

Ochratoxin and Ochratoxicosis

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INTRODUCTION

Ochratoxins are metabolites of *Aspergillus*, *Fusarium*, and *Penicillium* species. The most commonly implicated species are *A. ochraceus* and *ostianus*, *P. viridicatum*, *griseofulvum* and possibly *solitum*.¹ Recent evidence shows that these mycotoxins are present in a variety of foods (cereals, pork, poultry, coffee, beer, wine, grape juice and milk. Analyses of these food products demonstrated that ochratoxins are also produced by *P. verucosum* and *A. niger* and *carbonarius*.^{2,3}

There are three generally recognized ochratoxins, designated A, B and C. Ochratoxin A (OTA) is chlorinated and is the most toxic, followed by OTB and OTC. Chemically they are described as 3,4-dihydro-methylisocoumarin derivative linked with an amide bond to the amino group of L-phenylalanine (Fig. 1)⁴

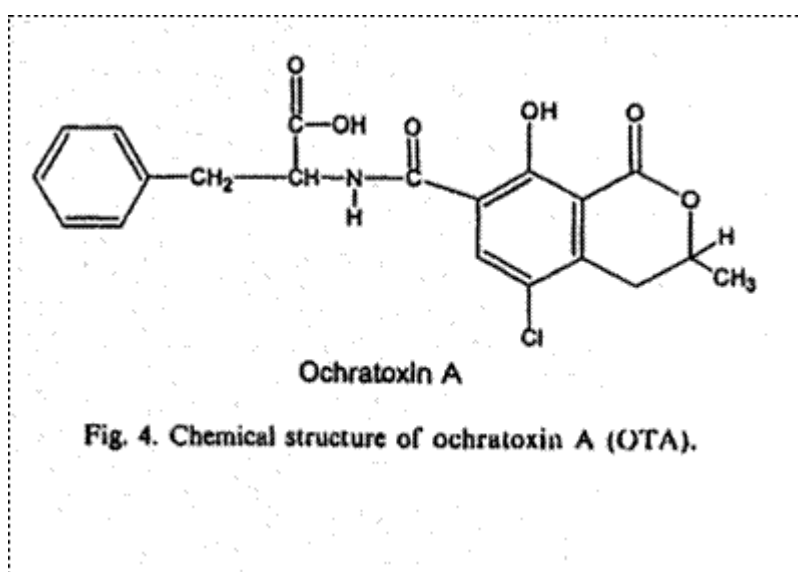


Figure 1 Chemical Structure of Ochratoxin A (OTA), adapted from Huessin et al., 2001, ref. 4

The role and risk assessment of OTA in animal and human disease has been reviewed. The estimated tolerable dosage in humans was estimated at 0.2 to 4.2 ng/kg body weight based upon NTP carcinogenicity study in rats. OTA is mutagenic, immunosuppressive and teratogenic in several species of animals. Its target organs are the kidneys (nephropathy) and the developing nervous system.^{5,6} Following intravenous administration OTA is eliminated with a half-life from body in vervet monkeys in 19-21 days.⁷ There is no reason to suspect that the elimination half-life would be significantly different in humans. OTA has been detected in human blood and milk. Other than nephropathy and urinary tumors, the evidence for human toxicology is scant. The toxicology of OTA will be briefly reviewed.

CARCINOGENESIS

There is inadequate evidence that OTA is carcinogenic in humans. There is sufficient evidence in experimental animals. Overall OTA is possibly carcinogenic to humans and is a Group 2B carcinogen.

Research Animals: Oral administration of OTA produced tubular cell carcinomas of the kidneys in male and female rats, and fibro adenomas of the mammary gland in the females.¹

Administration of OTA in the diet of ddy mice produced tumors of kidneys: solid renal tumors and cystic adenomas. Also, hepatic-tumors and hyperplastic liver nodules were found and unspecified lung tumors. In addition, solid renal-cell tumors occurred in ddd mice fed OTA in their diet.¹

Male and Female Fischer rats given oral doses of OTA had does related increased in kidney tumors: renal-cell adenomas, and renal-cell adenoma-carcinomas. Metastasis of the renal cell tumors occurred in 17 males and one female.¹

Humans: Balkan endemic neuropathy (BEN) associated with OTA occurs in Europe (Bulgaria, Croatia, Turkey, Egypt, and Yugoslavia) where OTA is relatively high in the diet. Individuals with BEN were surveyed for the presence of urinary tract tumors. The incidence of tumors in the urinary system was elevated in both men and women. Furthermore, the observations suggested that individuals with urinary tract tumors had elevated levels of OTA in the blood and urine. Approximately one-third of patients dying from BEN have papillomas and/or carcinomas of the renal pelvis, ureter or bladder.^{1,8-12}

Recently, it has been suggested that OTA can cause testicular cancer in humans.¹³ The hypothesis that consumption of foods contaminated with OTA causes testicular cancer was tested. The incidence of rates of testicular cancer in 20 countries was significantly correlated with the per-capita consumption of coffee and pig meat. Scwhartz concluded: "*Thus, OTA is a biologically plausible cause of testicular cancer. Future epidemiologic*

f OTA-containing foods such as cereals, pork products, milk, and coffee by mothers and their male children."

