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MYCOTOXINS OF ASPERGILLI; EXPOSURE AND HEALTH EFFECTS

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1. ABSTRACT

Mycotoxins derived from Aspergilli can be encountered both in domestic and occupational environments, and the exposure may lead to severe health hazards. Several *Aspergillus* species are associated with mycotoxin production: *A. ochraceus* with ochratoxin A, *A. fumigatus* with fumitremorgins, gliotoxin and verrucologen, *A. versicolor* with sterigmatocystin, and *A. flavus* and *A. parasiticus* with aflatoxins. Sterigmatocystin may also be produced by *A. flavus*, *A. nidulans*, *A. rugulosus*, and *A. unguis*. Exposure to mycotoxin may occur via enteric, inhalation or direct contact to skin and mucosa. Acute and chronic disorders, irritation, systemic reactions and even cancer may develop after the exposure to these toxins. Mycotoxins act as immunosupressants which may be in association with an increased prevalence of repeated infections found among the inhabitants of buildings with moisture problems.

2. INTRODUCTION

Mycotoxins are "natural products of fungi that evoke a toxic response when introduced in low concentrations to higher vertebrates by a natural route" (1). Mycotoxins are able to cause acute and chronic health effects in humans and animals that cannot be attributed to fungal infection or allergic reactions (2). The over 400 known mycotoxins are all complex organic compounds, most with molecular weights between 200 to 800 kD (3), and are not volatile at ambient temperatures.

All known mycotoxins are fungal secondary metabolites, which means that mycotoxin production need not be correlated with the growth and proliferation of the producing species. However, mycotoxin production is determined by the factors like induction, end-product inhibition, catabolite repression and phosphate regulation (4, 5). Therefore, even though some fungi can grow on almost any natural or synthetic materials, mycotoxin production occurs preferentially on materials that both allow these fungi to grow and provide the conditions for mycotoxin production.

3. PRODUCTION OF MYCOTOXINS

The production of mycotoxins has been long established in fungal isolates derived from agricultural environments. There is abundant data about the fungal species that are capable of producing each known mycotoxin, and about the growth media and conditions that induce production (6-9). It is known that the same sub-species might include both strains that produce mycotoxins and strains that lack this ability (4, 5). It has also been established that many of the known mycotoxin-producers are frequent colonizers in indoor environments (10-12). However, only very little is known about the presence of mycotoxins in indoor environments. In recent years the presence of a few selected mycotoxins have been verified in crude building materials contaminated with fungi (8, 13-17). In fact, most mycotoxins have yet to be extracted from either air samples or bulk materials derived from indoor environments.

Risk-assessment on the inhalation of mycotoxins cannot be made from the analysis of bulk samples of construction materials, in spite of that dose-response of airborne mycotoxin to humans is known. However, as most of the fungi isolated from damp indoor environments can elicit allergenic reactions in addition to being toxic (18), it seems that care should be exercised when moisture-damaged sites are torn down or renovated. Sterigmatocystin is an IARC class 2B carcinogen (9) and also has immunotoxic properties (7). In a recent study (19), *Aspergillus versicolor* and *Stachybotrys chartarum* were implicated as causes for building associated pulmonary disease in three adjacent office buildings. *A. versicolor* was found to be the predominant microbe in the indoor air while *S. chartarum* was isolated from bulk samples containing satratoxins (2-5 µg/g) . Unfortunately, sterigmatocystin

could not be isolated in that study, due to peak-interference in HPLC-UV. Previous to this study, satratoxins have been found in building materials by Johanning *et al*. (16 µg/g), Croft *et al.* (not quantified) and Anderson *et al.* (17 µg/g) (8,13,20). Recently, we extracted sterigmatocystin from water-damaged building materials contaminated by fungi (21).

4. MYCOTOXIN ANALYSES

Mycological analyses of air and crude building materials are routinely performed in environmental laboratories to evaluate the extent and spread of damage in buildings with moisture-problems, and to assess the risk to residents. The isolation of toxigenic species does not substantiate the presence of mycotoxins. Even though strains of fungi probably responsible for producing the mycotoxin are not recovered in culture, the isolation of related microbes and the demonstration of mycotoxins in bulk materials should be considered significant (22). In this context, it is essentila that the sources of mycotoxic fungal contamination should be removed and necessary precautions should be taken to prevent exposure to potentially harmful aerosolized particles during renovation of buildings with moisture-damage.

