CHAPTER 3

Mycotoxins as Causal Factors of Diseases in Humans

This chapter highlights the threat that mycotoxins hold for humans. It was communicated by Prof. Steyn at the Third Symposium of Monastir, Tunisia: Environmental Toxicants and Pathologies and was published in *Journal of Toxicology - Toxin Reviews* **18**, 229-244 (1999).

Contribution made by the candidate

The candidate played the major role in the literature search, design and writing of the manuscript.

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ABSTRACT

The criteria of human mycotoxicoses are discussed and the role of certain mycotoxins in human diseases highlighted, *e.g.* ergotoxins (ergotism), trichothecenes, T2-toxin (alimentary toxic aleukia), aflatoxins (primary liver cancer), ochratoxins (Balkan Endemic Nephropathy and chronic interstitial nephropathy) and fumonisins (oesophogeal cancer). The chemical properties and biochemical mechanism of action of aflatoxin B_1, ochratoxin A and fumonisin B_1 are discussed.

Keywords: Mycotoxins, Aflatoxin, Ochratoxin, Fumonisin, T2-toxin, Ergotamine, Patulin, Zearalenone, Cyclopiazonic acid, Mycotoxicoses

INTRODUCTION

Mycotoxins are a structurally diverse group of mostly low molecular weight compounds produced by the secondary metabolism of fungi such as species of *Aspergillus, Penicillium, Claviceps, Fusarium* and *Alternaria*. In nature most cereal grains, oil seeds, tree nuts and dehydrated fruits are susceptible to contamination by toxigenic fungi and mycotoxin formation, dependant on growth and storage conditions. Mycotoxicoses are the diseases caused by the ingestion of mycotoxins in man and animals. Mycotoxins induce powerful biological effects, *e.g.*: carcinogenic, mutagenic, teratogenic, estrogenic, immunotoxic, nephrotoxic and neurotoxic effects (See [1] and [2] for reviews). The human ingestion of mycotoxins is mainly due to the consumption of mycotoxin contaminated plant-based foods and to the residues and metabolites in animal-products, such as aflatoxin M_1. Since humans normally avoid foods visually contaminated by moulds, human health problems resulting from acute mycotoxicoses are relatively rare. The greatest human health concern related to mycotoxins is a cancer risk based on long term, low level exposures to carcinogenic toxins, such as the aflatoxins, ochratoxin A and fumonisins. Hsieh [3] proposed that the following criteria should be met to link a mycotoxin to a specific disease: occurrence of the mycotoxin in food samples; human exposure and incidence; reproducability of the characteristic symptoms in experimental animals; similar mode of action in human and animal models. In addition, climate seems to play a major role in the incidence of a mycotoxicoses; some

seasonal changes in the occurrence of mycotoxicoses have been reported, in particular animal mycotoxicoses, *e.g.* photosensitization diseases, diplodiosis and lupinosis [4].

AFLATOXINS

The aflatoxins are a group of hepatocarcinogenic bishydrofurano mycotoxins produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus*. Among the group of mycotoxins, aflatoxin B_1 is the most abundant and widely studied compound. The liver and kidneys are the target organs for organ toxicity and lesions are characterised mainly by carcinomas. Serious aflatoxin contamination of harvested crops has been reported on all continents and aflatoxin is found in food commodities such as corn, peanuts, cotton seed, sunflower seed and other energy rich products. When the feed consumed by cows contains aflatoxin B_1, the toxic metabolite aflatoxin M_1 is excreted in their milk. Aflatoxin M_1 has similar effects as aflatoxin B_1 on experimental animals and is a cause of great concern, especially in infant foods containing dairy products [5]. Aflatoxin contamination is related in the etiology of human primary liver cancer in countries in Africa [6] and South-East Asia [7](although other factors such as hepatitus B virus infection, alcohol consumption and smoking may also play a role) and is considered by the International Agency for Research in Cancer, Lyon, as a human carcinogen. Many countries have legislation to control the aflatoxin content in food and feed products. The mutagenic and carcinogenic effects of AFB_1 arise from metabolic activation by cytochrome P450s of the electron-rich dihydrobisfuran to the corresponding epoxide [8- 9]. AFB_1 -epoxide occurs in an *endo-* and *exo-*form: *exo-*form is a 500 times more potent mutagen than the *endo-*form [10-11] (See **Figure 1**). The phenomenon may be explained by the covalent binding of the *exo-*epoxide to the *N-*7 of the guanine residues of the DNA helixes, thereby leading to depurination and strand scission events [12-13]. These bond formations take place at DNA regions which are rich in guanine, such an important region occurs on codon 249 of the p53 tumour suppressor gene, at the third base position where a G $\rightarrow$ T transversion has been identified in hepatocellular carcinoma patients from China [14] and from sub-Saharan Africa [15]. A metabolite of AFB_1-epoxide, 8,9-dihydro-8,9 dihydroxy-aflatoxin B_1, may bind to cellular proteins *via* a Schiff base formation with primary amino groups to induce cellular injury and eventually cell death [16].

