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MICROBIAL NATURE AND BIOLOGICAL PROPERTY OF OCHRATOXINS

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ABSTRACT

Ochratoxin A is a fungal metabolite (mycotoxin) with pronounced nephrotoxic potency in all species of animals studies. It has shown teratogenic properties in three rodent species, and is a renal carcinogen in the mouse. Protein synthesis and RNA synthetase is competitively inhibited by the toxin; and renal phosphoenol pyruvate carboxykinase appears to be specifically inhibited by the compound. Ochratoxin A is a major disease determinant of mycotoxic porcine nephropathy, a disease occurring endemically in several countries. This disease resembles Balkan endemic nephropathy in man, suggesting a common causal relationship. Ochratoxin A has been found in foodstuffs in many countries, and the highest contamination frequency in foods has been encountered in an area of Yugoslavia, where Balkan endemic nephropathy is prevalent. The presence of ochratoxin A in human blood in that area is further proof of human exposure. Thus evidence is provided supporting the suggestion, that ochratoxin A is a nephrotoxin in man.

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INTRODUCTION

Ochratoxin A, the leading member of the ochratoxin group, was discovered 15 years ago in South Africa during laboratory screening for toxicity of a number of molds isolated from cereal and legume crops (van der Merve and co-workers, 1965). The compound was encountered as a naturally occurring contaminant of foodstuffs (maize) four years later (Shotwell and co-workers, 1969), and has since been found in foodstuffs in many countries in Europe and North America. Ochratoxin A is a potent nephrotoxin, and has been observed as a major disease determinant of mycotoxic porcine nephropathy, a chronic renal disease in pigs endemic in Scandinavia and probably also in Central Europe (Krogh, 1978). The toxin has been suggested as a disease determinant of Balkan endemic nephropathy (Krogh, 1974), a chronic renal disease in man occurring in certain areas of the Balkan peninsula. Lately increasing evidence for a causal role of ochratoxin A in this disease has been obtained, through the observation that locally grown foodstuffs in endemic areas are frequently contaminated with ochratoxin A (Krogh and co-workers, 1977) indicating pronounced human exposure. The observation that 7% of the population in an endemic areas appears to have ochratoxin A in the blood (Plestina and co-workers, 1981) is further documentation of a pronounced human exposure to this food contaminant.

Fungal Formation and Environmental Occurrence of Ochratoxin

The ochratoxins constitute a group of closely related

derivatives of isocoumarin linked to L- -phenylalanine (Fig. 1). The acids, the methyl and ethyl esters, and the isocoumarin part of ochratoxin B (ochratoxin) have all been isolated from fungal cultures under experimental conditions (Review by Steyn, 1977; Scott, 1977). Ochratoxin , the isocoumarin part of ochratoxin A, has been found in the intestine, urine, feces and liver of rodents (mouse, rat) experimentally fed ochratoxin A-containing feed, and is the cleavage product formed in the alimentary tract (Galtier and Alvinerie, 1976). In urine of rats fed ochratoxin A a metabolite, 4-hydroxyochratoxin A, has been identified, apparently a result of liver microsomal hydroxylation involving cytochrome P-450 (Størmer and Pedersen, 1980). In contrast, only ochratoxin A and very rarely ochratoxin B are produced under natural conditions, in plant products. The ochratoxin-producing fungi are included in the genera Aspergillus and Penicillium. The toxins were first isolated from Aspergillus ochreceus (hence the name), but the main producers, at least under colder climatic conditions, are found among the Penicillia, with Penicillium viridicatum being the leading producer (Krogh, 1978). Toxin production can occur in most plant products and is primarily governed by two factors: temperature and water, i.e. water activity (a_w) of the substrate (plant product). The temperature and a_w conditions for growth and ochratoxin A production have been determined for several fungal species (Northolt, van Egmond and Paulsch, 1979). Although optimal conditions for ochratoxin production by Penicillium viridica-

