The significance of sphingoid bases and its 1-phosphates as endogenously bioactive molecules in fumonisin cytotoxicity

Hwan-Soo Yoo
College of Pharmacy, Chungbuk National University

Introduction

Fumonisins are a family of naturally occurring, structurally related compounds (Bezuidenhout *et al.*, 1988) produced by *Fusarium moniliforme*, and related fungi that are prevalent on corn, sorgham, millet and other agricultural products grown throughout the world. Apparent target organs of fumonisin-induced toxicoses are species-specific. There are currently several animal diseases and toxicities associated with fumonisins: equine leukoencephalomalacia (ELEM) in equids, porcine pulmonary edema (PPE) syndrome in pigs, and liver cancer, hepatotoxicity and nephrotoxicity in rats. Fumonisins have been shown to be cytotoxic in a variety of mammalian cell lines (Shier *et al.*, 1991). Cell death caused by fumonisins B_1 and B_2 was observed in some rat hepatoma cell lines (H4TG, H4-II-E, H4-II-E-C3, Fao, MH1C1, MCA-RH 7777 and MCA-RH 8994) and MDCK cells derived from dog kidney distal tubules. Structurally similar to sphingoid bases, fumonisins are potent inhibitors of ceramide synthase in a variety of cell types (Wang *et al.*, 1991). Ceramide synthase (sphingosine and sphinganine N-acyltransferase) is a key enzyme in *de novo* sphingolipid biosynthesis and in the reacylation of free sphingoid bases derived from dietary sources and complex sphingolipid turnover. *In vivo* and *in vitro* studies have shown that fumonisins disrupt sphingolipid metabolism resulting in (i) inhibition of *de novo* sphingolipid biosynthesis, (ii) increased intracellular concentration of free sphinganine and its 1-phosphate, (iii) depletion of complex sphingolipids, and (iv) an increase in sphingoid base degradation products. Sphingolipids can be found in all eucaryotic cells, where they are especially abundant in the plasma membrane and related cell membranes, such as golgi apparatus and lysosomes. There are currently over 300 known sphingolipids with distinct head groups (Bell *et al.*, 1993). In mammalian cells, the most common sphingoid base is D-erythro-sphingosine, and typically, free sphingoid bases are present in very low concentrations, because sphinganine biosynthesized *de novo* is rapidly acylated with a long-chain fatty acid to form N-acylsphinganine (dihydroceramide). Sphingosine and its metabolites have been implicated as

modulators of membrane signal transduction systems and shown to be involved in diverse cellular processes. Sphingosine-1-phosphate, formed from sphingosine by sphingosine kinase, has recently been added to the list of these bioactive sphingolipids. Sphingosine-1-phosphate stimulates cell proliferation in Swiss 3T3 fibroblasts, possibly via induction of intracellular Ca^{2+} mobilization, enhanced phosphatidic acid synthesis or mitogen-activated protein kinase activation (Wu *et al.*, 1995), whereas this phosphorylated sphingoid base inhibits cell motility and invasiveness of certain tumor cells and PDGF-induced chemotaxis of human arterial smooth muscle cells. S1P is recognized as a major mediator of regulation of intracellular calcium. S1P also acts as pro-survival mediator either intracellularly or extracellularly through Edothelial Differentiation Gene receptor (S1P receptor). The purpose of this study was to investigate the mechanistic relationship between the fumonisin-induced cytotoxicity and the altered sphingolipid metabolism in culture cells and mice.

Results and Discussions

Fumonisins are cytotoxic to culture cells and mice. Fumonisin B_1 (35μM) significantly decreased the rate of increase in cell proliferation of LLC-PK_1 cells. There was a lag period of approximately 24-48 hr preceding the inhibition of cell proliferation after treatment with fumonisin B_1. During this lag period cells appeared normal. After 24-48 hr, cells treated with 35 μM fumonisin B_1 began to develop a fibroblast-like appearance (loss of cell-cell contact, elongated, spindle shaped). Unlike studies with CHO cells and Chang liver cells, the toxicity of fumonisin B_1 was easily observed in proliferating LLC-PK_1 cells. Other proliferating cell lines are also sensitive to fumonisins. The rat hepatoma (H4TG) and dog kidney cell lines (MDCK) are highly sensitive to inhibition of cell proliferation by fumonisins B_1 and B_2 (Shier *et al.*, 1991). Thus, in these cultured cell lines, the ability to undergo cell division appears to be a prerequisite for the occurrence of cytotoxicity. A corollary to this is the fact that there is at least a 24-hr lag period from the time that cells are exposed to fumonisin B_1 and inhibition of proliferation, irrespective of the fumonisin B_1 concentrations. The doubling time for LLC-PK_1 cells is approximately 24 hr. In a separate experiment, exposure of confluent monolayers of LLC-PK_1 cells to fumonisin B_1 up to 1 mM for 29 hr had no effect on domes or the normal epithelial-like morphology compared to control cultures. Taken together, these results suggest that either cell division or the buildup of some toxic principle is required for toxicity in LLC-PK_1 cells. ICR mice treated with 10mg/kg fumonisin B1 showed the severe tissue damage in liver and kidney from the light microscopic and electron microscopic observations.