exo-epoxide

endo-epoxide
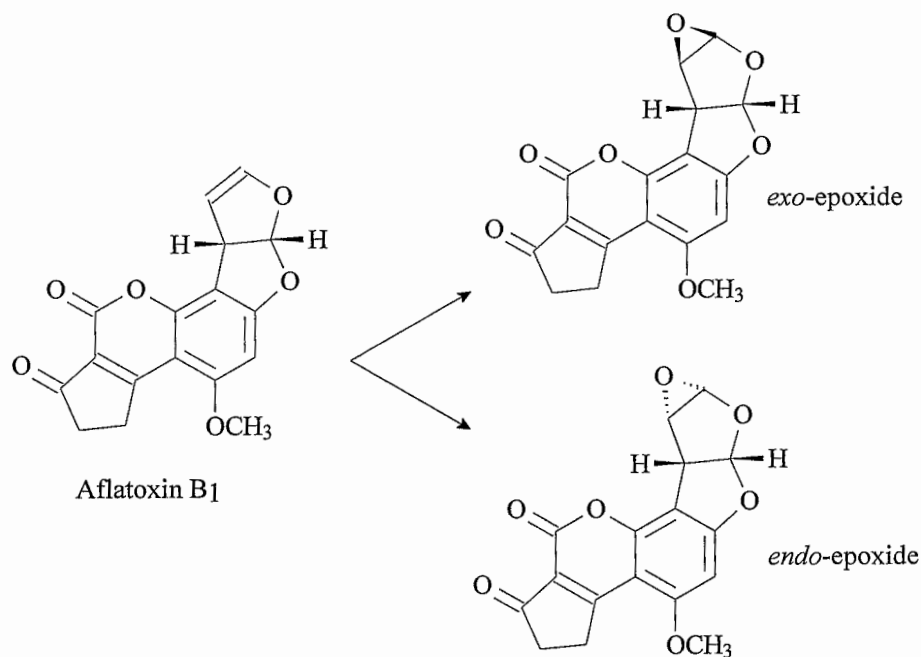
Aflatoxin B₁

Figure 1: Metabolism of aflatoxin B₁

FUMONISINS

The high incidence of human esophageal cancer in the Transkei [17] and parts of China [18-19] has been causally linked to the consumption of corn contaminated with high levels of *Fusarium moniliforme* and its toxins (particularly fumonisins). These toxins are unambiguously associated with equine leukoencephalomalacia (LEM) [20-21] better known as 'hole in the head disease', a fatal neurological disease in horses fed feed contaminated with the fungus *F. moniliforme*. Fumonisins have also been reported to cause pulmonary oedema in pigs [22] and a nondescribed poultry disease (ill thrift). FB₁ is hepatocarcinogenic [23], fetotoxic [24] and non-genotoxic to rats [25] (See **Figure 2**). FB₁ inhibits the conversion of sphinganine and sphingosine to ceramide, by ceramide synthetase in neuronal cells [26]. This leads to the accumulation of sphingoid bases and is reported to be connected with the diseases associated with fumonisins [26]. The disruption of the mechanism of sphingolipids, which are important components of cell membranes, could have serious effects on cell growth, differentiation and behaviour [27]. Apart from the inhibition of hepatocyte proliferation, FB₁ also effects the synthesis of cellular lipids by altering the incorporation of palmitic acid [28]. Cell proliferation, which plays an important role in cancer initiation and promotion, is controlled by long chain fatty acids *via* their modulation of prostaglandin levels [29]. This

was supported by an accumulation of polyunsaturated fatty acids in primary hepatocytes which were exposed to FB_1. Gavino and coworkers [30] reported that increased levels of polyunsaturated fatty acids have been associated with lipid peroxidation in normal and cancer cells. This implies that FB_1 can indirectly cause lipid peroxidation which could affect cells by damaging cellular membranes [25].
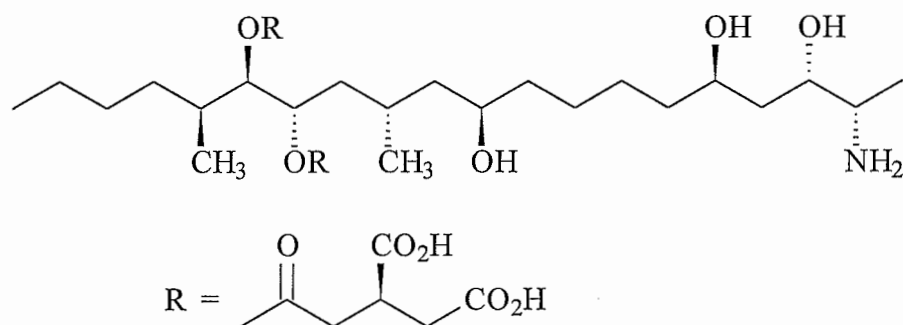

Figure 2: The structure of fumonisin B_1

OCHRATOXIN

The ochratoxins are metabolites of *Aspergillus ochraceus* and *Penicillium verrucosum*. The ochratoxins comprise a dihydro-isocoumarin moiety linked to the L-amino-acid, phenylalanine through an α-amide bond. Ochratoxin A (OTA) has been shown to be nephrotoxic, carcinogenic, immunotoxic and teratogenic to all animal species tested so far, and induces experimental liver and kidney tumours (see **Figure 3**). It affects mainly the kidneys through degeneration of the proximal tubules, interstitial fibrosis in the renal cortex, hyalinisation of the glomeruli and atrophy in the tubular epithelium [31-33]. Danish Porcine Nephropathy, a kidney disease amongst pigs fed mouldy cereal feeds, was discovered in Denmark in 1928. This disease was positively linked to the frequency of OTA contamination in feed samples and has since been found in Sweden, Norway, Finland, Hungary and Great Britain. Pigs fed OTA showed reduced feed intake, loss of body weight, increased water consumption followed by polyurea diarrhoea, polydipsia and dehydration [34]. In the 1950's a similar disease was discovered amongst humans in various rural areas in Bulgaria, Romania and Yugoslavia. The disease called Balkan Endemic Nephropathy (BEN) is an invariably fatal chronic disease and is characterised by contracted kidneys and features changes exclusively in the renal cortex of

the kidney. OTA has been found more frequently in food samples and in the serum of people taken from villages with BEN, than in areas where the disease is unknown [32, 35-37]. OTA is also suspected to play a role in Chronic Interstitial Nephritis (CIN) in North Africa [38]. Krogh *et al.,* [39] reported nephropathy in pigs fed diets containing 0,2-4 mg.kg^{-1} OTA for a period of four months; all lesions found, were confined to the kidney.
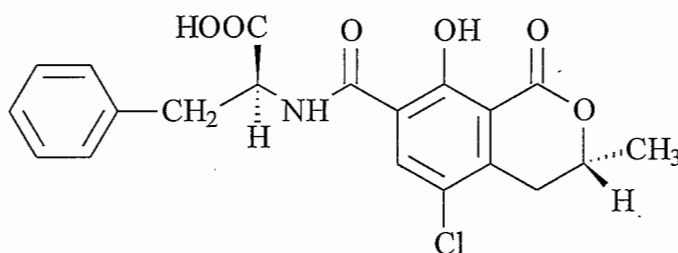