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tum are found at a_w = 0.95 and 24°C, the toxin is still being produced at a temperature as low as 4°C. The a_w values, determined under laboratory conditions, can be converted to the more practical parameter, water content (water %), specific for each commodity. Thus the lowest water % allowing ochratoxin A production by Penicillium viridicatum is 18.5% in wheat and 21.6% in maize (at 24°C). Water content of cereals and other plant products is dependent on a number of factors, such as climatic conditions (precipitation) during growth and harvest period, and harvest and storage treatment. Thus submarginal agricultural techniques are conducive to high water content in plant products and subsequent ochratoxin A production by the ubiquitously occurring fungi. Surveys of foodstuffs, mainly cereals, have revealed the presence of ochratoxin A in the following geographical areas: Europe: Czechoslovakia, Denmark, France, Germany, Hungary, Poland, Sweden, United Kingdom, Yugoslavia; North America: Canada, U.S.A. Actually, no survey for ochratoxin A appears to have been conducted without finding the contaminant. In foods the frequency of contamination varies from 1% to 18.8% with levels in the range 5-360 g/kg; in feeds up to 100% of samples have been found contaminated, with levels in the range 5-27,500 g/kg (Review: Krogh, 1978). Although the mean level of ochratoxin A in all reported surveys is 1035 g/kg, 83% of the samples contains less than 200 g/kg (Krogh, 1980). When food animals (pigs, poultry) are exposed to ochratoxin A-contaminated feed, a part of the ingested toxin will be retained, as resi-

dues, in the tissues. Thus carry-over of ochratoxin A from feed to food of animal origin is possible, and surveys at meat plants in Denmark and Sweden have revealed that 25-35% of pigs suffering from nephropathy contains residues of ochratoxin A (Krogh and co-workers, 1977; Rutqvist and co-workers, 1977).

Biological Effects of Ochratoxin A

Ochratoxin A is resorbed from the alimentary tract, starting already in the stomach and continuing in the small intestin (Galtier, 1979). By passing the intestine a part of ochratoxin A is cleaved forming ochratoxin and phenylalanine, due to the hydrolytic activity of carboxypeptidase A and chymotrypsinogen (Suzuki, Satoh and Yamazaki, 1977) and of the intestinal bacteria and protoxoa (in ruminants) (Galtier and Alvinerie, 1976). In the blood ochratoxin A is present as bound to serum albumin (Chu, 1971; Galtier, 1974a), and as free ochratoxin A; saturation in the rat occurs at 70 g ochratoxin A/ml plasma.

As indicated in table 1 the binding of ochratoxin A to serum albumin is particularly strong in cattle, pig and man. Apart from the blood the highest tissue concentrations of ochratoxin A are found in the kidney and the liver in rats (Galtier, 1974b; Chang and Chu, 1977; Lillehøj and co-workers, 1979) and in pigs (Krogh, 1978; Rutqvist and co-workers, 1978), pointing to the target organs of this toxin. Under experimen-

Table 1. In Vitro Binding of Ochratoxin A to Serum Albumin of Several Species*

Animal Species	Number of Binding Sites	Intrinsic Association Constant (M^{-1})
Cattle	0.58	94,600
Pig	0.56	71,100
Man	0.58	63,500
Horse	0.57	57,400
Chicken	0.51	52,700
Rat	0.68	40,100
Sheep	0.88	22,600

* Adapted from Galtier (1979).

tal conditions ochratoxin A has revealed a pronounced acute toxic effect in a number of mammal, bird and fish species, with peroral LD_{50} in the range 3.4 - 62.4 mg/kg bw. The main pathologic changes observed during LD_{50} experiments are necrosis of the renal tubules and of periportal liver cells: additional findings (in the pig, rat and dog) are enteritis and necrosis of lymphoid tissue (Review: WHO, 1979). When lower doses of ochratoxin A have been employed, corresponding to feed levels below 5-10 mg/kg, the effect (in pigs) has been confined to the kidneys (Krogh, 1978). The effect of ochratoxin A above 5-10 mg/kg is only of marginal interest as such

