depending on the species. OTA is biotransformed and the slow elimination of OTA metabolites in animals and humans play an important role in the toxicity, carcinogenicity, and organ specificity of OTA (Zepnik et al., 2003).

The toxic effect of OTA can be potentiated by certain drugs or synergy with other mycotoxins or other contaminants. Potential synergic effects which multiple mycotoxins might have are thought to make the task of delineating the role of OTA far more complex and any long-term effects are considered to be difficult to assess precisely (Erkekoglu et al., 2010).

Studies have shown that the exposure of the human population to OTA is a world-wide problem (Baudrimont, 1995; Märtlbauer et al., 2009; Pfohl-Leszkowicz et al., 2007; Pfohl-Leszkowicz, 2009; Duarte et al., 2011).

It is known that the dietary exposure of humans to OTA can be assessed by estimating daily intake by analyzing OTA in food using the known amount of the consumed diet (Malir et al., 2006). This method has, however, many disadvantages. It is necessary analyze a wide range of food types, analyst is confronted with often sporadic occurrence and low levels at which OTA is found, and there is a problem how to obtain a representative sampling. Measurement of exposure through only foodstuffs is not always able to provide a full picture of human exposure to OTA completely (Duarte et al., 2011).

The dietary exposure of humans to OTA can be also estimated by analyzing levels of OTA in a biological materials (Malir et al., 2006; Duarte et al., 2011).

Biomonitoring using the biomarkers

Biomarkers are essential instruments employed in order to measure the exposure to a toxic agent or the extent of any toxic response to such agent and also to predict the likely response (Timbrell, 1998). Biomarkers are generally divided into three categories, namely biomarkers of exposure (themselves divided into markers of internal dose and markers of effective dose), of response or toxic effect, and of susceptibility (Timbrell, 1998, Ostry et al., 2010b). Nevertheless, regardless of the category, any biomarker should be quantitative, sensitive, non invasive, specific, easily measurable, related to the appropriate biochemical mechanism. It should also work at realistic doses. The category of biomarker selected should, in theory, depend on many factors, including the information, that is sought, availability, and validity (Timbrell, 1998).

Biomarkers of internal dose indicate that exposure to a particular compound has taken place by measuring the compound or its metabolite(s) in body fluids or tissues (Timbrell, 1998), e.g. in milk, blood, urine, kidney (target dose).

In the Czech Republic, OTA has been traditionally used biomarkers of internal dose which means most available data reflect findings of these biomarkers.

Monitoring OTA by using biomarkers of internal dose

OTA in milk, serum or urine qualifies as a biomarker of exposure due to its slow excretion from the body as exemplified by its half-life in blood of several weeks. A major drawback is, however, still the incomplete knowledge of OTA pharmacokinetic profile (Larsen, 1995). Other disadvantages that biomonitoring of OTA exposure

raises include the necessity of invasive collection in case of monitoring its presence in blood, necessity of medical personnel assistance, and especially exposure assessment based on assumptions of bioavailability and plasma clearance. In case of urine, generally OTA lower levels compared with serum and incomplete knowledge about indication of time are thought to pose a problem (Duarte et al., 2011). On the other hand, benefits from OTA analysis in biological materials cannot be overlooked. It must be mentioned in particular that the OTA presence in these materials reflects exposure of organism to OTA stemming from all routes (see supra), exposure from all sources and also the bioavailability of OTA. Moreover, only one type of sample needs to be analyzed which makes individual and group risk assessments easier (Duarte et al., 2011).

OTA in human milk

Since OTA is excreted via breastfed children including babies are also exposed to OTA (Gareis et al., 1988).

In Norway, the relationship between OTA contamination of human milk and dietary intake was examined (Skaug et al., 2001). These results confirm the occurrence of OTA in human milk and its likely association with maternal dietary habits. The strongest associations were observed with foodstuff sources of vegetable origin and, to a lesser extent, with food of animal origin (Skaug et al., 2001). The OTA presence in human milk has been reported in different countries (Jonsyn et al., 1995; El-Sayed et al., 2002; Turconi et al., 2004; Hassan et al., 2006; Dostal et al., 2007; Gürbay et al., 2009; Muňoz et al., 2010; Biasucci et al., 2011). High values (in the range of 0.62–13.11 μg/1) were observed in all samples tested in a Turkish survey (Gürbay et al., 2009). However, the highest values of OTA were observed in Egypt and in Sierra Leone. In Egypt, OTA was found in 43 (35.8%) of 120 tested human milk samples (in the range from 5.1 to 45 μg/1) (El-Sayed et al., 2002), and as for Sierra Leone, OTA was found in 35 of 40 tested human milk samples (in the range from 0.2 to 337 μg/1) (Jonsyn et al., 1995).