As the techniques to collect and analyze airborne propagules improve, mycotoxins can be analyzed more precisely from indoor air, which may enable us to assess the possible health consequences of mycotoxins to residents of water-damaged buildings. In future, the ubiquitous nature of mycotoxins in indoor environments can be established with added techniques to study more mycotoxins and their effects. There are specific techniques available to analyze most fungi present in environmental samples. Identifying the fungi responsible for producing mycotoxins in building materials will require using different techniques in combination with the enrichment and purification of fungi from building materials, and extracting toxins from the isolates for mycotoxin analyses.

5. ASPERGILLUS AND MYCOTOXINS

Several *Aspergillus* species are associated with mycotoxin production: *A. ochraceus* Wilhelm (ochratoxin A), *A. fumigatus* Fresenius (fumitremorgins, gliotoxin, verrucologen), *A. versicolor* (Vuillemin) Tiraboschi (sterigmatocystin), *A. flavus* Link (aflatoxins), and *A. parasiticus* Speare (aflatoxins) (2, 23-25). Sterigmatocystin may also be produced by *A. flavus*, *A. nidulans*, *A. rugulosus*, and *A. unguis* (6, 7).

Aflatoxins (AFs) are a group of heterocyclic, oxygen-containing mycotoxins that possess the bisdifurano ring system common to aflatoxin-precursors including sterigmatocystin (26). AFs are produced by certain strains of *A. flavus* and *A. parasiticus* via a biosynthesis route including sterigmatocystin (AFB_1, AFG_1) or dihydrosterigmatocystin (AFB_2, AFG_2) as the immediate precursor (26). *A. versicolor* lacks the enzymatic pathway necessary to convert sterigmatocystin and dihydrosterigmatocystin to the corresponding AFs. Therefore, strains of *A. versicolor* are known to emit sterigmatocystin in large quantities (7, 23).

Other potent mycotoxins characteristic to *Aspergillus* spp. are the ochratoxins, particularly ochratoxin A. Citrinine has also been reported to occur in *Aspergillus* spp., some of which may be from misidentified strains of *Penicillium citrinum*, which is a potent producer of this mycotoxin (7, 23).

Production of AFs is known to coincide with the occurrence of *A. flavus* and *A. parasiticus* in damp, warm conditions (7, 27). Storage of corn and other food and feed in such conditions is known to be susceptible to AF production (27). *A. versicolor*, on the other hand, seems to be better adapted to conditions prevailing in damp building materials in water-damaged buildings, and has frequently been reported to coincide with the occurrence of sterigmatocystin in such residences (14, 19, 28). There are also indications that ochratoxin A and citrinine can occur in indoor environments (29), but as of yet, food and feed has been the source of most ochratoxin A and citrinine, as well as AFs (9, 30).

Airborne concentration of aflatoxin B1 found in dust collected during harvest and grain unloading has ranged from 0.04 to 92 ng/m^3. Higher levels of aflatoxin B1 has been detected in the airborne dust samples collected from enclosed animal feeding buildings (5-421 ng/m^3) and during bin cleaning (124-4849 ng/m^3). Aflatoxin B1 up to 5100 ng/g were found in settled dust collected from an enclosed animal feeding building (31).

The presence of aflatoxin in respirable airborne peanut dust has been investigated (32). Dust concentrations varied from 10.5 to 65.1 mg/m^3, and airborne aflatoxin B1 concentrations reached a maximum of 7.6 ng/m^3.

Aflatoxins are potent carcinogens, produced by *A. flavus* and *A. parasiticus* in grains and seeds such as peanuts, maize, nuts and oilseeds. Ochratoxin A is nephrotoxic and probable carcinogen as well. It is produced by *Penicillium verrucosum* in cereal grains in cold climates, by *A. carbonarius* in grapes, wines and vine fruits, and by *A. ochraceus* occasionally in coffee beans.

In indoor climates, potentially toxinogenic strains belonging to different *Aspergillus* species commonly occur simultaneously, particularly in buildings with a history of water damage (28).

6. INHALATION EXPOSURE

Inhalation exposure to mycotoxins occurs by inhaling airborne particulates containing mycotoxins, including dust and fungal components. In agricultural settings, mycotoxicoses in both farm animals and humans can result from oral, dermal or inhalation exposure of mycotoxin-contaminated grain or dust (3, 33-38). In laboratory mammals, symptoms can be induced by systemic, oral, dermal, subcutaneous or inhalation exposure (9, 39), with inhalation exposure often being several orders of magnitude more toxic than dermal or even systemic administration (40-42).