Figure 3: The structure of ochratoxin A

The three main known effects of OTA seem to be centered on enzymes involved in phenylalanine metabolism, particularly phenylalanyl-tRNA synthetase which catalyse the Phe-tRNA aminoacylation reaction, consequently effecting protein synthesis [40]; secondly the disruption of hepatic microsomal calcium homeostasis by impairment of the endoplasmic reticulum membrane, probably *via* lipid peroxidation [41]; and lastly OTA inhibits mitochondrial state 3 and 4 respiration [42] by acting as a competitive inhibitor of mitochondrial transport carrier proteins located in the inner mitochondrial membrane [43-44]. OTA enhances the rate of lipid peroxidation by chelating ferric ions (Fe^{3+}) and facilitating their reduction to ferrous ions (Fe^{2+}). The reoxidation to Fe^{3+}- OTA is accompanied by the consumption of O_2 [45] and the subsequent formation of extremely damaging hydroxyl radicals in the presence of the NADPH-cytochrome-P-450 reductase system and NADPH [46].

The metabolism of OTA differs both in different animal species and different cellular systems (See **Figure 4**). It is hydrolysed to the non-toxic OTα (7-carboxy-5-chloro-3,4-dihydro-8-hydroxy-3-methylisocoumarin) *in vivo* and *in vitro* in rats [47-48] and hydroxylated to (4*R*)-OH-OTA in human and rat liver microsomal systems under the influence of cytochrome P-

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450's [49-48], while the (4S)-OH-OTA- epimer is more prevalent in pig liver microsomes [50]. Both (4R)-OH-OTA and (4S)-OH-OTA were found in rat and rabbit liver [49] and rat kidney [51]. The 10-OH metabolite of OTA was formed from OTA with rabbit liver microsomal system [48].

(4S)-OH-OTA: R$_1$ = OH, R$_2$ = H   10-OH-OTA
(4R)-OH-OTA: R$_1$ = H, R$_2$ = OH
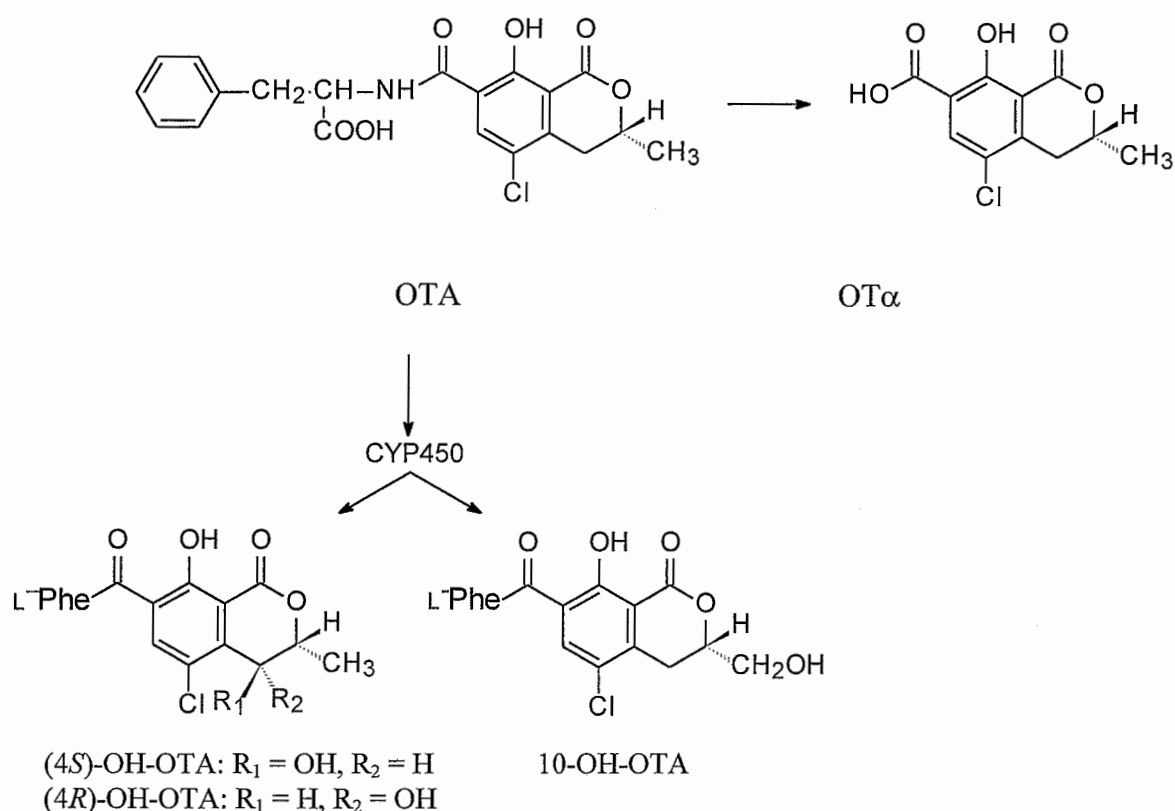
Figure 4: Metabolism of OTA

OTA contamination is widespread in cereals, coffee, pulses, feedstuffs and other plant products. Raw agricultural products, contaminated with OTA and used as feed, can also lead to contamination of meat and meat products of non-ruminant animals such as poultry and pigs [5,4].

