ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXIII, N 1, 14 SECTIO D 2008

[1]Medical Chemistry Department, [2]Department of Rehabilitation, Physioteraphy and Balneotherapy Medical University of Lublin, [3]Centre of Occupational Medicine of Gniezno

MAŁGORZATA SZTANKE[1], KAZIMIERZ PASTERNAK[1], WŁODZIMIERZ BULIKOWSKI[2], MAYSAM BACHER[1], KATARZYNA KUCHARSKA[1], WOJCIECH JANKOWSKI[3]

The influence of a high dose of ochratoxin A on some elements concentrations in skins with the fur of female rats

The toxic metabolites of fungi called mycotoxins are highly dangerous and potentially hazardous to the health of humans and animals (1).

Ochratoxin A (OTA, L-phenylalanine-N-[(5-chloro-3,4-dihydro-8-hydroxy-3-methyl-1-oxo-1*H*--2-benzopyren-7-yl)carbonyl]-(R)-isocoumarin) (Fig. 1) is one of the most common and toxicologically important naturally occurring secondary metabolites of two fungal genera: *Aspergillus* and *Penicillium* (1–4). OTA was first isolated in 1965 from *Aspergillus ochraceus* (5). The production of this mycotoxin is dependent on different factors such as temperature, water activity (a_w) and medium composition, which affect the physiology of fungal products (4). At a moderate temperature the main producer of OTA is *Aspergillus ochraceus*, whereas in cool and temperate regions OTA is mainly produced by *Penicillium verrucosum* or *Penicillium nordicum;* however, in tropical and semitropical regions – by *Aspergillus carbonarius* (4, 6).

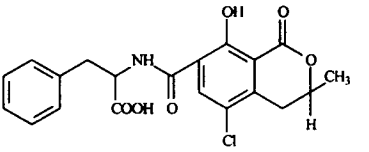

Fig. 1. Chemical structure of ochratoxin A

This ubiquitous mycotoxin is found as a frequent contaminant of a large variety of food, feed and beverage. Contamination of OTA takes place in cereals such as wheat, maize, rye, barley and oats. It also occurs in peanuts, coffee, beer, wine, bread, rice and dried fruits. OTA is widely found in improperly stored food products, particularly in northern climates of Europe and North America. This mycotoxin was also detected in animal and human blood and human milk samples in countries throughout the world (5, 7, 8, 9).

OTA has a relative chemical stability during industrial processing and a long serum half-life of 35 days (20–50 days) in humans, 72–120 h in pigs and 77 h in preruminant calves (1, 5, 7). Due to OTA occurrence in food and feedstuffs of plants and animal origin and its slow elimination and tissue-specific retention, an acceptable daily intake of this mycotoxin in humans was suggested by

several international committees (1, 6, 10). In 1991 it was established at a tolerable daily intake of 16 ng·kg^{-1} of b.w., which was reduced to 14 ng·kg^{-1} of b.w. in 1995. Currently an acceptable level for daily exposure of humans is up to 5 ng·kg^{-1} of b.w. (5, 11).

OTA has been linked to the genesis of several states in both animals and humans. This mycotoxin has been proposed as the etiologic agent in the development of nephropathies (Balkan Endemic Nephropathy – BEN and Chronic Intestitial Nephropathy – CIN) and might be implicated in the onset of urinary tract tumours, although other factor may be involved (2, 6, 9, 11, 12). OTA also has immunotoxic, hepatotoxic, teratogenic, neurotoxic, genotoxic, cytotoxic, mutagenic and carcinogenic properties and is classified as a possible human carcinogen (group 2B) (1, 10–13).

Based on the literature data, the toxicity of ochratoxin A may be a result of protein synthesis inhibition, ATP synthesis inhibition, membrane peroxidation promotion, calcium homeostasis disruption and DNA damage by this mycotoxin (3, 4, 7).

The concentrations of macro- and microelements in skins with the fur of animals can reflect these bioelement content in the organism (14) and therefore, the purpose of this study was to determine the magnesium, calcium, zinc and copper concentrations in skins with the fur of female rats exposed to a high dose of OTA.