Despite the fact that OTA levels in milk are much lower than levels of OTA in blood (down to 10x) (Breitholtz-Emanuelsson et al., 1993), OTA contamination of human breast milk presents a potentially serious health hazard (Kuiper-Goodman et al., 2010).

OTA in human blood

The most studied approach for biomonitoring of OTA exposure evaluation is certainly the measurement of OTA concentrations in blood specimens. In the past decades, OTA has been detected in human blood samples on worldwide level. Consequently, OTA levels in blood are considered to be one of the essential indicators of human exposure to OTA.

The highest value which has been found in blood serum was 1800 μg/l (Steyn, 1993). OTA levels ≥10 μg/l of serum are generally thought to induce nephropathies (Fournier, 1993).

Scott has described OTA in blood serum as uniquely useful biomarker of OTA exposure due to its high-affinity binding to serum albumin or to other small proteins which should result into long persistence of OTA in blood serum (Scott, 2005). However, as for the exact correlation between OTA intake and its findings in blood (both in serum and plasma), opinions differ. According to Breitholtz et al. (1991a), OTA serum levels reflect the dietary intake of this toxin. In another study conducted in the UK, however, no correlation between plasma concentrations and consumption of OTA was observed (Gilbert et al., 2001). Using the same protocol, these data were confirmed in Bulgaria and in Serbia (Petkova-Bocharova et al., 2003; Pfohl-Leszkowicz et al., 2004; Pfohl-Leszkowicz et al., 2006; Pfohl-Leszkowicz, 2009). Moreover, regional variations in OTA plasma concentrations in healthy humans have been reported from many countries (Breitholtz et al., 1991b; Creppy et al., 1993; Zimmerli and Dick, 1995; Scott et al., 1998; Gareis et al., 2000; Scott, 2005; Karima et al., 2010). These variations might be explained by differences in the local diet. In general, there were significantly higher OTA plasma concentrations in regions where more local food products are consumed (Breitholtz et al., 1991b, Creppy et al., 1993). Climate may also play a role. Apart from regional variations, seasonal variability in OTA levels has also been recorded. In this regard, OTA blood concentration has been shown to be higher in summer e.g. in Italy (Palli et al., 1999) in Spain (Burdaspal and Legarda, 1998) or in Croatia (Peraica et al., 2001).

But the determinations of OTA in blood remain the basic method how to monitor human exposure to OTA. Variations of serum OTA concentrations over time have been noted in several countries. This contributes to the unreability of correlations of dietary intake and serum levels (Scott, 2005; Castegnaro et al., 2006; Pfohl-Leszkowicz et al., 2006; Pfohl-Leszkowicz and Manderville, 2007).

Part of the variation may be explained by toxicokinetical properties. Once OTA reaches the bloodstream, it is bound to serum proteins (>99%), which facilitates its passive absorption in the nonionised form, but hinders its glomerular filtration. OTA binds strongly to albumin (binding saturation above several hundred micrograms per mililitre of serum (Schwerdt et al., 1999), but also strongly to other small proteins (20,000 Da), for which binding saturation is reached with an OTA concentration of 10–20 ng/ml (Stojkovic et al., 1984). The fraction of OTA bound to proteins constitutes a mobile reserve of OTA that can be released as soon as the free OTA fraction decreases. This delays elimination and thus increases the risk of accumulation of OTA in tissues (Pfohl-Leszkowicz and Manderville, 2007).

In a study, OTA consumption, blood concentration and excretion were monitored in Bulgarian volunteers for a 1-month period (Pfohl-Leszkowicz et al., 2004; Castegnaro et al., 2006). For some individuals, regular

and continuous OTA intake equivalent to 1.2–2.2 ng/kg bw/day led to a relatively high steady state OTA blood concentration (1.46 ng/l) and low OTA elimination. For others, variable intake (1 week high, 1 week low) led to a constant relatively low OTA blood concentration (0.5 ng/ml), which was in the same range as volunteers having a regular very low OTA intake (in some cases below the LOD). Taken together, these data indicate that OTA blood level is relatively stable over a period of 1 month for a given individual and is regulated by urinary excretion. Thus, variation of OTA food intake is not directly reflected by a variation of OTA blood concentration: high OTA intake is not always reflected by high serum OTA concentration and the highest serum concentration is not related to the highest OTA consumption (Pfohl-Leszkowicz and Manderville, 2007).

Interesting conclusions were published by StuderRohr who have shown in kinetic experiments in humans that during the first 6 days since intake OTA is mainly distributed within the body and only a minor fraction is excreted. During the next 69 days, the OTA plasma concentration decreases while a larger amount of OTA (and metabolites) is excreted via urine. In the same study, the plasma half-life of OTA in one individual has been determined at about 20 h in the first 6 days and 35.6 days from the 6th day onwards (Studer-Rohr et al., 2000).