levels are rarely encountered in foodstuffs. The nephropathy induced in pigs during 4 months of exposure to ochretoxin A in the range 200-4000 g/kg feed is characterized by impairment of proximal tubular function, indicated by a decrease of Tm_{PAH}/C_{In}; of the ability to concentrate urine, and by an increased urinary excretion of glucose, leucine aminopeptidase and protein. The changes of renal structure include atrophy of the proximal tubules, interstitial formation of connective tissue in the renal cortex, and in advanced cases sclerotized glomerular tufts (Krogh and co-workers, 1974). In a long-term study in pigs fed 1 mg ochratoxin A/kg feed the renal impairment aggravated slightly during the two year period, without reaching a state of terminal renal failure (Krogh and co-workers, 1979). Teratogenic effects of ochratoxin A have been demonstrated in the mouse (Hayes, H od and Lee, 1974), the hamster (Hood, Naughton and Hayes, 1976) and the rat (Brown, Szczech and Purmalis, 1976). Renal carcinoma and liver tumors were induced in mice (Kanisawa and Suzuki, 1978). Ochratoxin A is an inhibitor of tRNA synthetase and protein synthesis in several microorganisms (Bacillus subtilis, Bacillus stearothermophilus, Streptococcus faecalis, yeasts) as well as in rat hepatoma cells (Konrad and Röschenthaler, 1977; Bunge, Dirheimer and Röschenthaler, 1978; Heller and Röschenthaler, 1978; Creppy and co-workers, 1979b). The competitive inhibitory effect of ochratoxin A on tRNA synthetase and protein synthesis in rat hepatoma cells can be prevented by phenylalanine (Creppy and co-workers, 1979b), an observation suggest-

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ing the possibility of preventive measures in ochratoxin A-induced diseases. Thus the acute intraperitoneal effect of ochratoxin A (LD_{100}) in mice can be prevented by concomittant injection of phenylalanine (Creppy and co-workers, 1980); it remains to be elucidated whether chronic effects (nephropathy) in animals also can be prevented by phenylalanine. Experiments in rats have shown, that ochratoxin A inhibits renal gluconeogenesis through an effect on renal phosphoenolpyruvate carboxykinase, whereas the corresponding hepatic enzyme is unaffected (Meisner and Selanik, 1979). Preliminary data indicate that renal phosphoenolpyruvate carboxykinase is inhibited by ochratoxin A also in the pig, an observation that may provide the basis for a specific diagnostic procedure for clinical use (Meisner and Krogh, unpublished).

Causal Role in Disease

Mycotoxic procine nephropathy is an endemically occurring disease in Denmark known for decades (Krogh, 1976), and recently the disease has been observed in Sweden (Rutqvist and co-workers, 1978) as well as in Central Europe (Krogh, 1980). At slaughter the kidneys appear pale and enlarged, with cortical fibrosis visible on the cut surface. Histologically atrophy of proximal tubules and interstitial cortical fibrosis is observed. Ochratoxin A is a major disease determinant of the disease (Krogh, 1978), but also other nephrotoxic mycotoxins, such as citrinin, might be involved. The prevalence rate of mycotoxic

porcine nephropathy ranges from 10 to 70 per cases per 10.000 pigs, heavely influenced by weather conditions (Krogh, 1976). Balkan endemic nephropathy is a chronic renal disease in man, with unknown etiology so far. Because of the similarity of this disease with mycotoxic porcine nephropathy (with partly known etiology) attempts have been made to elucidate a possible causal role for ochratoxin A in this human condition. Surveys of locally grown foodstuffs in an endemic area of Yugoslavia, covering a 5-years period, have revealed that 8-12% of the cereals are contaminated with ochratoxin A, compared to an average of 3% for foods in all other European and North American countries (Krogh and co-workers, 1977; Pavlovic, Plestina and Krogh, 1979). These observations indicate an increased exposure of the inhabitants in endemic areas to ochratoxin A, although the intake of this compound has not yet been measured in any part of the world. Indirect evidence for an increased exposured was recently provide by the observation that 7% of a fraction of the population (201 persons) in the endemic area of Yugoslavia mentioned above had ochratoxin A in the blood (Maximal concentration 1.8 g/ml) compared to none in the control group (Plestina and co-workers, 1981).

REFERENCES

Brown, M.H., G.M.Szczech, and B.P.Purmalis (1976). Teratogenic and toxic effects of ochratoxin A in rats. Toxicol. and App. Pharm, 331-338.

Bunge, I., G.Dirheimer, and R.Röschenthaler (1978). In vivo and in vitro inhibition of protein synthesis in Bacillus stearothermophilus by ochratoxin A. Biochem.Biophys.Res. Comm. 83, 398-405.

Chang, F.C. and F.S. Chu (1977). The fate of ochratoxin A in rats. Fd. Cosmet.Toxicol. 15, 199-204.

