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Ochratoxin A in cereals, foodstuffs and human plasma

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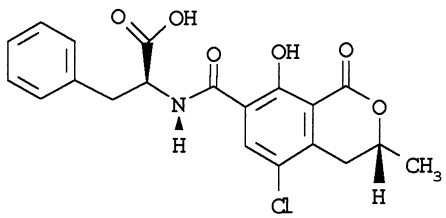
Abstract

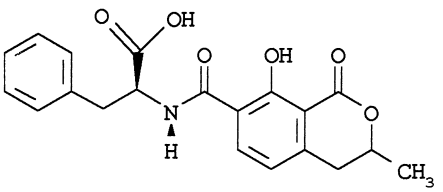
Aspergillus and *Penicillium* fungi contaminate cereals and foodstuffs, and can thus introduce ochratoxin A (OTA) into the food chain. In this work, five new isolates of Aspergillus: *A. albertensis*, *A. auricomus*, *A. wentii*, *A. fumigatus* and *A. versicolor,* were found to produce ochratoxin A. Data on the occurrence and the concentration levels of ochratoxin A in European food of vegetable and animal origin are reported. Furthermore, data on the concentration of ochratoxin A in blood of citizens of Western Europe are compared with those of some areas where Balkan endemic nephropathy is endemic. The results of the studies of Stoev and co-workers are reviewed and the possibility that OTA alone cannot be the cause of the Balkan endemic nephropathy is discussed.

Introduction

Ochratoxin A (OTA) is a mycotoxin produced by several species of *Aspergillus* and *Penicillium* fungi. These fungi are ubiquitous, occurring primarily in temperate climates. Their potential for the contamination of cereals and foodstuffs is widespread. The accumulation of OTA by *Aspergillus* and *Penicillium* is affected by the amount of inoculum, the substrate, water activity, moisture content, temperature, incubation time and by the species of the fungus itself. Ochratoxin A was isolated for the first time by van der Merwe et al. (1965) as a metabolite of *Aspergillus ochraceus.* Ochratoxin A is a dihydromethyl-isocoumarin (ochratoxin α) moiety linked via the 7-carboxy group, by an amidic bond, to a molecule of L-β-phenylalanine (Figure 1). Because of its chemical structure, the best method for the analysis of OTA is HPLC linked to a fluorescence detector. Confirmatory methods are (1) formation of the OTA-methylester by reaction of boron trifluoride in methanol; (2) degradation of OTA by carboxypeptidase with formation of ochratoxin α.



OCHRATOXIN A



OCHRATOXIN B

Figure 1. Molecular structures of ochratoxin A and B.

Biological activity of ochratoxin A

Kidneys are probably the target organ for OTA, where it causes nephrotoxic effects, and liver damage in

monogastric animals. Mycotoxic porcine nephropathy (MPN) was first recognised in Denmark where it was associated with the use of mouldy rye in feed, and in the Balkan States where human endemic nephropathy is a chronic fatal disease. Aside from nephrotoxicity, OTA has immunosuppressive properties resulting in higher susceptibility to infections, and it is teratogenic and carcinogenic. Furthermore, OTA inhibits protein synthesis by competing with phenylalanine in the phenylalanyl-tRNA synthase-catalysed reaction. The inhibition can be reversed by the presence of phenylalanine (Betina, 1989).