Nevertheless, human exposure to OTA has been estimated on the ground of blood plasma levels (Scott, 2005). Most studies have employed two versions of the Klaassen equation (Breitholtz et al., 1991a; Miraglia et al., 1996) relating the continuous dietary intake of OTA with plasma level, plasma clearance (considering only renal filtration) and bioavailability. The renal filtration rates have been calculated according to a study of Hagelberg et al., 1989 or Gilbert et al., 2001. Estimation of exposure based only on assumptions of bioavailability and plasma clearance seems to involve a disadvantage. The calculation of OTA intake from plasma level using the Klaassen equation can underestimate the intake because only clearance (i.e. OTA elimination) is assumed to involve filtration, and an approximated rate, just as the bioavailability coefficient (Duarte et al., 2011).

OTA is ubiquitous in human blood serum/plasma and indicates continuous exposure to the toxin, originating mainly from food intake (Scott, 2005). Advantages of monitoring OTA in blood of healthy persons consist in relatively high OTA levels found compared to OTA determinations in urine. As a result, monitoring OTA in blood requires less sensitive analytical methods (Duarte et al., 2011). At the same time, precise knowledge of the normal exposure of the general population is a prerequisite with a view of assessing the role of OTA in human pathologies or in potential bioterrorist attack.

OTA has been repeatedly found in the blood serum of the healthy population of both Czechoslovakia (Fukal and Reisnerova, 1990) and the Czech Republic since the 1990s (Fukal and Reisnerova, 1990; Ruprich and Ostrý, 1993a,b).

Table 1. The prevalence of OTA in the blood serum of adult donors in Czech Republic – results from monitoring (Malir et al., 2006; Malir et al., 2008).

Year	1994–2002		2005	
OTA level range (μg/l)	n+[a,b]	n+ (%)	n+	n+ (%)
0.1–0.2	1181	56.9	58	34.3
0.2–1	860	41.4	110	65.1
1–2	34	1.6	0	0
2	2	0.1	1	0.6
Total	2077	100.0	169	100.0

n+, numbers of positive samples; n+ (%), percentage of positive samples; [a], all findings ≥ 0.1 μg/l included as positive; [b], OTA levels < 0.1 μg/l considered as ½ LOQ (limit of quantification) = 0.05 μg/l.

In 1994–2002 OTA was systematically monitored in serum from several districts in the Czech Republic. The detected OTA levels are also summarized in Table 1. (Malir et al., 1998b; Malir et al., 2001a,b; Malir et al., 2006; Malir et al., 2008).

The data obtained in the Czech Republic may be, thus, compared with data on OTA occurrence in blood samples (serum or blood plasma) of healthy humans in other countries, see Table 2 (Märtlbauer et al., 2009; Ostry et al., 2010b; Duarte et al., 2011).

OTA in patients with renal disorders

The presence of OTA in food samples from areas of BEN and high incidence of urinary tract tumours occurred was found and published (Petkova-Bocharova and Castegnaro, 1985). Subsequently it was found high levels of OTA in blood of individuals with BEN associated with tumors of the urinary tract compared with healthy individuals (Petkova-Bocharova et al., 1988). On this basis, it was stated that the prevalence of OTA presence in the blood of people with BEN and urinary tract tumours supported the hypothesis that OTA, nephrotoxic a carcinogenic mycotoxin, is involved in the etio-pathogenesis of these diseases (Özçelik et al., 2001). Later the same was found and confirmed for CIN, chronic interstitial nephropathy. Here several studies attempted to establish a relationship between blood biomarker and general nephropathic conditions, both in the endemic and nonendemic regions. The higher incidence in Algeria and mean OTA levels in Tunisia and in Turkey (Khalef et al., 1993; Maaroufi et al., 1995; Özçelik et al., 2001) were reported. OTA positive blood samples were found in the group of nephropathic individuals in several countries indicate that OTA plays the role in the human urinary pathology in affected studied patients. Because urinary diseases develop over a long period of time should be the concentration of OTA in serum has traditionally been monitored for many years that could be the role of OTA in the pathology of renal tumors and urinary tract evaluated (Özçelik et al., 2001). The data OTA levels in blood samples of patients with various renal disorders in different countries can be seen in Table 3.

Table 2. International overview of published data on ochratoxin A in blood samples from healthy persons.