ERGOTOXINS

Ergotism is probably the oldest and best known mycotoxicosis. It was one of the recurring calamities of the Middle Ages and approximately 40 000 people died of this disease in an epidemic in the year 944 in central and northern Europe. Two characteristic forms of ergotism can be distinguished, namely gangrenous ergotism and convulsive ergotism. The symptoms of gangrenous ergotism are vomiting, diarrhoea and a burning pain in the limbs. In severe

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cases dry gangrene of entire limbs can cause them to become completely detached from the body. Ergotism is caused by a number of *Claviceps* species that infect grass species, particularly rye, wheat and pearl millet [52]. The latter is a staple in India, where a number of ergot outbreaks have been reported in the late 1950s and 1975 [53]. Two groups of mycotoxins are associated with ergotism: alkaloids of the clavine group and the ergotamine group. Ergotamine (See **Figure 5**) and related alkaloids are derivatives of lysergic acid and are associated with amino acids [54]. Ergotism has no predominance among sex, but children are more susceptible, particularly to convulsive ergotism [55]. As a disease ergotism occurs very rarely today, primarily because most of the ergots are removed in the cleaning and milling process and furthermore, most ergot alkaloids are destroyed in the cooking and baking process [56], but cases of similar diseases are often reported in animals *e.g.* in cattle [57-58]. Subclinical ergotism was diagnosed in a number of patients treated for migraine headaches with ergotamine tartrate, bringing the continued use of this compound into disrepute [59].
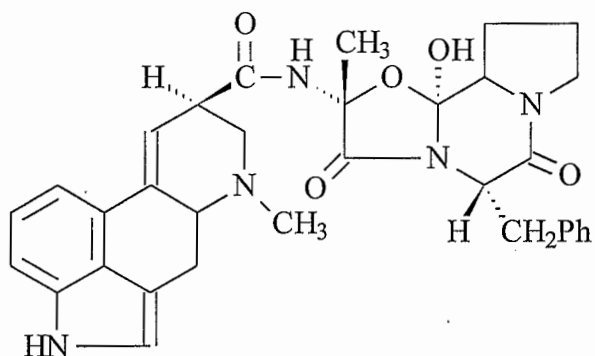

Figure 5: The structure of ergotamine

PATULIN

Patulin is an α,β-unsaturated lactone and is one of a group of mycotoxins that contains a 5-membered cyclic ring system [See **Figure 6**]. Patulin contamination occurs in fruit, especially apples and their processed products. Patulin levels in apples is regulated in several European countries at a tolerance level of 50 μg.kg^{-1}. It possesses powerful mutagenic and cytotoxic properties and causes immunotoxic, neurotoxic and gastrointestinal effects in experimental

animals and inhibits protein synthesis by inhibition of amino acid uptake and their incorporation into proteins [60]. No diseases have been linked to patulin consumption.
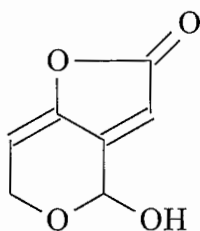
Figure 6: The structure of patulin

TRICHOTHECENES

The trichothecenes are a group of approximately 148 related sesquiterpenoids [61-62] produced by various *Fusarium* species. Of the simple trichothecenes the ones most frequently detected in agricultural commodities are T-2 toxin [**Figure 7**], diacetoxyscirpenol, nivalenol and deoxynivalenol (DON). When feedstuffs for swine are contaminated with DON (also known as vomitoxin), economic losses may occur, because these animals may refuse the feed or show weight loss, suffering from vomiting and diarrhoea [63]. Ueno and coworkers demonstrated that the T-2 toxin was the etiological agent in ATA (alimentary toxic aleukia), it has also been shown to alter brain neurochemistry and eating behaviour in animals [64]. After consumption of food made from grain which remained unharvested under snow, people showed symptoms characteristic of ATA including vomiting, diarrhoea, skin inflammation, leukopenia, multiple haemorrhage ext. This disease has been widely reported in the former USSR since 1913, especially during the Second World War when food was scarce and people were forced to eat grain that had been left unharvested in the field throughout the winter [52]. People on a balanced died were less likely to be severely affected than those who were subsisting on a staple diet of overwintered grain and the disease was more prevalent in middle-aged women [65]. T-2 toxin produces chemically-induced pathologic alterations in the mitotic gastrointestinal tract and lymphoid cells [66]. Lafarge-Frayssinet and co-workers [67], found T2-toxin to induce single-strand DNA breaks in cultured splenic and thymic cells and DNA breaks in splenic and thymic lymphocytes *in vivo* in mice [52].

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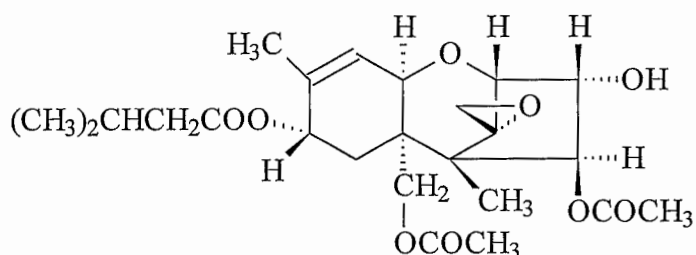
Figure 7: The structure of T2-toxin

ZEARALENONE

Zearalenone, also commonly known as F2 toxin, is a non-steroidal product of several *Fusarium* species, particularly occurring in maize and wheat, and often found together with deoxynivalenol [See **Figure 8**]. Zearalenone shows strong estrogenic effects (hyperestrogenism leading to infertility) in swine, cattle and poultry [68] and causes problems with the reproductive organs of farm animals, especially swine. It also affects the liver and kidneys of the animals. It has also been shown to be genotoxic as well as inducing hepatocellular adenomas in mice [69]. Although zearalenone has a low acute toxicity, it is linked to various estrogenic diseases that affect children.
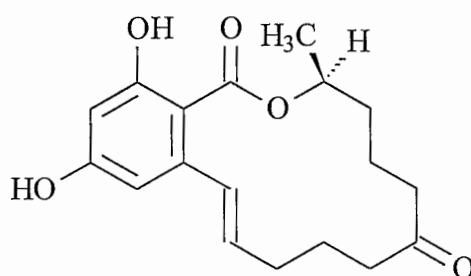