New ochratoxigenic Aspergillus *species*

Recently, Varga et al. (1996) reported three more *Aspergillus* species to be ochratoxigenic. They are: *A. albertensis*, *A. auricomus* and *A. wentii*. Besides producing OTA, all three strains produced ochratoxin B. High-performance thin-layer chromatography was used to analyse cleaned concentrated mycelial extracts. Ochratoxin A was identified under UV light (360 nm) as a bluish-green fluorescent spot with the same R_f value to that of an OTA reference standard. The fluorescence of OTA spots, of both the standard and the extracts, changed to deep blue on treatment of the chromatographic plate with $NaHCO_3$ (5% $NaHCO_3$ in 17% ethanol). The identity of OTA was confirmed by HPLC, by diode array detection and by an immunochemical test (ELISA). *Aspergillus albertensis* was isolated from Canadian cereals, *A. auricomus* from Australian peanuts and *A. wentii* from Indonesian soybeans. One of the three strains of *A. wentii* (IMI 017295), did not produce OTA, even though it had the same origin as that of a toxin-producing strain (ATCC 1023). It is possible that *A. wentii* ATCC 1023 had lost the ability to produce OTA during prolonged maintenance of the culture by periodic transfer on agar slants. Abarca et al. (1997) screened 176 isolates of *Aspergillus*, cultivated on sterilised corn, for their ability to produce OTA. The strains were isolated by the dilution plating technique during the course of a mycological study on animal mixed feeds and raw materials (corn, soybeans and peas). The strains were cultivated on yeast extract-sucrose broth and on sterilised corn. The cultures were extracted with chloroform and analysed by TLC and HPLC. The identity of OTA in samples was confirmed by preparing its methyl ester. One isolate of *A. fumigatus* and one of *A. versicolor* were able to produce this mycotoxin (Abarca et al., 1997). These two species had not previously been reported as ochratoxigenic fungi.

Occurrence of ochratoxin A in cereals and foodstuffs

Ochratoxin A is produced by *Penicillium* species in temperate or cold climates and by a number of *Aspergillus* species in warmer and tropical parts of the world. The best known species of *Aspergillus* that produces OTA in food is *A. alutaceus* (syn. *A. ochraceus*), but OTA is also produced by *A. sulphureus*, *A. sclerotium* or *A. melleus*, which, however, are not very often found in cereals and feeds (Madhyasta et al., 1990; Moss, 1996). *Aspergillus alutaceus* is more common on green coffee beans, cocoa beans, soybeans, peanuts, rice and corn (Kuiper-Goodman and Scott, 1989). *Penicillium verrucosum* is commonly associated with stored cereals and is frequently detected in northern Europe and Canada. Ochratoxin A has been detected in practically all cereals, including corn, barley, wheat, sorghum, rye, oats and rice, and its occurrence is related to climate and especially to harvest and post-harvest conditions. Animal feeds may be contaminated, sometimes at concentrations exceeding 5000 $\mu g\,kg^{-1}$ (Council for Agricultural Science and Technology Cast, 1989). Recently, OTA was detected at high concentrations in dried vine fruits originating from Greece and Turkey (Ministry of the Agriculture, Fisheries and Foods (MAFF), 1997). The concentrations of OTA in European food commodities have been reported by van Egmond and Speijers (1994) and by Pittet (1998). Some of their data are shown in Tables 1–3, while the occurrence of OTA in blood samples of healthy humans is reported in Table 4.

Discussion

The data reported on cereal samples analysed from 1989 to 1991 (Table 1) show rather high concentrations with maximum concentrations reaching the level of milligrams per kilo sample (2400–5410 $\mu g\,kg^{-1}$). The data concerning analyses of OTA in cereals produced during years 1995–1997 (Table 2) show an average incidence of 35%. The highest concentrations are considerably lower than those reported in Table 1. However, the data in Table 2 show that OTA was present in several commodities other than cereals. From the data shown in Table 3, it is also evident that OTA was

Table 1. Ochratoxin A in food commodities of vegetable origin in Europe (from van Egmond and Speijers, 1994)