Toxigenic fungi have been isolated from building materials and air samples in buildings with moisture problems, where the residents have suffered from non-specific symptoms possibly related to mycotoxin production, such as cough, irritation of eyes, skin and respiratory tract, joint ache, headache and fatigue (11, 14, 19, 43-48).

Aspergillus toxins may have an important role in causing direct effects in respiratory tract: damage of epithelia and mucous membranes can lead to pulmonary disorders. Amitani *et al*. (49) purified and characterized cilioinhibitory factors of *A. fumigatus*. A low-molecular weight cilioinhibitory factor was characterized by mass spectrometry as gliotoxin. Gliotoxin significantly slowed ciliary beat frequence in association with epithelial damage at concentration above 0.2 µg/ml. In the same investigation, fumagillin and helvolic acid from *A. fumigatus* were also cilioinhibitory but at much higher concentarion. This may have an impact in developing respiratory distress during *Aspergillus* contamination in respiratory tract.

Mycotoxins can also provoke systemic reactions. Tremorgenic toxins from Aspergilli are able to cause symptoms via the central nervous system. Nausea, fever and fatigue are often associated with exposure to toxic fungi (14). In addition, some toxins are able to cause immunosuppression which in turn may lead to repeated infections (42).

Nasal exposure with aflatoxin B1 aerosol in rats suppressed alveolar macrophage phagocytosis at an estimated dose of 16.8 ug/kg with the effect persisting for approximately 2 weeks (50). Renal failure associated with respiratory distress was demonstrated in two farmers who had been exposed to ochratoxin A produced by *A. ochraceus* (51).

7. FUTURE DIRECTIONS

Exposure to microbes occurs both in occupational settings and in domestic environments. Bioaerosols are able to become a health risk to exposed persons who can develop allergic or toxic symptoms in respiratory tract, eyes or skin. Systemic reactions, disorders in central nervous systems and even cancer may develop due to the mycotoxin exposure. Exposure to toxin producing microbes increase the health risk and even small amounts of microbial contamination may lead to fatal outcomes. Development of new techniques to determine mycotoxin producing microbes and measure different toxins in work environments are utmost important in the near future. Risk assessment of mycotoxin exposure has to be more precise both in occupational and domestic settings. More information has to be achieved from research to practice about the pathogenesis of toxin-induced diseases in order to improve the diagnosis of these health outcomes and to prevent further new cases of disorders.

8. REFERENCES

1. Bennett J: Mycotoxins, mycotoxicoses, mycotoxicology and Mycopathologia [editorial]. *Mycopathologia* 100(1), 3-5 (1987)

2. Hendry K & E. C. Cole EC: A review of mycotoxins in indoor air. *J Toxicol Environ Health* 38(2), 183-98 (1993)

3. Samson R: Mycotoxins: a mycologist's perspective. *J Med Vet Mycology* 30, Suppl 1, 9-18 (1992)

4. Bu´Lock J: Mycotoxins as secondary metabolites. Academic Press NY (1980)

5. Betina V: Mycotoxins as secondary metabolites. In: Developments in food science 8 - Mycotoxins. Production, isolation, separation and purification. Ed: Betina V, Elsevier, Amsterdam (1984)

6. Kurata H & Y. Ueno: Developments in Food Science 7 - Toxigenic fungi - their toxins and health hazard. Elsevier Publishing Ltd, Amsterdam (1983)

7. Betina V: Developments in Food Science 8 - Mycotoxins, production, isolation, separation and purification. Elsevier, Amsterdam (1984)

8. Croft W, B. B. Jarvis & C. S. Yatawara: Airborne outbreak of trichothecene toxicosis. *Atm Environ* 20, 549-552 (1986)

9. IARC: Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins. WHO, Lyon, France (1993)

10. Pasanen A, T. Juutinen, M. Jantunen & P. Kalliokoski: Occurrence and moisture requirements of microbial growth in building materials. *International Biodeterioration and Biodegradation* 30, 273-283 (1992)

11. Rautiala S, T. Reponen, A. Hyvärinen, A. Nevalainen, T. Husman & A. Vehviläinen A: Exposure to airborne microbes during the repair of moldy buildings. *Am Ind Hyg Assoc* J 57(3), 279-84 (1996)