MATERIAL AND METHODS

The experiment was carried out on Wistar female rats, weighing 150–170 g. The animals were divided into 4 groups, 6 rats each. Groups I and II of animals were controls. Group I received redistilled drinking water and group II – magnesium chloride ($MgCl_2$) in a dose of 5 mg·kg^{-1} of b.w. The other groups of animals (groups III and IV) obtained intragastrically OTA in a dose of 1 mg·kg^{-1} of b.w., whereas group III additionally received magnesium chloride ($MgCl_2$) in a dose of 5 mg·kg^{-1} of b.w. All animals were fed a standard diet (LSM dry food) and drank redistilled water. Food and water were given *ad libitum*. The experiment was conducted for 30 days and then animals were sacrificed under ketamine anaesthesia (100 μg·kg^{-1} of b.w. via intraperitoneal injection) and the skins with the fur were collected for further examinations. The skins were kept at the temperature of 0–4°C and then dried at the temperature of 22°C with relative humidity of 56% for 24 h. Next the skins were rinsed in Wacker drums in redistilled water at the temperature of 25°C and dried for 24 h at the temperature of 25°C against air flow of 0.2-0.8 m/s. After drying the skins were cut into pieces and incinerated at the temperature of 550°C for 2 h. The obtained white ash was dissolved in 25 ml of 2M HCl and copper concentrations were assayed by AAS method (15) with the usage of atomic absorption spectrophotometer AAS-3. The magnesium and calcium concentrations were measured in the presence of strontium chloride correction buffer. The wavelengths were 285.2 nm and 422.7 nm, whereas the slit widths were 0.2 mm and 0.5 mm, respectively. However, for zinc and copper, the wavelengths were 213.9 nm and 324.8 nm, whereas the slit widths were 0.2 mm and 0.3 mm, respectively. The obtained results were submitted to statistical analysis with the Cochran-Cox test. The differences at $p < 0.05$ were considered as statistically significant. The study was approved by The Local Ethical Commission of the Medical University of Lublin, acceptance 271/2001.

RESULTS

The application of OTA at a dose of 1 mg·kg^{-1} of b.w. for 30 days to female rats influenced some elements concentrations in skins with the fur. These changes are shown in Table 1. It was noticed that magnesium, calcium, zinc and copper concentrations in skins with the fur of female rats exposed

to OTA were increased as compared to control groups (groups I and II). These alterations were statistically significant in the case of magnesium, calcium and copper levels, but not significant in the case of zinc concentrations. The administration of OTA together with magnesium chloride at a dose of 5 mg·kg^{-1} of b.w. to animals (group IV) also caused an increase of tested element concentrations in comparison to group II, but these changes were less important, except magnesium, than in a group exposed to OTA only (group III).

Table 1. Magnesium, calcium, zinc and copper concentrations in skins with the fur of female rats receiving OTA

Groups	Concentration (μg·g^{-1} of skin)							
	Mg		Ca		Zn		Cu	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Control (Group I)	261.1	7.2	182.8	10.3	164.7	7.2	2.18	0.33
• $MgCl_2$ (Group II)	286.6	8.1	e.5	8.1	167.8	9.0	3.91	0.39
OTA (Group III)	306.9*	14.4	335.6*	13.3	189.3	10.6	5.85*	0.46
OTA + $MgCl_2$ (Group IV)	321.3**	10.1	216.4**	6.4	170.2	8.0	4.96**	0.44

* Statistical significance vs. group I ($p<0.01$), ** Statistical significance vs. group II ($p<0.01$)
$\bar{x}$ – mean value for an animal' group, SD – standard deviation

DISCUSSION

The literature data include some reports concerning OTA toxicity (4, 5, 9, 10, 11, 13). Only few findings that show mutual interactions between bioelements and this mycotoxin are available in the literature (14, 16–23). A correct balance of macro- and microelements is needed for a normal function of a living organism. Disturbances in the metabolism of these elements may be induced by administration of various compounds, i.e. mycotoxins (OTA). Under our experimental conditions, the application of OTA at a dose of 1 mg·kg^{-1} of b.w. influenced the tested bioelement homeostasis. The concentrations of magnesium, calcium, zinc and copper in skins with the fur of female rats exposed to this mycotoxin were significantly increased, except zinc – where these alterations were rather slight, in comparison to the control groups. Moreover, it was shown that the supplementation of magnesium chloride to rats exposed to OTA also raised all the above-mentioned element concentrations in skins with the fur, but to a smaller extent than OTA only, except magnesium.

There are some studies which demonstrated the influence of OTA on various element concentrations (14, 16–19, 21–23), as well as on antioxidant defence system (24).

Medjugorac-Popovski et al. (21) investigated the concurrent effects of this mycotoxin on iron, zinc and copper concentrations in rats. They confirmed that a high dose of OTA (1 mg·kg^{-1} of b.w.) decreased zinc and copper concentrations in kidneys and increased zinc concentration in urine.

