Fumonisins are water soluble mycotoxins produced by several species of *Fusarium* fungi. The toxins are often found as contaminants of corn, apparently as a result of the endophytic relationship between corn and *F. moniliforme* (Bacon and Hinton, 1996), i.e. the fungus lives within the tissues of the corn plant, and may actually impart some beneficial qualities to the corn, such as enhanced disease or insect resistance. Fumonisins, however, are harmful to animals that consume contaminated corn, in particular horses and swine, and may be related to the high rates of esophageal cancer that occur in some parts of the world where corn is consumed as a staple.

Fumonisins are specific inhibitors of the enzyme ceramide synthase (sphinganine and sphingosine N-acyltransferase), which is a key enzyme in the pathway leading to formation of sphingomyelin and complex sphingolipids (Wang et al., 1991). The disruption of this pathway has several important implications for cellular function and there exists strong evidence suggesting that the toxicological properties of fumonisins are actually due to altered sphingolipid metabolism. In this paper, the nature of fumonisin toxicity, and how these effects may be related to altered signaling pathways that are mediated through sphingoid bases and their metabolites, ceramides and more complex sphingolipids will be discussed.

**Toxicity of Fumonisins**

*Leucoencephalomalacia.* Fumonisins, or at least the predominant molecular forms, fumonisins B₁ and B₂ (FB₁, FB₂), have been shown to be causative agents of equine leucoencephalomalacia (LEM), a fatal disease of horses and other equines. The disease has been recognized at least since the early 1900's as 'moldy corn poisoning', and is characterized by lethargy, head pressing, circling, staggering, profuse sweating, and finally death. Areas of hemorrhage and liquefactive necrosis are observed in the brain at necropsy. The syndrome has been reproduced experimentally by feeding corn naturally contaminated with fumonisins (Wilson et al., 1992), as well as by the administration of purified FB₁ (Kellerman et al., 1990). LEM was thought to only occur in horses and other equines, but recently signs of leucoencephalomalacia and hemorrhage were observed in the brains of rabbits dosed with FB₁ (Bucci et al., 1996).

*Porcine Pulmonary Edema.* Porcine pulmonary edema (PPE) is another unique toxicological response to fumonisins, apparently occurring only in swine. This fatal disease develops when pigs consume diet containing *F. moniliforme* or fumonisins, and is characterized by pulmonary edema and hydrothorax (Harrison et al., 1990). The conspicuous presence of lamellar whorls in pulmonary interstitial macrophages (PIMs) of affected pigs led Hashem et al. (1992) to propose that the pulmonary edema was secondary to phagocytosis of membranous material that originates in fumonisin damaged liver or other organs and enters the circulation. Since pigs have large numbers of PIMs relative to other species, this theory was hypothesized to account for the species specificity of PPE.

*Hepatosis, Nephrosis and Hepatocarcinoma.* In addition to the species-specific effects of fumonisins, the toxins cause effects that are common for many animals. The most common target organ of all species studied is liver, and kidney also appears to be a target organ in several species, including rats, mice, horses, swine, sheep and rabbits. The lesions
observed in tissues of fumonisin-treated animals include degenerated hepatocytes, single cell necrosis, increased numbers of mitotic cells, focal necrosis, inflammatory infiltration, bile duct proliferation, fibrosis, and cirrhosis (Voss et al., 1994). Fumonisin also induces apoptosis in vivo in liver and kidney of rats fed the toxin (Tolleson et al., 1996). Although fumonisin is not genotoxic (Norred et al., 1992a), it does have promoting activity and may be an initiator as well (Gelderblom et al., 1992). Fumonisin Bₕ is a potent inducer of gamma glutamyltranspeptidase-positive foci, both in the livers of rats initiated with diethylnitrosamine and in rats exposed to no known initiating substances (Gelderblom et al., 1988). In one study in which 50 ppm FBₕ was fed to rats in a diet that was deficient in several vitamins and lipotropes, liver tumors developed in two thirds of the experimental animals. Long term, multiple dose feeding studies of purified FBₕ to rats and mice are currently underway at the United States National Center for Toxicological Research, and should help more clearly define the carcinogenicity of this mycotoxin.