Commodity	Country	Number of samples analysed	% contamination	Range of concentration ($\mu g\,kg^{-1}$)	Reference
Wheat	Yugoslavia[a]	130	8.5	14–135	Pavlovic et al. (1979)
Wheat	Denmark	194	37	0.8–37	Tholstrup and Rasmussen (1990)
Wheat (ecologically grown)	Denmark	36	46	1.2–21	Tholstrup and Rasmussen (1990)
Wheat	Poland	239	11.7	5–2400	Golinski et al. (1991)
Wheat	Germany	64	1.6	0.4[c]	Frank (1991)
Wheat[b]	Netherlands	38	15	0.1–4.2	van Egmond and Sizoo (1984)
Wheat bran	Germany	84	10.7	6.8[c]	Frank (1991)
Wheat bran	Denmark	57	10.5	5–20	Pedersen and Hansen (1981)
Wheat bran	Denmark	57	68	0.5–12	Tholstrup and Rasmussen (1990)
Wheat bran	Denmark	15	66	0.1–26	Tholstrup and Rasmussen (1990)
Wheat flour	Poland	137	19.7	3700[c]	Golinski et al. (1991)
Rye	Denmark	267	37	2.5–120	Tholstrup and Rasmussen (1990)
Rye (ecologically grown)	Denmark	53	81	0.7–120	Tholstrup and Rasmussen (1990)
Rye flour	Poland	78	26.9	5410[c]	Golinski et al. (1991)
Rye	Poland	228	27.2	5–2400	Golinski et al. (1991)
Rye	Germany	64	1.6	0.4[c]	Frank (1991)
Rye[b]	the Netherlands	14	36	0.1–16.8	van Egmond and Sizoo (1984)

[a]Area with endemic nephropathy. [b]Imported grain. [c]Average value.

Table 2. Recent data on the occurrence of ochratoxin A in foods (from Pittet, 1998)

Commodity	Country	Publication year	Number of samples	Incidence (%)	Range of concentration ($\mu g\,kg^{-1}$)
Barley	Germany	1997	39	31	0.1–2.7
Wheat	Denmark	1996	520	32	0.05–51
Rye	Denmark	1996	616	42	0.05–121
Oats	Denmark	1996	92	44	0.05–5.6
Wheat	Switzerland	1996	28	54	0.1–10.0
Wheat, barley	UK	1995	1061	4	1–33
Green coffee	Japan	1997	47	30	0.1–17.4
Green coffee	UK	1996	291	38	0.2–27.3
Cocoa powder	UK	1997	20	85	0.2–1.1
Dried vine fruits[a]	UK	1997	60	88	0.2–53.6
Wine	Germany	1996	144	42	0.01–7.0
Spices & herbs[b]	UK	1996	29	86	1.2–50.4

[a]Including raisins, currants and sultanas. [b]Including chilli powder, curry powder, tandoori, ginger, garlic and five spice powders.

present in animal feeds, since 39% of kidney pig samples contained the toxin. The highest concentration of OTA detected was of 240 $\mu g\,kg^{-1}$ tissue.

Since OTA is present in several food commodities of vegetable and animal origin, humans and animals can be exposed to OTA through the consumption of contaminated foodstuffs. Ochratoxin A has been detected in human blood and human milk samples and there has been an apparent association between the presence of OTA and Balkan endemic nephropathy. Ochratoxin A has also been associated with urinary tract tumours (Breitholtz-Emanuelsson et al., 1993; Miraglia et al., 1993; Creppy et al., 1993). In 1982, OTA was for the first time detected in blood of inhabitants of regions in Croatia where nephropathy is endemic (Hult et al., 1982). Blood samples, taken

Table 3. Ochratoxin A in food commodities of animal origin in Europe (from van Egmond and Speijers, 1994)