Country	Collecting period	n+ (%)	OTA min-max (μg/l)	OTA mean (μg/l)	Reference
Europe					
Bulgaria	1984,1986 1989–1990	10	—	12.0	Petkova-Bocharova et al., 1988; Petkova-Bocharova and Castegnaro, 1991
Bulgaria	—	100	max. 8.4	1.59	Petkova-Bocharova et al., 2003
Croatia	1997–1998	59.4	max. 15.9	0.30	Domijan et al., 1999; Peraica et al., 1999, 2001
Czechoslovakia	1990	21	0.5–12.0	0.37	Fukal and Reisnerova, 1990
Czechoslovakia	1990–1991	40	0.5–19.4	0.63	Ruprich and Ostry, 1993b
Czech Republic	1994–2002	94.2	0.1–13.7	0.24	Malir et al., 1998a; Malir et al., 2001a,b; Malir et al., 2006
Czech Republic	2005	83.7	0.1–2.3	0.21	Malir et al., 2008
Denmark	1990	54.2	0.1–13.2	1.8	Hald, 1991
France	1991–1992	18.1	0.1–161	0.4	Creppy et al., 1993; Benford et al., 2001
Germany	1977–1985	56.5	0.1–14.4	0.6	Bauer and Gareis, 1987
Germany	1988	68	0.1–8.4	0.75	Hadlok and Wagner, 1993
Germany	1999	98.1	0.06–2.03	0.27	Rösner et al., 2000
Germany	2005–2006	100	0.05–0.75	0.75	Degen et al., 2007
Hungary	1995	82	0.2–10.0	—	Solti et al., 1997
Hungary	1997	77	0.1–1.4	—	Tapai et al., 1997
Italy	1992	100	0.1–2.0	0.53	Breitholtz-Emanuelsson, 1994
Italy	1994–1996	97	0.1–57.2	0.56	Palli et al., 1999
Norway	1998	100	0.05–0.42	0.18	Thuvander et al., 2001; EU, 2002
Poland	1983–1985	7.2	1–40	0.28	Golinski et al., 1991
Poland	2005	100	0.1–0.4	0.37	Grajewski et al., 2007
Spain	1996–1998	53.3	0.5–4.0	0.71	Jimenez et al., 1998
Spain	1996–1997	72	0.21–6.96	0.63	Pérez de Obanos et al., 2001
Sweden	1989	12.8	0.3–7.0	0.20	Breitholtz et al., 1991b
Sweden	1997	100	0.01–0.48	0.21	Thuvander et al., 2001; EU 2002
Switzerland	1992–1993	100	0.06–6.02	ca 0.4	Zimmerli and Dick, 1995
UK	2000	100	0.4–3.11	1.09	Mac Donald et al., 2001; EU 2002
Former Yugoslavia	1980	7.8	max. 8.0	5.4	Hult et al., 1982; Hald, 1991
	1981–1989	0–3.7	max. 50.0	—	Fuchs et al., 1991
Africa					
Algeria	—	66.9	max. 9.0	2.8	Khalef et al., 1993
Egypt	—	2.9	max. 0.91	0.08	Wafa et al., 1998
Marocco	2000	60	0.08–6.59	0.2	Filali et al., 2002
Tunisia	—	62	max. 3.2	1.22	Maaroufi et al., 1995a
	—	71	max. 7.5	2.6	Hassen et al., 2004
	—	—	max. 3.4	0.49	Hmaissia Khlifa et al., 2008
	—	66	max. 2.3	1.1	Maaroufi et al., 1995b
	1991–2000	62–82	0.1–5.5	2.0	Abid et al., 2003
	1996, 1998	100	0.1–8.06	0.53	Grosso et al., 2003
	—	62	max. 3.2	1.22	Hassen et al., 2004
Ivory Coast	2001, 2004	34.9	max. 11.62	0.58	Sangare-Tigori et al., 2006
Sierra Leone	1996	33	max. 18.2	—	Jonsyn, 1999
Asia					
Japan	1992–1996	85	max. 0.28	0.07	Ueno et al., 1998
Lebanon	2001–2002	33	max. 0.87	0.17	Assaf et al., 2004
Pakistan	—	97	max. 1.24	0.31	Aslam et al., 2005
Turkey	—	—	max. 1.43	0.44	Ozçelik et al., 2001
The Americas					
Argentina (2 regions)	2004–2005	63.8	0.19–47.6	0.15	Pacin et al., 2008
		62.3	0.19–74.8	0.43	
Chile (2 regions)	2004	54	0.4–2.75	0.44	Munoz et al., 2006
		91	0.4–2.12	0.77	
Costa Rica	—	95	max. 1.91	0.62	Guzman et al., 2007
Canada	1991	38.3	max. 9.0	1.29	Kuiper-Goodman et al., 1993
	1994[a]	100	max. 2.37	0.88	Scott et al., 1998

n+ (%), percentage of positive samples; [a], study included persons working at grain storage facilities.