12. Miller J, A. M. Laflamme, Y. Sobol, P. Lafontaine & R. Greenhalg: Fungi and fungal products in some canadian houses. *International Biodeterioration* 24, 103-120 (1988)

13. Andersson M, M. Nikulin, U. Köljalg, M. C. Andersson, F. Rainey & K. Reijula K: Bacteria, molds, and toxins in water-damaged building materials. *Appl Environ Microbiol* 63(2), 387-93 (1997)

14. Johanning E, R. Biagini, D. Hull, P. Morey, B. Jarvis & P. Landsbergis: Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. *Int Arch Occup Environ Health* 68(4), 207-18 (1996)

15. Tuomi T, L. Saarinen, S. Lappalainen, O. Lindroos, M. Nikulin & K. Reijula: Trichothecene mycotoxins in some water-damaged buildings. In: Proceedings of the 3rd International Conference on Bioaerosols, Fungi and Mycotoxins; 1999. Ed: Johanning E, Saratoga Springs, NY (1999)

16. Gravesen S, P. A. Nielsen, R. Iversen & K. F. Nielsen: Microfungal contamination of damp buildings. Examples of risk constructions and risk materials. *Environ Health Perspect* 107 (3), 505-508 (1999)

17. Smoragiewicz W, B. Cossette, A. Boutard & K. Krzystyniak: Trichothecene mycotoxins in the dust of ventilation systems in office buildings. *Int Arch Occup Environ Health* 65(2), 113-7 (1993)

18. Day J: Allergic respiratory responses to fungi. In: The mycota VI. Human and animal relationships. Heidelberg: Springer-Verlag Berlin (1996)