Commodity	Country	Number of samples analysed	% contamination	Range of concentration ($\mu g\,kg^{-1}$)	Reference
Kidney (pig)[a,d]	the Netherlands	46	71	0.2–2.0	van Egmond et al. (1984)
Kidney (pig)[d]	the Netherlands	6	100	0.2–1.0	
Kidney (pig)[a]	the Netherlands	29	7	0.2–0.4	van Egmond et al. (1984)
Kidney (pig)	the Netherlands	6	17	0.2–0.8	
Kidney (pig)[a,e]	the Netherlands	24	71	0.2–240	van Egmond et al. (1984)
Kidney (pig)	Hungary	122	39	2–100	Sandor et al. (1982)
Kidney (pig)	Sweden	129	25	2–104	Rutqvist et al. (1977)
Kidney (pig)	Sweden	90	27	2–88	Josefsson and Moller (1979)
Kidney (pig)	Poland	113	24	1–23	Golinski et al. (1984)
Kidney (pig)	Belgium	95	9.8	0.2 to >80	Rousseau and Van Peteghem (1989)
Kidney (pig)[a]	Czechoslovakia	96	79.2	1–20	Fukal and Marek (1991)
Kidney (pig)	Czechoslovakia	63	1.6	1–5	Fukal and Marek (1991)
Kidney (chicken)[c]	Denmark	—	—	19[b]	Krogh et al. (1976)
Kidney (chicken)[c]	Poland	—	—	1–6	Juskiewicz et al. (1982)
Kidney (quail)[c]	Poland	—	—	80–110	Piskorska-Pliszczinska and Juszikiewicz (1990)
Liver[c]	Germany	—	—	9.1–1.8	Bauer (1988)
Egg (chicken)[c]	Poland	—	—	0.7–13	Juszkiewicz et al. (1982)

[a]From animals with nephropathy. [b]Maximum level detected. [c]Accumulation study. [d]Pigs used for breeding. [e]Originated from Denmark.

from people living in regions where Balkan endemic nephropathy was endemic and from regions where the syndrome was absent, have been investigated for the last ten years. The results of those studies show that OTA was present, not only in blood of people living in regions where nephropathy is endemic, but also in blood of people from regions with no records of nephropathy (Radic et al., 1997). Recently, a new investigation of the occurrence of OTA in human blood of healthy inhabitants living in different regions of Croatia has been carried out by Peraica et al. (1999). The blood samples of the inhabitants of five cities were analysed for the presence of OTA. Positive samples were confirmed by formation of the methyl ester of the toxin. The mean concentration in all samples was $0.39\,ng\,ml^{-1}$ plasma. A concentration of OTA above $0.2\,ng\,ml^{-1}$ (detection limit) was found in 148 of 249 samples (59.4%). In most of the samples, the OTA concentration was between 0.2 and $1.0\,ng\,ml^{-1}$ plasma. The highest value detected was $15.9\,ng\,ml^{-1}$. Considering the data in Table 4, which reports results from investigations carried out on human blood in several European countries, 53% of samples were positive and the average concentration of OTA was $0.5\,ng\,ml^{-1}$. The average value of OTA concentration for the Croatian samples was $0.39\,ng\,ml^{-1}$. According to Peraica et al. (1999), the mean daily intake of OTA in Croatia was $0.53\,ng\,kg^{-1}$ bw/day, which is below the European mean daily intake ($0.9\,ng\,kg^{-1}$ bw/day) (Hoehler, 1998). These results suggest that there is a need to reduce the contamination in cereal-based foodstuffs, and hence decrease the exposure of humans and animals to OTA, not only in areas where Balkan endemic nephropathy occurs, but also in other European countries where the syndrome is absent.

The key question is: if the mean daily intake of OTA in Croatia is lower than that of the Western European countries, then why does the endemic nephropathy exists only in Balkan countries? The answer could be found, perhaps, in the work of Stoev et al. (2001). In that study naturally-occurring porcine nephropathy in Bulgaria was characterised by vascular lesions, renal haemorrhages and enlargement of the renal nodes. These symptoms differ from those of classic MPN, as described in Denmark (Krogh, 1976). Furthermore, the incidence of MPN is higher in Bulgaria than in Denmark by one to two orders of magnitude, even though the overall concentrations of OTA in animal feed (100–200 ppb) and in blood samples from affected animals are substantially lower than those (1–2 ppm) required to reproduce classical MPN of a severity similar to that observed in Bulgaria (Stoev et al., 1998,

Table 4. The occurrence of ochratoxin A in blood samples of healthy humans[a] (from Peraica et al., 1999)