Chu, F.S. (1971). Interaction of ochratoxin A with bovine serum albumin. Arch.Biochem.Biophys. 147, 359-366.

Creppy, E.-E., A.A.J. Lugnier, G. Beck, R.Röschenthaler, and G.Dirheimer (1979b). Action of ochratoxin A on cultured hepatoma cells - reversion of inhibition by phenylalanine. FEBS Letters 104, 287-290.

Creppy, E.-E., A.A.J.Lugnier, F.Fasiolo, K.Heller, and R.Röschenthaler (1979a). In vitro inhibition of yeast phenylalanyl-tRNA synthetase by ochratoxin. A. Chem.-Biol.Interact. 24, 257-261.

Creppy, E.-E., M.Schlegel, R. Röschenthaler, and G.Dirheimer (1980). Phenylalanine prevents acute poisoning by ochratoxin A in mice. Toxicol. Lett. 6, 77-80.

Galtier, P. (1974a). Devenir de l'ochratoxine A dans l'organisme animal. I. Transport sanguin de la toxine chez le rat. Ann.Rech.Veter.5, 311-318.

Galtier, P. (1974b). Devenir de l'ochratoxine A dans l'organisme animal II. Distribution tissulaire et elimination chez le rat. Ann. Rech.Veter.5, 319-328.

Galtier, P. (1979). Etude toxicologique et pharmacocinetique d'une mycotoxine, l'ochratoxine A. These d'Etates Sciences pharmaceutiques, Toulouse, 1-50.

Galtier, P. and M. Alvinerie (1976). In vitro transformation of ochratoxin A by animal microbial floras. Ann.Rech. veter. 7, 91-98.

Hayes, A.W., R.D. Hood, H.L. Lee (1974). Teratogenic effects of ochratoxin A in mice. Teratology, 9, 93-97.

Heller, K. and Röschenthaler (1978). Inhibition of protein synthesis in Streptococcus faecalis by ochratoxin A. Can. J. Microbiol. 24, 466-472.

Hood, R.D., M.J. Naughton and A.W. Hayes (1976). Prenatal effects of ochratoxin A in hamsters. Teratology 13, 11-14.

Kanisawa, M. and S.Suzuki (1978). Induction of renal and hepatic tumors in mice by ochratoxin A, a mycotoxin. Gann, 69, 599-600.

Konrad, I. and R.Röschenthaler (1977). Inhibitation of phenylalanine tRNA synthetase from Bacillus subtilis by ochratoxin A. FEBS Letters 83, 341-347.

Krogh, P. (1974). Mycotoxic porcine nephropathy - a possible model for Balkan (endemic) nephropathy. In: Endemic nephropathy (A. Puchlev), Bulgarian Academy of Science, Sofia, 266-270.

Krogh, P. (1978). Causal associations of mycotoxic porcine nephropathy. Acta Path. Microbiol. scand. Sect. A.Suppl. No. 269, 28.

Krogh, P. (1980). Ochratoxins: Occurrence, biological effects and causal role in diseases. In: Natural Toxins (D. Eaker and T. Wadström), Pergamon Press, Oxford, 673-680.

Krogh, P., N.H. Axelsen, F. Elling, N. Gyrd-Hansen, B. Hald, J. Hyldegaard-Jensen, A.E. Larsen, A. Madsen, H.P. Mortensen, T. Møller, O.K. Petersen, U. Ravnskov, M. Rostgaard, and O. Aalund (1974). Experimental porcine nephropathy. Changes of renal function and structure induced by ochratoxin A-contaminated feed. Acta path. microbiol. scand., Section A, Supplementum No. 246, 21.

Krogh, P., F. Elling, Chr. Friis, B. Hald, A.E. Larsen, E.B. Lillehøj, A. Madsen, H.P. Mortensen, F. Rasmussen and U. Ravnskov (1979). Porcine nephropathy induced by long-term ingestion of ochratoxin A. Vet. Pathol. 16, 466-475.

Krogh, P., B. Hald, R. Plestina and S. Ceovic (1977). Balkan (endemic) nephropathy and foodborn ochratoxin A: Preliminary results of a survey of foodstuffs. Acta path. microbiol. scand Sect. B, 85, 238-240.