Figure 8: The structure of zearalenone

CYCLOPIAZONIC ACID

Cyclopiazonic acid, an indole tetrameric acid, frequently produced by *Penicillium cyclopium,* is a potent inhibitor of calcium uptake and Ca^{2+}-ATPase activity in sarcoplasmic and endoplasmic reticulum [70] (See **Figure 9**). It causes muscular incoordination, hypokinesia,

catelepsy, gait disturbances, tremor, opisthotonus, convulsions and neurochemical changes [71]. *Aspergillus flavus* and *Aspergillus parasiticus* produce both the cyclopiazonic acids and the aflatoxins, with the to be expected synergistic effects. The related tetramic acid, tenuazonic acid was tentatively linked to Onyalai a trombocytopenic purpura among people living in the northern part of Namibia.
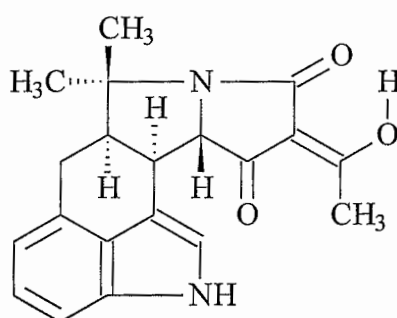
Figure 9: The structure of cyclopiazonic acid

DISEASES OF UNKNOWN ETIOLOGY: MSELENI JOINT DISEASE AND KASHIN-BECK DISEASE

Mseleni Joint Disease (MJD) is a precocious degenerative osteoarthiopathic disease that affect several hundred people in the Mseleni region of northern Zululand, South Africa. Handigodu joint disease (HDJ) is a very similar disease in the Shimaga district of southern India and it is possible that they have the same etiology [72]. These diseases are characterised by articular discomfort in the hip, knee and ankle joints and adults may be seriously handicapped; prosthetic hip joint replacements are often necessary. In severe cases brachydactylous dwarfism can occur in the case of MJD [73]. Although these diseases affect kindreds, no evidence of genetic imprint has been found to date [74]. The etiology of these diseases is unknown but the possibility exist that mycotoxins may be involved in the etiology.

Kashin-Beck is an endemic disease in parts of China and Russia named after two nineteenth century Russian scientists, Kashin and Beck. Symptoms include symmetric stiffness, pain and swelling of the joints, until in later stadia symptoms of generalised osteoarthritis manifest themselves. The disease affect an estimated two million people that consume maize or wheat in China alone. Pathological changes include degeneration and necrosis of chondrocytes in the growth plates and articular cartilages and proliferation or repair of cartilage following

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chondronecrosis [52]. The etiology of the disease is unknown but many possible causes have been identified including trace metal toxicity, selenium deficiency, hereditary factors, chelating agents such as humic acid, decaying plant material from walnut tree forests and mycotoxins associated with *Fusarium*-contaminated foods [75-76]. Zhai and coworkers [76] found that certain combinations of food substances high in protein had a protective effect and that boys are twice as susceptible as girls to Kashin-Beck disease.

CONCLUSION

Since 1960, spectacular progress has been reported on the taxonomy of toxigenic fungi, the analysis of mycotoxins in complex matrices of foodstuffs, the mechanism of action of mycotoxins with special reference to chemical carcinogenesis, and the chemistry and biogenesis of fungal secondary metabolites.

The current challenges are directed at obtaining a much better understanding of the mechanism of action of mycotoxins at molecular and cellular levels; application of the current knowledge to the developing area of molecular etiology; the application of molecular biology to the rapid identification of toxigenic fungi and to the development of cereals and groundnuts which are resistant to pre- and post-harvest infestation by toxigenic fungi; a better understanding of the genetics and enzymology of fungal secondary metabolism and mycotoxin production; developing technology for the analysis of mycotoxins in complex mixtures and most importantly to quantify the real threats associated with mycotoxin food intake by the development of models for quantitative risk analysis. The foregoing lofty objectives should enable scientists to unambiguously link some neglected human diseases to the consumption of mycotoxin-contaminated foods.

REFERENCES

1. Smith, J.E., and Henderson, R.S., *Mycotoxins and Animal Foods*. CRC Press, Boston, 1991.
2. Miller, J.D., and Trenholm, H.L. *Mycotoxins in Grain: Compounds other than aflatoxin*. Eagan Press. St. Paul, Minnesota, 1994.
3. Hsieh, D.P.H., *Health risks posed by mycotoxins in foods*. Korean J. Toxicol., 6 (2): 159-166, 1990.
4. Steyn, P.S., and Stander, M.A. *Mycotoxins with special reference to the carcinogenic mycotoxins: aflatoxins, ochratoxins and fumonisins*. in *General and*

Applied Toxicology, 2nd edition, 1999, 2145-2177.

5. Van Egmond H.P. and Speijers G.J.A., *Survey of data on the incidence and levels of ochratoxin A in food and animal feed worldwide*. Journal of Natural Toxins, 3 (2): 125-143, 1994.

6. Van Rensburg, S.J., *Role of mycotoxins in endemic liver and oesophageal cancer*. in *Mycotoxins and phycotoxins*, edited by P.S. Steyn and R. Vleggaar, p. 483-494, Elsevier, Amsterdam, 1989.

7. Wang, L-Y., Hatch, M., Chen, C-J., Levin, B., You, S-L., Lu, S-N., Wu, M-H., Wu, W-P., Wang, L-W., Wang, Q., Huang, G-Y., Yang, P-M., Lee, H-S. and Santella, R.M., *Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan*. International Journal of Cancer, 67 (5): 620-625, 1996.