Country	Collection period	Number of positive/analysed (%)	Mean concentration (ng ml^{-1})	Range of concentration (ng ml^{-1})	Reference
Bulgaria	1984–1990	9/125 (7)	0.2	1.0-10.0	Petkova-Bocharova and Castegnaro (1991)
Canada	1989–1990	45/90 (50)	0.45	0.20–35.33	Frochlich et al. (1991)
	1994	144/144 (100)	0.88	0.29–2.37	Scott et al. (1998)
Czechoslovakia	1990	35/143 (24)	0.14	0.1–1.3	Fukal and Reisnerova (1990)
Czech Republic	1994	734/809 (91)	0.23	0.1–13.7	Malir et al. (1998)
	1995	404/413 (98)	0.24	0.1-1.9	
Croatia	1997	148/249 (59)	0.39	0.2–15.9	Peraica et al. (1999)
Denmark	1986–1988	78/144 (54)	1.8	0.1–13.2	Hald (1991)
France	1991–1992				
Alsace		97/500 (19)		0.1–11.8	Creppy et al. (1993)
Aquitaine		385/2055 (19)		0.1–16.0	
Rhone-Alpe		75/515 (15)		0.1–4.3	
Germany	1977	84/165 (51)	0.79	0.1–14.4	Bauer and Gareis (1987)
	1985	89/141 (68)	0.42	0.1–1.8	Bauer and Gareis (1987)
	1988	142/208 (68)	0.75	0.1–8.0	Hadlock (1993)
Hungary	1995	291/355 (82)		0.2–10.0	Solti et al. (1997)
	1997	213/277 (77)		0.1–1.4	Tapai et al. (1997)
Italy	1992	65/65 (100)	0.53	0.1–2.0	Breitholtz-Emanuelsson et al. (1994)
Japan, Tokyo	1992–1996	156/184 (85)	0.068	0.004–0.278	Ueno et al.1998
Poland	1983–1984	25/397 (6)	0.21	1.0–13.0	Golinski (1987)
	1984–1985	52/668 (8)	0.31	1.0–40.0	
Spain	1996–1998	40/75 (53)	0.71	0.5–4.0	Jimenez et al. (1998)
Switzerland					
North of the Alps		251/252 (100)		0.06–2.14	Zimmerli and Dick (1995)
South of the Alps		116/116 (100)		0.11–6.02	
Sweden	1989				Breitholtz-Emanuelsson et al. (1991)
Visby		29/99 (29)	0.26	0.3–7.0	
Uppsala		3/99 (3)	0.02	0.3–0.8	
Ostersund		6/99 (6)	0.03	0.3–0.8	

[a]Mean concentration is calculated in all samples, and concentration range only in positive samples.

1998a,b). It therefore, appears that Bulgarian MPN cannot be explained by the concentration of OTA alone, but it may be caused by the consumption of multiple mycotoxins. Penicillic acid and other secondary metabolites are produced by fungi of the *A. ochraceus* group, which are the major OTA producers in warm areas, and by *P. verrucosum* which is the main ochratoxigenic fungus in colder areas, such as Scandinavia (Ciegler, 1972; Marquardt and Frolich, 1992). It seems that the penicillic acid has an inhibitory effect on carboxypeptidase activity, an enzyme that is involved in the primary detoxification of OTA in the intestinal tract. In this way penicillic acid could impair the natural detoxification of OTA, thus enhancing its toxicity (Parker et al., 1982). According to the study of Stoev et al. (2001), the synergistic effects between OTA and penicillic acid and possibly other fungal metabolites, may be responsible for the Balkan endemic nephropathy in Bulgaria, which is associated with relatively low levels of OTA in feed ($207.10 \pm 65.14\ \mu g\ kg^{-1}$ and $114.06 \pm 35.79\ \mu g\ kg^{-1}$, Stoev et al., 1998, 1998b). Clearly the interactions between different mycotoxins deserve further study.

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