TERATOGENESIS and MUTAGENESIS

OTA crosses the placenta and is also transferred to newborn rats and mice via lactation.¹⁴ In addition, OTA-DNA adducts are formed in liver, kidney and other tissues of the progeny.^{15,16} This is significant in light of the fact that OTA causes birth defects in rodents. In mice damage to the neural plates and folds, mid-brain and forebrain was reported in one study while a second one showed cell death in the telencephalon.¹ Other abnormalities included necrosis of the brain (mice);¹ fetal resorption and visceral and skeletal defects (rats, mice and hamster);^{1,17} craniofacial (exencephaly, midfacial and lip clefts, hypotelorism and synophthalmia and body wall malformations in mice;¹⁸ and, a reduction of synapses per neuron in the somatosensory cortex of mice.¹⁹ Finally, prenatal exposure of rats results in suppressed lymphocyte mitogenic response to lipopolysaccharide and Con A that lasts through at least 13 weeks of age.²⁰ Thus, sufficient

experimental evidence exists in the scientific literature to classify OTA as a teratogen, affecting both the nervous system, skeletal structures and immune system of research animals.

IMMUNOSUPPRESSION

OTA causes immunosuppression following prenatal (see above), postnatal and adult-life exposures. These effects include reduced phagocytosis and lymphocyte markers (pig weaners),²¹ and increased susceptibility to bacterial infections and delayed response to immunization in piglets.²² In adult mice NK cell activity is suppressed by OTA.²³

Purified human lymphocyte populations and subpopulations are adversely affected by OTA in vitro.²⁴ Both IL-2 production and IL-2 receptor expression on activated T cells are severely impaired by OTA and not OTB. The inhibitory action of OTA is reversed in the presence of OTB. Further, B cells do not respond to polyclonal activators following a brief exposure to OTA. The authors suggest that the toxin causes immunosuppression through interference with essential processes of cell metabolism (see mitochondria below) irrespective of lymphocyte population or subpopulation

MITOCHONDRIA

Several lines of experimental observations demonstrate that OTA effects mitochondrial function and causes mitochondrial damage. The reader is referred to Wallace²⁵ for background information on mitochondrial DNA in aging and disease.

In chicks and quail OTA causes pathological changes in the ultra structure of mitochondria in proximal convoluted tubules and glomeruli of kidneys and liver. These changes include abnormal shapes, enlarged mitochondrial matrix, and excessive lipid droplets.²⁶⁻²⁸

OTA causes oxidative stress and production of free radicals in rat hepatocytes and proximal tubules of the kidneys. Lipid peroxidation preceded cell death in cells of the proximal tubules.^{29, 30}

OTA is a noncompetitive inhibitor of both succinate-cytochrome c reductase and succinate dehydrogenase. It impairs mitochondrial respiration and oxidative phosphorylation through impairment of the mitochondrial membrane and by inhibition of succinate-supported electron transfer activities of the respiratory chain.³¹ It also inhibits glutamate/malate substrate respiration of Site I and causes lipid peroxidation leading to cell death.³² Another mechanism appears to be the activation of mitochondrial NHE interfering with Ca²⁺ homeostasis. This induces extracellular acidification leading to cell death in renal proximal tubules.^{33,34}

DNA, PROTEIN and RNA

OTA is mutagenic and carcinogenic.¹ It causes DNA single-stranded breaks and DNA adducts in the DNA of spleen, liver and kidney in OTA treated mice.^{35,36}

OTA inhibits bacterial, yeast and liver phenylalanyl-tRNA synthetases. The inhibition is competitive to phenylalanine and is reversed by an excess of this amino acid. It also inhibits phenylalanine hydroxylase and lowers the concentration of phosphoenolpyruvate carboxykinase.

It appears that an inhibition of protein and RNA synthesis is the end result of these toxic effects.³⁷ Inhibition of protein and RNA synthesis is considered one of the toxic effects of OTA.¹

APOPTOSIS

OTA induces apoptosis (programmed cell death) in a variety of cell types in vivo and in vitro. The mechanisms include caspase 3 activation, mitogen-activated protein kinases (MAPK) family, and c-jun amino-terminal kinase (JNK). The apoptosis is also mediated through cellular processes involved in the degradation of DNA. Finally, the mechanisms leading to cell death may be inhibited by various antioxidants.³⁸⁻⁴¹

BALKAN ENDEMIC NEPHROPATHY

OTA is nephrotoxic in all animals studied and has been implicated in the etiology of Balkan endemic nephropathy (BEN).¹ The clinical picture of BEN is that of a slowly progressing tubulo-interstitial chronic nephritis and urethral tumors are frequent, occurring in 2-47 % of cases.⁴² The proximal tubule cells are the primary target for OTA toxicity. BEN is an end-stage renal disease.

Epidemiological investigations have shown that BEN and dietary exposure are associated, leading to the conclusion that OTA is one of the causative agents in the

and the identification of DNA-ochratoxin A adducts in urinary tract tumors in patients from areas with BEN add support to this conclusion.

CONCLUSIONS

OTA is a mycotoxin produced by species of *Aspergillus*, *Fusarium* and *Penicillium*. It is found in many food crops, including cereals, coffee, cocoa and dried vine fruits. OTA is mutagenic, carcinogenic, teratogenic and immunosuppressive in a variety of animal species. It has been implicated in the etiology of BEN and urinary tract tumors in humans. It is a mitochondrial poison causing mitochondrial damage, oxidative burst, lipid peroxidation and interferes with oxidative phosphorylation. In addition, OTA increases apoptosis in several cell types.

The UK's Joint Expert Committee on Food Additives has set a provisional tolerable dietary intake (TDI) of 0.2 mg/kg body weight per week. OTA has been found in human and cow milk samples in European countries.¹ In Norway the concentrations found in the human and cow milk were sufficient to suggest that the TDI of 5 ng/kg body/day would be exceeded in small children who consume large quantities of milk.⁴⁸⁻⁵⁰

Finally, airborne exposure to OTA can occur, adding to the daily intake of the mycotoxin via the respiratory tract. Thus, OTA has been demonstrated in dust and fungal conidia in samples taken from cowsheds. Furthermore, OTA was detected in dust samples from the heating ducts of a house where animals showed signs of ochratoxicosis.^{51,52}

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