Lillehøj, E.B., W.F. Kwolek, F. Elling and P. Krogh (1979). Tissue distribution of radioactivity from ochratoxin A - ^{14}C in rats. Mycopathol. Mycol. Applic. 68, 175-177.

Meisner, H. and P. Selanik (1979). Inhibition of renal gluconeogenesis in rats by ochratoxin. Biochem. J. 180, 681-684.

Van der Merwe, K.J., P.S.Steyn, L. Fourie, Scott De B, and J.J. Theron (1965). Ochratoxin A, a toxic metabolite produced by Aspergillus ochraceus Wilh. Nature 205, 1112-1113.

Northolt, M.D., H.P. Egmond, and W.E. Paulsch (1979). Ochratoxin A production by some fungal species in relation to water activity and temperature. J. Food Protect. 42, 485-490.

Pavlovic, M., R. Plestina and P. Krogh (1979). Ochratoxin A contamination of foodstuffs in an area with Balkan (endemic) nephropathy. Acta path. microbiol. scand. Sect. B 87, 243-246.

Rutqvist, L., N.-E. Björklund, K. Hult, E. Hökby, and B. Carlsson (1979). Ochratoxin A as the cause of spontaneous nephropathy in fattening pigs. Appl. Env. Microbiol. 36, 920-925.

Rutqvist, L., N.-E. Björklund, K. Hult, and S. Gatenbeck (1977). Spontaneous occurrence of ochratoxin residues in kidneys of fattening pigs. Zbl. Vet. Med. A 24, 402-408.

Plestina, R., S. Geovic, S. Gatenbeck, V. Habazin-Novak, K. Hult, E. Hökby, P. Krogh, and B. Radic (1981). Human exposure to ochratoxin A in areas of Yugoslavia with endemic nephropathy. In: Proceedings of IV IUPAC Symposium of Mycotoxins and Phycotoxins (P. Krogh), Pathotox Publishing Inc., Park Forest South, Illinois, USA (in press).

Scott, P.M. (1977). Penicillium mycotoxins. In T.D. Wyllie and L.G. Morehouse (Eds.), Mycotoxic Fungi, Mycotoxins, Mycotoxicoses, Vol. 1, Marcel Dekker, New York, 283-356.

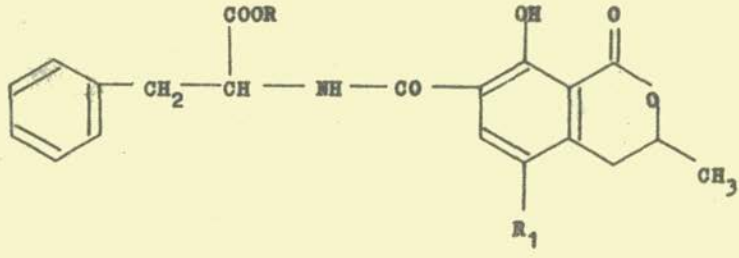
Shotwell, O.L., C.W. Hesseltine and M.L. Goulden (1969). Ochratoxin A: Occurrence as natural contaminant of a corn sample. Appl. Microbiol. 17, 765-766.

Steyn, P.S. (1977). Mycotoxins, excluding aflatoxin, zearalenone and the trichothecenes. In J.V. Rodricks, C.W.Hesseltine and M.A. Mehlman (Eds.), Mycotoxins in Human and animal Health, Pathotox Publ., Illinois, 419-467.

Størmer, F.C. and J.I. Pedersen (1980). Formation of 4-hydroxyochratoxin A from ochratoxin A by rat liver microsomes. Appl. Environ. Microbiol. 39, 971-975.

Suzuki, S., T. Satoh and M. Yamazaki (1977). The pharmacokinetics of ochratoxin A in rats. Japan. J. Pharmacol. 27, 735-744.

World Health Organization (1979). Environmental Health Criteria 11, Mycotoxins, Geneva, 86-98.



Ochratoxin A	R = H	R_1 = Cl
Ochratoxin B	R = H	R_1 = H
Ochratoxin C	R = C_2H_5	R_1 = Cl
Methyl ester of Ochratoxin A	R = CH_3	R_1 = Cl
Methyl-ethyl ester of Ochratoxin B	R = CH_3 or C_2H_5	R_1 = H

Fig.1. Chemical structure of ochratoxins.

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