8. Ishii, K., Maeda, K., Kamataki, T. and Kato, R., *Mutagenic activation of aflatoxin B_1 by several forms of purified cytochrome P450*. Mutat. Res., 174: 85-88, 1986.

9. Forrester, L.M., Neal, G.E., Judah, D.J., Glancey, M.J., and Wolf, C.R., *Evidence for involvement of multiple forms of cytochrome P450 in aflatoxin B_1 metabolism in human liver.*, Proc. Natl. Acad. Sci. USA, 87: 8306-8310, 1990.

10. Raney, K.D., Coles, B., Guengrich, F.P. and Harris, T.M., *The endo-8,9-epoxide of aflatoxin B_1: a new metabolite*. Chem. Res. Toxicol., 5: 333-335, 1992.

11. Iyer, R.S., Coles, B.F., Thier, K.D., Guengerich, F.P. and Harris, T.M., *DNA adduction by the potent carcinogen aflatoxin B_1: Mechanistic studies*. J. Am. Chem. Soc., 116: 1603-1609, 1994.

12. Essigmann, J.M, Croy, R.G., Nadzan, A.M., Busby, W.F., Jr., Reinhold, V.N., Büchi, G. and Wogan, G.N. *Structural identification of the major DNA adduct formed by aflatoxin B_1 in vitro*. Proc. Natl. Acad. Sci. USA, 74: 1870-1874, 1977.

13. Essigmann, J.M, Green, C.L., Croy, R.G., Fowler, K., Büchi, G. and Wogan, G.N., *Interactions of aflatoxin B_1 and alkylating agents with DNA: structural and functional studies*. Cold Spring Harbor Sympos. Quant. Biol., 47: 327-337, 1982.

14. Hsu, I.C., Metcalf, R.A., Sun, T., Welsch, J.A., Wang, N.J. and Harris, C.C., *Mutational hotspot in the p53 gene in human hepatocellular carcinomas*, Nature, 350: 427-428, 1991.

15. Bressac, B., Kew, M., Wands, W. and Ozturk, M., *Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa*. Nature, 350: 429-431, 1991.

16. Fink-Gremmels, personnal communication, 1996.

17. Rheeder, J.P., Marasas, W.F.O., Thiel, P.G., Sydenham, E.W., Shephard, G.S and van Schalkwyk, D.J., *Fusarium moniliforme and fumonisins in corn in relation to human esophageal cancer in Transkei*. Phytopathology, 82 (3): 353-357, 1992.

18. Yang, C.S., *Research on esophageal cancer in China: A review*. Cancer Res., 40: 2633-2644, 1980.

19. Chu, F.S. and Li, G.Y., *Simultaneous occurrence of fumonisin B_1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with high incidences of esophageal cancer*. Appl. Environ. Microbiol., 60 (3): 847-852, 1994.

20. Bezuidenhout, G.C., Gelderblom, W.C.A., Gorst-Allman, C.P., Horak, R.M., Marasas, W.F.O., Spiteller, G. and Vleggaar, R., *Structure elucidation of the fumonisins, mycotoxins from Fusarium moniliforme. J. Chem. Soc., Chem. Commun.*, 743-745, 1988.

21. Gelderblom, W.C.A., Jaskiewicz, K., Marasas, W.F.O., Thiel, P.G., Horak, R.M., Vleggaar, R. and Kriek, N.P.J., *Fumonisins-novel mycotoxins with cancer-*

promoting activity produced by Fusarium moniliforme, Appl. Environ. Microbiol., 54 (7): 1806-1811, 1988.

22. Ross, P.F., Nelson, P.E., Richard, J.L., Osweiler, G.D., Rice, L.G., Plattner, R.D., and Wilson T.M., *Production of fumonisins by Fusarium moniliforme and Fusarium proliferatum isolates associated with equine leukoencephalomalacia and a pulmonary endema in swine.* Applied Environmental Biology, 56: 3225-3226, 1990.

23. Gelderblom, W.C.A., Kriek, N.P.J., Marasas, W.F.O., and Thiel, P.G., *Toxicity and carcinogenicity of the Fusarium moniliforme metabolite, fumonisin B$_1$ in rats.*, Carcinogenesis, 12: 1247-1251, 1991.

24. Lebepe-Mazur, S., Bal, H., Hopmans, E., Murphy, P. and Henrich, S. *Fumonisin B$_1$ is fetotoxic in rats.* Vet. Human Toxicol., 37 (2): 126-130, 1995.

25. Gelderblom, W.C.A., Snyman, S.D., Abel, S., Lebepe-Mazur, S., Smuts, C.M., van der Westhuizen, L., Marasas, W.F.O., Victor, T.C., Knasmulle, R.S. and Huber, W., *Hepatotoxic - and carcinogenecity of the fumonisins in rats: a review regarding mechanistic implications for establishing risk in humans*, in: *Fumonisins in Food*, edited by Jackson, L.S., De Vries, I.W. and Bullerman L.D., p. 279-296, Plenum Press, New York, 1996.

26. Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T. and Merrill, A.H., *Inhibition of sphingolipid biosynthesis by fumonisins: implications for diseases associated with Fusarium moniliforme.* J. Biol. Chem., 266: 14486-14490, 1991.

27. Merrill, A.H., *Cell regulation by spingosine and more complex sphingolipids*, J. Bioenerg. Biomembr., 23: 83-104, 1991.

28. Gelderblom, W.C.A., Smuts, C.M., Abel, S., Snyman, S.D., Cawood, M.E., van der Westhuizen, L. and Swanevelder, S., *Effects of Fumonisin B$_1$ on Protein and Lipid Synthesis in Primary Rat Hepatocytes*, Fd. Chem. Toxic., 34: 361-369, 1996.