Some authors (20, 23) showed that OTA disrupts calcium homeostasis. Rahimtula and Chong (23) observed that a single high dose or multiple lower doses of OTA administered to rats resulted in an increase of renal endoplasmic reticulum calcium pump activity, and that this mycotoxin also decreased renal mitochondrial respiration and calcium uptake. These authors suggested that it might lead to an increase in cytosolic calcium level. Moreover, the increase in microsomal calcium uptake activity may be attempted to restore calcium homeostasis. Repeated moderate doses of OTA led

to the eventual decrease in microsomal calcium pump activity, and this could lead to even higher cytosolic calcium levels. Khan et al. (20) demonstrated that disruption of microsomal calcium homeostasis by an impairment of the endoplasmic reticulum membrane may be caused by enhanced lipid peroxidation. It was previously reported (25, 26) that OTA increased malonyldialdehyde (MDA) formation, a biomarker of lipid peroxidation *in vitro* upon incubation with rat liver microsomes in the presence of NADPH.

The study carried out on male rats exposed to a high dose of OTA (1 $mg \cdot kg^{-1}$ of b.w.) demonstrated that this mycotoxin increased calcium concentrations in rats' skins as compared to the control (19). Similar results were obtained during administration of a low dose of this mycotoxin (25 $\mu g \cdot kg^{-1}$ of b.w.) to rats (17). These observations confirm the results of current findings.

Previously conducted investigation results proved that OTA influenced various micro- and macroelement concentrations in rats' skins. The application of a high dose of this mycotoxin (1 $mg \cdot kg^{-1}$ of b.w.) to male rats for 30 days increased zinc and copper levels in the skins of these animals (14, 22). Similar results were observed in the case of copper in rats exposed to a medium (250 $\mu g \cdot kg^{-1}$ of b.w.) dose of OTA; however, in pregnant female rats increases in copper concentrations and decreases in zinc concentrations were noticed as compared to the control (18). It is possible that these alterations were connected with oxidative damage and stress response to OTA exposure in rats. It is known that zinc and copper play a very important role in oxidative processes in a living organism, so these changes can result from their antioxidative property (2). However, in the case of magnesium, OTA at a dose of 50 $\mu g \cdot kg^{-1}$ of b.w. increased concentrations of this macroelement in male rats' skins (17).

In the present study, the supplementation of magnesium chloride to female rats exposed to OTA caused a rise of tested element concentrations, but to a smaller extent than OTA only, except magnesium concentration. Similar results were noticed in other authors' findings (14, 16, 17, 19, 22). Undoubtedly, OTA influenced some bioelement concentrations in a living organism, which was confirmed in some studies.

CONCLUSIONS

1. The application of a high dose (1 $mg \cdot kg^{-1}$ of b.w.) of OTA for 30 days to female rats influenced some element concentrations in skins with the fur.

2. Statistically important increases of magnesium, calcium and copper concentrations were noticed in rats exposed to OTA as compared to control groups. However, in the case of zinc levels these changes were not statistically significant.

3. The supplementation of magnesium chloride at a dose of 5 $mg \cdot kg^{-1}$ of b.w. to exposed to OTA rats raised magnesium, calcium, zinc and copper concentrations in skins with the fur, but to a smaller extent than OTA only, except magnesium concentration.

REFERENCES

1. Solti L. et al.: Analysis of serum and seminal plasma after feeding ochratoxin a with breeding boars. Anim. Reprod. Sci., 56, 123, 1999.
2. Gautier J.-C. et al.: Oxidative damage and stress response from ochratoxin A exposure in rats. Free Radic. Biol. Med., 30, 10, 1089, 2001.
3. Hoehler D. et al.: Free radical generation as induced by ochratoxin A and its analogs in bacteria (*Bacillus brevis*). J. Biol. Chem., 271, 44, 27388, 1996.