In Vitro Toxicology. The actions of fumonisins have been investigated in a number of cultured cell lines, as well as in primary hepatocytes and liver slices. In the original studies to ascertain the effects of FBₕ on sphingolipid biosynthesis, Wang et al. (1991) observed the apparent lack of overt cytotoxicity of fumonisin to rat primary hepatocytes. Even when cells were exposed for 4 days to 1 μM FBₕ, there was no decrease in the number of viable hepatocytes relative to controls. The resistance of hepatocytes to fumonisins has also been noted in other studies. Exposure of primary hepatocytes to a very high (10 mM) concentration of FBₕ for 2 hr did not cause release of lactate dehydrogenase, and caused only partial inhibition of valine incorporation (Norred et al., 1991). In rat liver slices, no toxicity was observed after exposure to 100 μM FBₕ for 20 hr, or after exposure to 1 μM for up to 72 hr (Norred et al., 1996). Fumonisin Bₕ does, however, induce apoptosis in vitro, and it is also anti-proliferative for a variety of human cell types (Tolleson et al., 1996).

In contrast to the lack of toxicity produced by fumonisin in liver, Yoo et al. (1992) reported that FBₕ inhibited (10-35 μM) cell proliferation and killed (>35 μM) cells when tested in LLC-PK₁ renal epithelial cells. Interestingly, the dose response for inhibition of ceramide synthase activity by FBₕ after 7 hr exposure to the toxin paralleled the cytotoxicity of FBₕ to the cells which occurred between 3-5 days of exposure. Since LLC-PK₁ cells are a dividing cell line, whereas hepatocytes generally do not divide in culture, Yoo et al. (1992) speculated that proliferating cells are more subject to the toxic effects of FBₕ than non-proliferating cells. A study by Abbas et al. (1993) supports this theory, since fumonisins B₁, B₂ and B₃ were all toxic to three different cell lines, all of which proliferate in culture. In the same report, fumonisin A₁ and A₂, which are N-acetylated forms of FB₁ and FB₂, respectively, had little or no cytotoxicity, whereas the aminopentols AP₁ and AP₂, which are the backbone structures of FB₁ and FB₂ with the tricarballylic acid moiety cleaved off had similar cytotoxicity to the intact fumonisins.

Relationship of Ceramide Synthase Inhibition to Fumonisin Toxicity and Immune Response

It is well established that FB₁, and other fumonisins, are specific, potent inhibitors of ceramide synthase (Wang et al., 1991; Merrill et al., 1993a). Can this fundamental biochemical effect be the underlying cause for the many toxic manifestations of fumonisins that appear in different animals and plants? Evidence is accumulating that suggests that such is the case - that disruption of sphingolipid metabolism can lead to alterations in cell function and regulation, and that the ramifications of these responses can account for the toxicities - even carcinogenicity - that are observed (Merrill, Jr. et al., 1995; Riley et al., 1996; Yoo et al., 1996; Schroeder et al., 1994). LEM, PPE, hepato- and nephrotoxicity, and other in vivo and in vitro lesions that result from fumonisin exposure could all be due to the changes in cell behavior, growth inhibition, increased apoptosis, and cytotoxicity that result from disruption of sphingolipid metabolism. The accumulation of sphingoid bases and their metabolites and the depletion of critical complex sphingolipids can result in inhibition of Na⁺/K⁺ ATPase, inhibition of protein kinase C, release of intracellular Ca²⁺, promotion of retinoblastoma protein dephosphorylation, and induction of apoptosis (Merrill et al., 1995).