29. Cornwell, D.G. and Morisaki, N., *FA paradoxes in the control of cell proliferation: prostaglandins, lipid peroxides and cooxidation reactions.* in *Free Radicals in Biology VI.*, edited by W. A. Proyor., p. 95-148, Academic Press, Orlando, FL, 1984.

30. Gavino, V.C., Miller, J.S., Ikharebha, S.O., Milo, G.E. and Maizewell, D.G., *Effect of polyunsaturated fatty acid and antioxidants on lipid peroxidation in tissue cultures.* J. Lipid Res., 22, 763-769, 1981.

31. Krogh P., *Mycotoxic porcine nephropathy - a possible model for Balkan (endemic) nephropathy.*, in *Endemic nephropathy*, edited by A. Puchlev, p. 266-270, Balgarian Academy of Sciences, Sofia, 1974.

32. Krogh P., Hald B., Plestina R. and Ceovic S., *Balkan (endemic) nephropathy and foodborne ochratoxin A: Preliminary results and foodstuffs*, Acta Pathologica Microbiologica et Immunologica Scandinavica Section B, 85: 238-240, 1977.

33. Krogh P., *Ochratoxin in foods*, in: *Mycotoxins in food*, edited by P. Krogh, p. 97.Academic Press, London, 1987.

34. Szczech G.M., Carlton W.W., Tuite J. and Caldwell R., *Ochratoxin A toxicosis in swine.* Vet. Pathol., 10: 347, 1973.

35. Pavlovic M., Plestina R. and Krogh P., *Ochratoxin A contamination of foodstuffs in an area with Balkan (endemic) nephropathy.* Acta Pathologica Microbiologica et Immunologica Scandinavica Section B, 87: 243-246, 1979.

36. Petkova-Bocharova T. and Castegnaro M., *Ochratoxin contamination of cereals in an area of high incidence of Balkan endemic nephropathy in Bulgaria.* Food. Addit. Contam., 2: 267-270, 1985.

37. Petkova-Bocharova T., Chernozemsky I.N. and Castegnaro M., *Ochratoxin A in human blood in relation to Balkan endemic nephropathy and urinary system tumours in Bulgaria*. Food. Addit. Contam., 5 (3): 299-301, 1988.

38. Bacha, H., Maaroufi, K., Achour, A., Hamammi, M., Ellouz, F., and Creppy, E.E., *Ochratoxins and ochratoxicoses in humans in Tunisia*. in *Human ochratoxicosis and its pathologies,* edited by E.E. Creppy, M. Castegnaro and G. Dirheimer. p. 111-121, John Libbey Eurotext Ltd., 1993.

39. Krogh P., Gyrd-Hansen N., Hald B., Larsen S., Nielsen J.P., Smith M., Ivanoff C. and Meisner H., *Renal enzyme activities in experimental ochratoxin A-induced porcine nephropathy: Diagnostic potential of phosphoenolpyruvate carboxykinase and gamma-glutamyl transpeptidase activity.*, J. Toxicol. Environ. Health, 23: 1, 1988.

40. Creppy E.-E., Röschenthaler R. and Dirheimer G. *Inhibition of protein synthesis in mice by ochratoxin A and its prevention by phenylalanine.*, Fd Chem. Toxic., 22 (11): 883, 1984.

41. Omar R.F., Rahimtula A.D. and Bartsch H., *Role of cytochrome P-450 in Ochratoxin A-stimulated lipid peroxidation*. J. Biochem. Toxicol., 6: 203-209, 1991.

42. Moore J.H. and Truelove B., *Ochratoxin A, inhibition of mitochondrial respiration*. Science (Washington DC), 168: 1102, 1970.

43. Meisner H. and Chan S., *Ochratoxin A an inhibitor of mitochondrial transport*, Biochem., 13: 2759, 1974.

44. Wei Y.-H., Lu C.-Y., Lin T.-N. and Wei R.D., *Effect of ochratoxin A on rat liver mitochondrial respiration and oxidative phosphorylation*. Toxicology, 36: 119, 1985.

45. Omar R.F., Hasinoff B.B., Mejilla F. and Rahimtula A.D., *Mechanism of ochratoxin A stimulated lipid peroxidation*, Biochem. Pharmacol., 40 (6): 1183, 1990.

46. Hasinoff B.B., Rahimtula A.D. and Omar R.F., *NADPH-cytochrome-P-450 reductase promoted hydroxyl radical production by the iron(III)-ochratoxin A complex*, Biochemica et Biophysica Acta, 1036: 78, 1990.

47. Hansen C.E., Dueland S., Drevon C.A. and Størmer F.C., *Metabolism of ochratoxin A by primary cultures of rat hepatocytes*. Appl. Environ. Microbiol., 43 (6): 1267-1271, 1982.

48. Størmer F.C., Støren O., Hansen C.E., Pederson J.I., and Aasen A.J., *Formation of (4R)- and (4S)-4-hydroxyochratoxin A and 10-hydroxyochratoxin A by rabbit liver microsomes*. Appl. Environ. Microbiol., 45: 1183-1187, 1983.

49. Størmer F.C., Hansen C.E., Pederson J.I., Hvistendahl G. and Aasen A.J., *Formation of (4R)- and (4S)-4-hydroxyochratoxin A by liver microsomes from various species*. Appl. Environ. Microbiol., 42: 1051-1056, 1981.

50. Moroi K., Suzuki S., Kuga T., Yamazaki M. and Kanisawa M., *Reduction of ochratoxin A toxicity in mice treated with phenylalanine and phenobarbital*, Toxicol. Lett., 25: 1-5, 1985.

51. Stein A.F., Phillips T.D., Kubena L.F. and Harvey R.B., *Renal tubular secretion and readsorption as factors in ochratoxicosis: Effects of probenecid on nephrotoxicity*. J. Toxicol. Environ. Health, 16: 593, 1985.