4. Ringot D. et al.: Toxicokinetics and toxicodynamics of ochratoxin A, an update. Chem.-Biol. Int., 159, 18, 2006.
5. Al-Anati L., Petzinger E.: Immunotoxic activity of ochratoxin A. J. Vet. Pharmacol. Therap., 29, 79, 2006.
6. Dortant P. M. et al.: Age-related differences in toxicity of ochratoxin A in female rats. Food Chem. Toxicol., 39, 55, 2001.
7. Creppy E. E., Baudrimont I.. et al.: How aspartame prevents the toxicity of ochratoxin A. J. Toxicol. Sci., 23, suppl. 2, 165, 1998.
8. Joosten H.M.L.J. et al.: Production of ochratoxin A by *Aspergillus carbonarius* on coffee cherries. Int. J. Food Microbiol., 65, 39, 2001.
9. Manderville R. A. et al.: Stoichiometric preference in copper-promoted oxidative DNA damage by ochratoxin A. J. Inorg. Biochem., 95, 87, 2003.
10. Mally A. et al.: Biotransformation and nephrotoxicity of ochratoxin B in rats. Toxicol. Appl. Pharmacol., 206, 43, 2005.
11. Alvarez L. et al.: Immunotoxic effects of ochratoxin A in Wistar rats after oral administration. Food Chem. Toxicol., 42, 825, 2004.
12. O'Brien E., Dietrich D. R.: Ochratoxin A: the continuing enigma. Crit. Rev. Toxicol., 35, 1, 33, 2005.
13. Marin-Kuan M. et al.: A toxicogenomics approach to identify new plausible epigenetic mechanisms of ochratoxin A carcinogenicity in rat. Toxicol. Sci., 89, 1, 120, 2005.
14. Pasternak K. et al.: The influence of a high dose of ochratoxin A on copper concentrations in rats' skins. Pol. J. Environ. Stud., 15, 2b, 559, 2006.
15. Marczenko Z., Balcerzak M.: Spektrofotometryczne metody oznaczania w analizie nieorganicznej. PWN Warszawa 1998.
16. Bulikowski W. et al.: Stężenie miedzi w skórze zwierząt przy małym narażeniu na ochratoksynę A. Probl. Hig. Prac. 13, 165, 2005.
17. Bulikowski W. et al.: Wpływ ochratoksyny A na stężenie wapnia i magnezu w skórze zwierząt doświadczalnych. Materiały II Międzynarodowej Konferencji „Obieg pierwiastków w przyrodzie" – Warszawa 27–29.10.1997. Instytut Ochrony Środowiska, Warszawa 1997.
18. Bulikowski W. et al.: Wpływ ochratoksyny na stężenie cynku i miedzi w skórze zwierząt doświadczalnych w okresie ciąży i porodu. Biul. Magnezol., 4 (2), 293, 1994.
19. Bulikowski W. et al.: Zawartość wapnia w skórze samców szczurzych eksponowanych na wysokie stężenia ochratoksyny A (OTA). Med. Pr., 56, 5, 363, 2005.
20. Khan S. et al.: Perturbation of liver microsomal calcium homeostasis by ochratoxin A. Biochem. Pharmacol., 38, 1, 67, 1989.
21. Medjugorac-Popovski M. et al.: Concentrations of trace elements in kidney and urine in ochratoxin A treated rats. Toxicol. Lett., 88, suppl. 1, 93, 1996.
22. Pasternak K. et al.: Zinc concentration in skins of rats exposured on a high dose of ochratoxin A. Pol. J. Environ. Stud., 15, 2b, 562, 2006.
23. Rahimtula A. D., Chong X.: Alterations in calcium homeostasis as a possible cause of ochratoxin A nephrotoxicity. IARC Sci. Publ., 115, 207, 1991.
24. Szpetnar M. et al.: Wpływ magnezu na aktywność enzymów przeciwutleniających w tkankach szczurów intoksykowanych ochratoksyną A. Bromat. Chem. Toksykol., 34, 1, 13, 2001.
25. Omar R. F. et al.: Mechanism of ochratoxin a stimulated lipid peroxidation. Biochem. Pharmacol., 40, 1183, 1990.
26. Rahimtula A. D. et al.: Lipid peroxidation as a possible cause of ochratoxin A toxicity. Biochem. Pharmacol., 37, 4469, 1988.

SUMMARY

Ochratoxin A (OTA) is one of the most dangerous mycotoxins, which causes toxic effects in living organisms. It was shown to be nephrotoxic, hepatotoxic, teratogenic, immunotoxic to several species of animals and to cause nephropathies and urothelial tumours in humans. The purpose of this study was to determine the influence of a high dose of OTA on magnesium, calcium, zinc and copper concentrations in skins with the fur of female rats. It has been shown that the application of this mycotoxin for 30 days to rats caused an increase in these element concentrations in the skins of animals. Moreover, it was demonstrated that the supplementation of magnesium chloride during exposure to ochratoxin A also raised all above-mentioned elements concentrations in skins, but to a smaller extent than OTA only, except magnesium concentration.

Wpływ wysokiej dawki ochratoksyny A na stężenia wybranych pierwiastków w skórach szczurzyc

Ochratoksyna A (OTA) jest jedną z najbardziej niebezpiecznych mykotoksyn, mających toksyczny wpływ na organizmy żywe. U wielu gatunków zwierząt wykazuje ona działanie nefrotoksyczne, hepatotoksyczne, teratogenne i immunotoksyczne, a także jest uważana za przyczynę nefropatii i guzów układu moczowego u ludzi. Celem pracy było określenie wpływu dużej dawki OTA na stężenie magnezu, wapnia, cynku i miedzi w skórach szczurzyc. Wykazano, że 30-dniowe podawanie tej mykotoksyny powodowało podwyższenie stężeń badanych pierwiastków w skórach zwierząt. Ponadto stwierdzono, że suplementacja chlorku magnezu podczas narażenia na tę mykotoksynę powodowała wzrost stężeń wymienionych pierwiastków w skórach, jednakże w mniejszym stopniu niż sama ochratoksyna A, poza stężeniem magnezu.