19. Hodgson M, P. Morey, W. Y. Leung, L. Morrow, D. Miller & B. Jarvis: Building-associated pulmonary disease from

exposure to *Stachybotrys chartarum* and *Aspergillus versicolor* . *J Occup Environ Med* 40(3), 241-9 (1998)
20. Johanning E, R. Biagini, D. Hull, P. Morey, B. Jarvis & P. Landsbergis: Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. *Int Arch Occup Environ Health* 68(4), 207-18 (1996)
21. Tuomi T, K. Reijula, T. Johnsson, K. Hemminki, E. Hintikka & O. Lindroos O: Mycotoxins in crude building materials from water-damaged buildings. *Appl Environ Microbiol* 66(5),1899-904 (2000)
22. Tuomi T, L. Saarinen, K. Reijula: Detection of polar and macrocyclic trichothecene mycotoxins from indoor environments. *Analyst* 123,1835-1841 (1998)
23. Frisvad J: The connection between the Penicillia and Aspergilli mycotoxins with special emphasis on misidentified isolates. *Arch Environ Contamin Toxicol* 18(3), 452-67 (1989)
24. Frisvad J & S. Gravesen: Penicillium and Aspergillus from danish homes and working places with indoor air problems: identification and mycotoxin determination. Elsevier Science BV, Amsterdam (1994)
25. Fischer G, T. Müller, R. Ostrowski & W. Dott: Mycotoxins of *Aspergillus fumigatus* in pure culture and in native bioaerosols from compost facilities. *Chemosphere* 38(8), 1745-1755 (1999)
26. Sweeney M, A. D. Dobson: Mycotoxin production by Aspergillus, Fusarium and Penicillium species. *Int J Food Microbiol* 43(3), 141-58 (1998)
27. Ellis W, J. P. Smith, B. K. Simpson, H. Ramaswamy & G. Doyon: Growth of and aflatoxin production by *Aspergillus flavus* in peanuts stored under modified atmosphere packaging (MAP) conditions. *Int J Food Microbiol* 22 (2-3), 173-87 (1994)
28. Tuomi T, T. Johnsson, E. Hintikka & K. Reijula: Detection of aflatoxins (G1-2, B1-2), sterigmatocystin, citrinine and ochratoxin A in samples contaminated by microbes. *Analyst* 126 (9), 1545-1550 (2001)
29. Richard J, R. D. Plattner, J. May & S. L. Liska: The occurrence of ochratoxin A in dust collected from a problem household. *Mycopathologia* 146(2), 99-103 (1999)
30. Trucksess M: Mycotoxins. *J AOAC Int* 84(1), 202-211 (2001)
31. Selim M, A.M. Juchems, W. Popendorf: Assessing airborne aflatoxin B1 during on-farm grain handling activities. *Am Ind Hyg Assoc* J 59(4), 252-256 (1998)
32. Sorenson W, W. Jones, J. Simpson & J. I. Davidson: Aflatoxin in respirable airborne peanut dust. *J Toxicol Environ Health* 14(4), 525-353 (1984)
33. Ciegler A & J. W. Bennet: Mycotoxins and mycotoxicoses. *BioScience* 30(8), 512-515 (1980)
34. Berry C: The pathology of mycotoxins. *J Pathol* 154(4), 301-311 (1988)
35. Corrier D: Mycotoxicosis: mechanisms of immunosuppression. *Vet Immunol Immunopathol* 30(1), 73-87 (1991)
36. Hintikka E & M. Nikulin: Airborne mycotoxins in agricultural and indoor environments. *Indoor Air* 4, 66-70 (1998)
37. Pohland A: Mycotoxins in review. Food Addit Contamin 10(1), 17-28 (1993)
38. Sorenson W: Mycotoxins toxic metabolites of fungi. In: Fungal infections and immune responses. Ed: Murphy J, Plenum Press, NY (1993)
39. WHO: Environmental health criteria 105. Selected mycotoxins: ochratoxins, trichothecenes, ergot. WHO, Geneva (1990)
40. Pang V, R. J. Lambert, P. J. Felsburg, V. R. Beasley, W. B. Buck & W. M. Haschek: Experimental T-2 toxicosis in swine following inhalation exposure: clinical signs and effects on hematology, serum biochemistry, and immune response. *Fundam Appl Toxicol* 11(1), 100-9 (1988)
41. Creasia D, J. D. Thurman, L. J. Jones, M. L. Nealley, C. G. York & R. W. Wannemacher: Acute inhalation toxicity of T-2 mycotoxin in mice. *Fundam Appl Toxicol* 8(2), 230-235 (1987)
42. Nikulin M, K. Reijula, B. Jarvis, P. Veijalainen, E. L. Hintikka: Effects of intranasal exposure to spores of *Stachybotrys atra* in mice. *Fundam Appl Toxicol* 35(2), 182-188 (1997)
43. Miller J: Fungi as contaminants in indoor air. *Atmos Environ* 26A, 2163-2172 (1992)
44. Auger PL, P. Gourdeau & J. D. Miller: Clinical experience with patients suffering from a chronic fatigue-like syndrome and repeated upper respiratory infections in relation to airborne molds. *Am J Ind Med* 25(1), 41-42 (1994)
45. MMWR: Acute pulmonary hemorrhage/hemosiderosis among infants - Cleveland, January 1993 - November 1994. *Morb Mortal Wkly Rep* 881-883 (1994)
46. MMWR: Acute pulmonary hemorrhage among infants - Chicago, April 1992 -November 1994. *Morb Mortal Wkly Rep* 67-74 (1995)
47. MMWR: Update: Pulmonary hemorrhage/Hemosiderosis among infants -Cleveland, Ohio, 1993-1996. *Morb Mortal Wkly Rep* 33-35 (1997)
48. Smith J, J. G. Anderson, C. W. Lewis & Y. M. Murad: Cytotoxic fungal spores in the indoor atmosphere of the damp domestic environment. Fems Microbiol Letters 79(1-3), 337-43 (1992)
49. Amitani R, G. Taylor, E. N. Elezis, C. Llewellyn-Jones, J. Mitchell & F. Kuze F: Purification and characterization of factors produced by *Aspergillus fumigatus* which affect human ciliated respiratory epithelium. *Inf Immun* 63(9), 3266-3271 (1995)
50. Jakab G, R. R. Hmieleski, A. Zarba, D. R: Hemenway & J. D. Groopman: Respiratory aflatoxicosis: suppression of pulmonary and systemic host defenses in rats and mice. Toxicol Appl Pharmacol 125(2), 198-205 (1994)
51. Di-Paolo N, A. Guarnieri, G. Garosi, G. Sacchi, A. M. Mangiarotti & M. Di-Paolo: Inhaled mycotoxins lead to acute renal failure. Nephrology, Dialysis, Transplantation : Official Publication of the European Dialysis and Transplant Association - European Renal Association 9(4), 116-20 (1994)

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