Table 3. OTA in blood samples of patients with various renal disorders – international comparison.

Country, collecting period (blood specimen)	Source	n/n + (%)	OTA min-max (μg/l)	OTA mean (μg/l)	Reference
Czech Republic, Hradec Kralove, 1998–1999 (serum)	Hospitalized patiens with pre-existing CHRI on the basis of chronic nephropathy (total), e.g.:	17/12 (70.6)	0.05–0.4 [a]	0.2	Malir et al., 2001a
	Tubulointerstitial chronic nephritis	5/3 (17.7)	0.05–0.3	0.1	
	Glomerulonephritis chronic	3/3 (17.7)	0.1–0.4	0.3	
	Other renal diseases in the CHRI stage	9/6 (35.2)	0.05–2.3	0.2	
	Outpatients with stabilized CHRI (total) e.g.:	103/96 (93.2)	0.05–3.1	0.4	
	Tubulointerstitial chronic nephritis	56/54 (52.4)	0.05–3.1	0.4	
	Glomerulonephritis chronic	15/14 (13.6)	0.05–2.2	0.4	
	Other renal diseases in the CHRI stage	32/28 (27.2)	0.05–2.3	0.5	
	Patients ESRD dialysis (total),	99/95 (96)	0.05–5.4	0.8	
Czech Republic, Hradec Kralove, 2006 (serum)	Kidney tumor patients (dg. Grawitz)	40/33 (82.5)	0.05*–0.3*	0.13	Malir et al., 2008
Egypt (serum)	ESRD medical treat	11/4 (36.4)	max. 3.8	1.0	Wafa et al., 1998
	ESRD dialysis	15/4 (26.7)	max. 2.2	0.3	
	Renal transplant recipients	15/2 (13.3)	max. 6.3	0.5	
	Patient nephrotic syndrome	15/8 (53.3)	max. 10.2	2.2	
	Urothelial tumours	15/3 (20)	max. 5.6	0.5	
Ivory coast (serum)	Nephropathy patiens undergoing dialysis	39/8 (20.5)	max. 2.4	1.1	Sangare-Tigori et al., 2006
Pakistan, Karachi (plasma)	Bladder cancer patients	96/87 (90.6)	max. 3.41	0.3	Aslam et al., 2005
Poland, Pomeranian region, Bydgoszcz, 2005 (serum), region	Kidney tumor patients	19/18 (94.7)	0.19–3.77	1.0	Grajewski et al., 2007
	Kidney cirrhosis	1/1 (100)	max. 1.9	1.9	
Portugal, Coimbra, Aveiro, 2002–2003 (serum)	Hemodialyzed patients	50/50 (100)	0.12–1.5	0.5	Dinis et al., 2007
		45/45 (100)	0.15–1.0	0.5	
Tunisia, Sahel (serum)	Hospitalized patients:Non-UTDUTD				Grosso et al., 2003
		62/62 (100)	—	0.5	
		47/38 (80.9)	—	1.0	
Tunisia, Monastir (plasma)	CIN patients[a]	-/20 (93)	max. 140.5	44.4	Hassen et al., 2004
	Nephro patients[b]	-/40 (83)	max. 73.2	8.1	
	CIN UA[c]	-/20 (100)	18.4–171.3	50.4	
	CIN KA[d]	-/20 (78)	max. 29	12.4	
Turkey, Sparta (serum)	Various urinary disorders	-/93 (-)	max: 5.5	0.7(- 2.1)	Ozçelik et al., 2001

ESRD medical treat, end-stage renal disease- patients under conservative medical treatment; ESRD dialysis, end-stage renal disease-patients under dialysis; Non UTD, hospitalized patients without urinary tract disease; UTD, hospitalized patients with dg. urinary tract disease; CIN[a], patients with chronic interstitial nephropathy (CIN) of unknown etiology; Nephro patients[b], chronic nephropathies of known etiologies; CIN UA[c], CIN of unknown etiology; CIN KA[d], CIN of known etiology; CHRI, chronic renal insufficiency.

Differences were found between levels of OTA in blood of healthy (blood donors) and that of nephropathy patients which can be explained by damages resulting into impaired renal excretory capacity in nephropathy patients. In patients with impaired renal function, retention of various substances and contaminants – including ochratoxin A – from the food chain occur and nitrogenous catabolites such as urea and creatinine accumulate in their blood.