52. Beardall, J.M., and Miller, J.D., *Diseases in humans with mycotoxins as possible causes*. In *Mycotoxins in grain: compounds other than aflatoxin.*, edited by J.D.

Miller and H.L. Trenholm., p. 487-539, Eagan Press, USA, 1994, and references cited therein.

53. Krishnamachari, K.A.V.R., and Bhat, R.V., *Poisoning by ergoty bajra (pearl millet) in man.*, Indian J. Med. Res., 64 (11):1624-1628, 1976.

54. Bhat, R.V., Roy, D.N., and Tulpule, P.G., *The nature of alkaloids of ergoty pearl millet and its comparison with alkaloids of ergoty rye and ergoty wheat.* Toxicol. Appl. Pharmacol., 36 (1): 11-17, 1976.

55. Van Rensburg, S.J., and Altenkirk, B., *Claviceps purpurea - Ergotism.* in *Mycotoxins.* edited by I.F.H. Purchase, p.69-96, Elsevier, Amsterdam, 1974.

56. Pohland, A.E., *Mycotoxins in review.* Food. Addit. Contam., 10 (1): 17-28, 1993.

57. Porter, J.K., *Ergot alkaloids and alkaloids from other endophytes, responsible for causing toxic syndrome in cattle after eating contaminated grass*, Prikl. Biokhim. Microbiol., 29 (1): 51-55, 1993.

58. Schneider, D.J., Miles, C.O., Garthwaite, I., Van Halderen, A., Wessels, J.C., Lategan, H.J., *First report of field outbreaks of ergot-alkaloid toxicity in South Africa.* Onderstepoort J. Vet. Res., 63 (2): 97-108, 1996.

59. Meyler, W.J., *Side effects of ergotamine.* Cephalalgia, 16 (1): 5-10, 1996.

60. Arafat, W., and Musa, M.N., *Patulin-induced inhibition of protein synthesis in hepatoma tissue culture.* Res. Commun. Mol. Pathol. Pharmacol., 87(2): 177-186, 1995.

61. Grove, J.F., *Non-macrocyclic trichothecenes,* Natural Products Reports, 187-209, 1988.

62. Grove, J.F., *Non-macrocyclic trichothecenes, Part 2,* Prog. Chem. Org. Nat. Prod., 69: 1-70, 1997 and references cited therein.

63. Van Egmond, H.P., *Aflatoxin M_1: occurrence, toxicity, Regulation.* in: *Mycotoxins in Dairy Products*, edited by H.P. van Egmond, p. 11-55, Elsevier Science Publishers LTD., England, 1989.

64. Meloche, J.L., and Smith, T.K., *Altered tissue amino acid metabolism in acute T-2 toxicosis.* Proc. Soc. Exp. Biol. Med., 210 (3): 260-265, 1995.

65. Joffe, A.Z., *Fusarium poae and F. sporotrichioides as principal causal agents of alimentary toxic aleukia.* in *Mycotoxic fungi, mycotoxins, mycotoxicoses - an encyclopedic handbook* vol. 3. p. 21-86, Marcel Dekker, New York, 1978.

66. Williams, P.P., *Effects of T-2 mycotoxin on gastrointestinal tissues: a review of in vivo and in vitro models.* Arch. Environ. Contam. Toxicol., 18(3): 374-387, 1989.

67. Lafarge-Frayssinet, C., Decloitre, F., Mousset, S., Martin, M., and Frayssinet, C., *Induction of DNA single strand-breaks by T2-toxin, a trichothecene metabolite of Fusarium. Effect on lymphoid organs and liver.* Mutat. Res., 88 (2):115-123, 1981.

68. Stob, M., Baldwin, R.S., Tuite, J., Andrews, F.N., and Gilette, K.G., *Isolation of an anabolic uterotropic compound from corn infected with Gibberella zeae.* Nature (London), 196: 1318, 1962.

69. Pfohl-Leszkowicz, A., Chekir-Ghedira, L., and Bacha, H., *Genotoxicity of zearalenone, an estrogenic mycotoxin: DNA adduct formation in female mouse tissues.* Carcinogenesis, 16(10): 2315-2320, 1995.

70. Riley, R.T., Goeger, D.E., Yoo, H., and Showker, J.L., *Comparison of three tetrameric acids and their ability to alter membrane function in cultured skeletal muscle cells and sarcoplasmic reticulum vesicles.* Toxicology and Applied Pharmacology, 114: 261-267, 1992.

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71. Porter, J.K., Norred, W.P., Cole, R.J., and Dorner, J.W., *Neurochemical effects of cyclopiazonic acid in chickens*. Proc. Soc. Exp. Biol. Med., 187: 335-340, 1988.
72. Agarwal, S.S., Phadke, S.R., Fredlund, V., Viljoen, D., and Beighton, P., *Mseleni and Handigodu familial esteoarthropathies: syndromic identity?*, Am. J. Med. Genet., 72 (4): 435-439, 1997.
73. Viljoen, D., Fredlund, V., Ramesar, R., and Bieghton, P., *Brachydactylous dwarfs of Mseleni*. Am. J. Med. Genet., 46 (6): 636-640, 1993.
74. Ballo, R., Viljoen, D., Machado, M., Keene, D., Horton, W., Fredlund, V., Jacobs, M., Martell, R., Beighton, P., and Ramesar, R., *Mseleni joint disease - a molecular genetic approach to defining the aetiology*. S. Afr. Med. J., 86 (8): 956-958, 1996.
75. Sokoloff, L., *Acquired chondronecrosis*. Ann. Rheum. Dis., 11 (2): 262-264, 1990.
76. Zhai, S.S., Kimbrough, R.D., Meng, B., Han., J.Y., LeVois, M., Hou, X., and Yin, X.N., *Kashin-Beck disease a cross-sectional study in seven villages in the People's Republic of China*. J. Toxicol. Environ. Health, 30 (4):239-259, 1990.