Elevated levels of free sphingoid bases, especially sphinganine, have been observed in ponies fed diets containing fumonisins. Furthermore, serum concentrations of complex sphingolipids were reduced (Wang et al., 1992). These changes occur within days of feeding diets containing 22-44 ppm FB₁, and precede the elevations in the serum enzymes aspartate aminotransaminase, gamma glutamyl transaminase and alkaline phosphatase that occur shortly before
death. Indeed, the elevation of serum sphingoid bases has been proposed as a biomarker of fumonisin or toxins with similar modes of action) exposure (Merrill et al., 1993b). Brain tissue is particularly rich in complex sphingolipids, which serve as important structural components of membranes, and it is possible that inhibition of brain ceramide synthase by fumonisins and the resultant depletion of sphingolipids could lead to the necrosis. However, the liquefactive necrosis observed is more likely an indirect effect of fumonisin exposure, since (1) fumonisin does not appear to cross the blood brain barrier, and (2) the lesion is grossly similar to that observed after cerebral hemorrhage, suggesting a vascular origin rather than a direct toxic effect on brain tissues. When ceramide synthase is inhibited by fumonisins, large quantities of sphinganine and sphingosine, which are quite lipophilic and normally occur at very low concentrations in cells, accumulate, cross membranes, and enter the circulatory system. The sphingoid bases are themselves cytotoxic, and thus could damage vascular epithelial cells. Additionally, the depletion of complex sphingolipids caused by fumonisins could damage epithelium, because complex sphingolipids are important in cell-cell communication, and loss of contact between cells could result. The end result of the impaired sphingolipid metabolism and subsequent accumulation of sphingoid bases could be a 'leaky' membrane, leading to spilling of vascular fluid into brain and regional tissue damage. Recent studies by Ramasamy et al. (1995) demonstrated the ability of fumonisin to disrupt the barrier function of cultured porcine pulmonary artery epithelial cells, as measured by increased albumin transfer across monolayers of the cells. Dosing the cells with D-erythro-sphinganine produced a similar increase in albumin transfer, suggesting that the disruption of the barrier function by fumonisin is due to its effects on sphingolipid metabolism. The authors propose that similar fumonisin-induced leaky membranes in vivo could be contributing factors in both PPE and LEM.

A number of studies have demonstrated the carcinogenicity of F. moniliforme to rats (Marasas et al., 1984; Wilson et al., 1985), and others have associated cases of human esophageal cancer to the presence of the fungus and fumonisins in corn and corn-based food (Rheebee et al., 1992; Sydenham et al., 1990). Gelderblom et al. (1988; 1992) reported both tumor promoting and initiating activity of FB1, and in feeding studies rats developed liver tumors when fed 50 ppm purified FB1 (Gelderblom et al., 1991). These effects, too, can be explained on the basis of sphingolipid metabolism disruption. DNA synthesis in cells is stimulated by the addition of sphingoid bases (Zhang et al., 1990), and by exposure to FB1 at doses that elevate sphingoid bases (Schroeder et al. 1994). Other mechanisms related to disruption of sphingolipid metabolism which could be associated with carcinogenesis include activation of growth factor receptors and mitogen activated kinases, induction of apoptosis and cellular differentiation, mobilization of intracellular calcium pools, stimulation/inhibition of protein kinases which regulate cell proliferation, activation of transcription factor AP-1, and cytotoxicity or inhibition of proliferation in normal cells (Riley et al., 1996).

There are many gaps in our knowledge of how cells receive the necessary messages and stimuli that regulate their physiology, growth and nondisruptive, programmed death. Although there is much evidence suggesting that fumonisins and related toxins cause their effects through disruption of sphingolipid biosynthesis, much work remains before such a scenario can be definitively declared as the toxins' mechanism of action. Greatly needed is a more thorough understanding of the ultimate consequences of altered sphingolipid biochemistry, and whether these alterations are the ultimate, underlying cause for the various toxic manifestations of fumonisins that are observed in different animal species.

REFERENCES


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