Therefore, in the Czech Republic, a study of OTA accumulation in outpatients with stabilized chronic renal insufficiency (CHRI) with mild to moderate creatinine retention and in patients in advanced renal insufficiency in the terminal stage of CHRI (who are regularly treated by dialysis) was conducted and compared with data from blood serum donors. The study demonstrated that unlike in the control group of blood serum donors (data from 1998 to 1999; OTA levels of the healthy population with normal creatinine in the blood serum), the retention of OTA was significant in outpatients with stabilized CHRI and in patients in the terminal stage of CHRI (requiring regular dialysis treatment). Because of the OTA nephrotoxicity, such retention may accelerate their disease. The study also showed that a standard dialysis did not result in the decrease of OTA serum levels (Malir et al., 2001a,b). The results obtained in this study are summarized in Figure 2. The dietary regimen of all patients was investigated too, with the help of a special questionnaire.

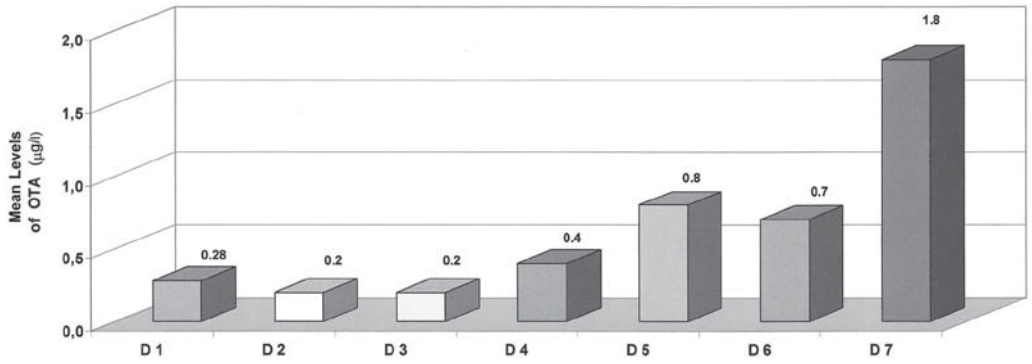

Figure 2. Findings of OTA in blood serum patients suffered renal insufficiency in the CR (Malir et al., 2001a,b).

1. D1 – control group (blood donors – results from monitoring)
2. D2 – hospitalized patients without demonstrable CHRI (with temporary renal insufficiency, group of 25 patients: 11 women, 14 men, mean age 65.5 years)
3. D3 – hospitalized patients with pre-existing CHRI (group of 17 patients: 15 men, 2 women, mean of 66.5 years)
4. D4 – outpatients with stabilized CHRI (group of 103 patients: 64 men, 39 women, mean age of 60.8 years)
5. D5 – total patients in RTD (regularly treated by dialysis), state before dialysis (group of 99 patients: 67 males, 32 females, mean age of 62 years)
6. D6 – patients in RTD, randomly chosen, state before dialysis (group of 10 patients: 4 men, 6 women, mean age of 69.3 years)
7. D7 – patients in RTD, selectively chosen, state before dialysis (group of 20 patients: 15 men, 5 women, mean age of 63.9 years)

In principle, the study showed that in stabilized, slightly or intermediately progressed, CHRI (D4), OTA serum levels were found to be significantly increased in comparison with the healthy population (D1). In patients regularly treated by dialysis, OTA serum levels were determined to be significantly higher in all three subgroups (D5, D6, D7) than in the healthy population (D1). Apart from that, in these patients standard dialysis did not result in a significant decrease in the OTA serum levels, see Tables 4 (Malir et al., 2001a) and 5 (Malir et al., 2001c). Further research showed that OTA bound to blood plasma proteins did not penetrate the dialysis membrane. The reason why OTA tends to accumulate in protracted chronic renal insufficiency (CHRI) has been attributed either to an excessive intake of OTA through foodstuffs or failures of biotransformation of OTA in CHRI or a combination of these two causes (Malir et al., 2001a,b).

In another clinical study carried out in 2006, the relationship between OTA levels in blood of the patients with kidney cancer (in majority, diagnosis of Grawitz tumor) was studied (Malir et al., 2008), see Table 3. Aims of study was to find out OTA levels in serum of the patients with kidney cancer and to compare their values with the those of the healthy population in the prospect of studying the relationship between the OTA levels in blood of patients with kidney cancer (Malir et al., 2008). The study concerned a group of 40 patients (28 males and 12 females) with the mean age of 56.9 years, the uncomplicated course of the operation, without metastases, overall metabolic disruption or cardiovascular disease. The blood samples were collected before the operation took place. This study did not demonstrate increased levels of OTA in blood of the selected persons. OTA was detected in 82.5% of samples with the mean of 0.13 µg/l. Unfortunately, only OTA was measured, not its metabolites due to unavailability of the corresponding standards.

OTA in urine

Although OTA levels in urine are very low in comparison to those in blood, under some authors, OTA in urine is thought to be a better indicator of exposure to OTA than OTA levels in plasma (Gilbert et al., 2001; Pfohl-Leszkowicz et al., 2006; Castegnaro et al., 2006), at least as far as dietary exposure is concerned. OTA could be found in urine several days after OTA ingestion (Pfohl-Leszkowicz and Manderville, 2007). The elimination of OTA through human urine has been reported to be low (mean value between 20 and 80 ng/day) and independent of the dose ingested (Castegnaro et al., 2006). The OTA uptake has been described as dependent on the free OTA concentration which is severely limited by binding of OTA to serum albumin (Pfohl-Leszkowicz and Manderville, 2007). Thus, the relationship between OTA in urine and OTA intake remains a complex issue as in case of OTA in blood. Moreover, the presence of OTA metabolites (e.g. nonchlorinated OTB metabolite and hydroxylated metabolites- 4-OH OTA; quinone derivatives) in human urine has been confirmed by various studies too (Jonsyn-Ellis, 2000; Castegnaro et al., 2006; Pfohl-Leszkowicz, 2009). Of interest only in the urine of rats were detected a total of 20 different metabolites of OTA, OP-OTA, OTB, OTA-OH, and OTα (Li et al., 2000).

Although OTA in urine is a promising alternative, it still waiting for further developments on the relationship at the individual level between OTA intake and urinary

Table 4. Patients in ESRD regularly treated by dialysis in Hradec Kralove, CR (1998–1999) (Malir et al., 2001a).

Number of patients (group)	Age (year)	Duration of RDT (month – mean)	Creatinine in serum before dialysis (avg.) (μmol/l)	Creatinine in serum after dialysis (avg.) (μmol/l)	OTA serum levels before dialysis (mean) (μg/l)	OTA serum levels after dialysis (μg/l)
99(M:F)67:32(gr.D5)	62	35.5	874.9	—	0.8 (0.05–5.4)[a]	—
10(M:F)(4:6)(gr.D6)	69.3	26.3	812.5	—	0.7 (0.2–1.9)	0.8 (0.2–2.2)
20(M:F)15:5(gr.D7)	63.9	37.9	819.6	346.7	1.8(0.2–5.4)	1.7 (0.2–5.2)

ESRD, end-stage renal disease; D5, total patients in ESRD dialysis, state before dialysis; D6, patients in ESRD regularly treated by dialysis (RTD), randomly chosen-with a previously uknown level of OTA; D7, patients in RTD, selectively chosen with a previously proven increased level of OTA; M, males; F, female; gr., group.
[a]OTA levels < 0.1 μg/l considered as ½ LOQ = 0.05 μg/l.

Table 5. Levels of OTA found in the serum of patients before and after dialysis and in the dialysate (Malir et al., 2001c).

Sex/Age (year)	Blood flow (ml/min)	Weight (kg)	Weight loss (kg)	OTA before dialysis (μg/l)	OTA after dialysis (μg/l)	OTA in dialysate (μg/l)[a]
Male (68)	270	67.2	–1.2	2.3	1.7	0.05
Female (72)	270	84.7	–0.2	0.5	0.5	0.05
Female (73)	270	58.0	–0.8	3.6	3.6	0.05
Male (75)	270	90.2	–3.2	2.3	2.0	0.05
Male (67)	270	87.1	–1.6	2.1	1.6	0.05

[a], OTA levels < 0.1 μg/l considered as ½ LOQ = 0.05 μg/l.

biomarker (the mother compound or its metabolites), as well as the temporal variations in the presented levels. It is important for defining whether OTA in urine can be approached as a biomarker of chronic or acute exposure, and if it suffers from the same within – subject variability as plasma (Duarte et al., 2011).

In the most notable Czech study, carried out in 2010, OTA was measured in total in 236 samples of urine collected from healthy persons within a 24-h cycle (males/females, 45–60 years old, two samples per person from non-consequative days at least 14 days time difference). 185 samples (78%) of these 236 samples were positive, with the limit of quantification (LOQ) of 2.0 ng/l, the mean of 7.32 ng/l and median of 4.47 ng/l (Ostry et al., 2010a,b). The detailed results concerning the total daily urinary excretion of OTA in men and women can be seen in Table 6. The total daily urinary excretion of OTA was determined with the mean of 0.3 ng/kg bw/day in men and of 0.15 ng/kg bw/day in women and with the median of 0.17 ng/kg bw/day in men and of 0.12 ng/kg bw/day in women (Ostry et al., 2010b).

These data were concluded to signalize the real exposure of the given population group to OTA, with higher percentage of positive urine samples in men (92%) than women (65%) (Ostry et al., 2010b). At the same time, these data do not seem to be substantially different from data known from other European countries although, contrary to the Czech study, OTA was usually determined in morning urine (non 24-h urine) in these countries, see Table 7.

Although the opinions on the role of this biomarker differ, it is promising. Nevertheless, the interpretation of this biomarker requires cooperation with physicians so that this biomarker could be used as reliable instrument for purposes of monitoring diseases associated with OTA presence.

Table 6. The results of total daily urinary OTA biomarker excretion (Ostry et al., 2010a, Ostry et al., 2010b).

Sex	Male		Female	
n /n +	116/107		120/78	
Biomarker	OTA (ng/day)	OTA (ng/kg bw/day)	OTA (ng/day)	OTA (ng/kg bw/day)
Mean[a]	25.4	0.30	9.27	0.15
Median[a]	14.7	0.17	7.62	0.12
90%[a]	32.5	0.43	20.2	0.30

[a]The concentration of OTA < 2.0 ng/l considered as ½ LOQ = 1.0 ng/l.

Table 7. The results of OTA in human urine from different populations in Europe.

Country	n	n+%	Mean (ng/l)	Reference
Hungary	88	61	13.0	Fazekas et al., 2005
Portugal	60	70	27.0	Pena et al., 2006
Portugal	30	43	19.0	Manique et al., 2008
Portugal	155	92	18.0	Duarte et al., 2010
Spain	30	81	30.0	Manique et al., 2008
Spain	72	12.5	237.0	Coronel et al., 2011
Turkey	233	90	14.3*	Akdemir et al., 2010

n, numbers of samples; n+ %, percentage of positive samples; *ng/g creatinine.

OTA in human kidneys

OTA presence in human tissues seems to be direct and definite proof of human exposure to OTA although practicability of such measurements "*in vivo*" is obviously limited.

One series of such measurements took place in thirty samples of human kidneys (without an exact diagnosis) collected "post-mortem" within the Czech biological monitoring program in 2001. OTA was present in 40% of samples (Ostry et al., 2005).

Table 8. The results of OTA in human kidney in the CR and in Poland.

Country	Incidence n/n +[a] (%)	OTA min-max (μg/l)	Mean[a,b] (μg/kg)	Reference
CR (without exact dg.)	30/12 (40)	0.1–0.2	0.07	Ostry et al., 2005
Poland (tumor)	19/15 (78.9)	0.15–0.39	0.26	Grajewski et al., 2007

n, numbers of samples; n+, numbers of positive samples; (%), percentage of positive samples.
[a]In the Czech Republic – all findings ≥ 0.1 μg/ kg included as positive; [b]the OTA < 0.1 μg/kg given as 1/2 limit of quantification = 0.05 μg/kg.

In another study OTA was analyzed in human serum and kidney samples, collected in Poland in the Pomeranian region in 2005. The samples were from patients after nephrectomy (Grajewski et al., 2007). The results of OTA in human kidney can be seen in Table 8.

Conclusions

The human exposure to OTA can be monitored also through determination of specific biomarkers. At present, there is clear evidence that the whole world population is exposed to OTA. The persuasive data in this regard come from monitoring the OTA presence in biological materials, in particular blood and urine but also other tissues. This is so despite the fact that due to the ambiguities concerning the exact value of OTA biomarkers, these data cannot be yet interpreted with the absolute certainty. This is supported by the fact that the high OTA intake is not always reflected by high serum OTA levels and the highest serum levels are not related to the highest OTA consumption renders blood of less use as a biomarker of the very recent exposure. In this regard, although daytime OTA urine excretion lacks a correlation with serum levels, it seems to be more valuable tool than the urinary concentration.

OTA which has been simultaneously shown to be present in foodstuffs available in all over the world is now suspected to be a "complete" renal carcinogen. As such, it has been linked to different renal tumours. Nephropathy, sometimes accompanied with malignant tumors, has been also observed in persons with increased levels of OTA. In this regard, it is interesting to note that in some countries including the CR, there is a high prevalence of renal carcinoma whose cause remain unknown which leads to ask which role OTA could play. OTA has been also suspected of inducing tumours of testicles.

Harmful effects of OTA can be potentiated by possible synergistic action with other mycotoxins or contaminants. The existence of these synergies makes assessment of the exact role of OTA in human and animal pathologies far more complex, especially when it comes to long-term perspective.

Continuous monitoring of OTA presence is, thus imperative, and so is the adequate response in term of legislative measures and good agricultural practices.

Declaration of interest

This paper was prepared with financial support by the project (no. NT 12051-3/2011) from the Czech Ministry of Health (IGA MZ CR). The authors have no conflict of interests.

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