Fumonisin B_1 inhibits *de novo* sphingolipid biosynthesis and induces the elevation of free sphinganine and their 1-phosphate. As the fumonisin B_1 concentrations were increased the incorporation of [^{3}H]serine into sphingosine decreased and incorporation into sphinganine increased. Incubation of LLC-PK_1 cells for 2 hr in [^{3}H]serine was based on preliminary studies in which we found that the ratio of [^{3}H]sphingosine/[^{3}H]sphinganine in untreated LLC-PK_1 cells increased over the first 2 hr of labeling to a ratio of approximately 2.0. A consequence of inhibition of sphinganine *N*-acyl transferase in the *de novo* pathway is an increase in the amount of free sphinganine. ICR mice were injected i.p. with 10mg/kg FB1, and kidney, liver, heart, lung, brain and serum were collected for sphingolipid analysis. The concentrations of sphingosine in control mice were approximately 1595pmol, 898pmol, 651pmol, 642pmol, 563pmol per 100mg wet weight in lung, kidney, liver, brain, heart, brain, liver and kidney, respectively. FB1-treated mice showed that the concentrations of elevated free sphinganine were 10.6nmol, 5.3nmol, 3.4nmol, 2.2nmol, 0.2nmol in kidney, liver, lung, heart and brain, respectively. Thus, these results indicate that 1) both de novo and turnover sphingolipid metabolisms are the most active in lung and 2) the most sensitive organ to FB1 is kidney, while the least sensitive one to FB1 is brain. Sphinganine 1-phosphate elevation following FB1 exposure to mice was highest in kidney and lung, and lowest in brain. FB1 increased sphinganine 1-phosphate concentration by 678pmol/100μl serum compared to 6pmol in control serum. In conclusion, FB1 sensitivity to sphingolipid metabolism is organ-specific and may be related to the fumonisin toxicity.

Fumonisin-induced cell death were related to the elevation of sphinganine 1-phosphate. The increase in intracellular sphinganine and its 1-phosphate after the fumonisin exposure is an early causal event in the fumonisin-induced inhibition of cell growth and increased cell death. Studies in F9 cells with the knock-out gene of sphingosine 1-phosphate lyase showed that the accumulated sphinganine 1-phosphate is essential for the fumonisin cytotoxicity. ISP treatment of LLC-PK_1 cells with the exposure of fumonisin B1 reduced the elevation of sphinganine as well as its 1-phosphate with the reversal of cytotoxicity.

Conclusion

The fumonisin-induced increase in sphinganine and sphinganine 1-phosphate appeared to be related to organ-specific toxicity and may the elevation of sphingolipids may be useful marker for fumonisin toxicity.

References

1. Bell, R.M., Hannun, Y.A., and Merrill, A.H., Jr. 1993. *Advances in Lipid Research: Sphingolipids and Their Metabolites*, Vols. 25-26. Academic Press, Orlando, FL.
2. Bezuidenhout, C.S., Gelderblom, W.C.A., Gorst-Allman, C.P., Horak, R.M., Marasas, W.F.O., Spiteller, G., and Vleggaar, R.1988. *Structure elucidation of the fumonisins, mycotoxins from Fusarium moniliforme.* J. Chem. Soc. Commun. 743-745.
3. Hannun, Y.A., and Bell, R.M. 1989. *Functions of sphingolipids and sphingolipid breakdown products in cellular regulation.* Science 243: 500-507.
4. Shier, W.T., Abbas, H.K., and Mirocha, C.J. 1991. *Toxicity of mycotoxins fumonisins B1 and B2 and Alternaria alternata f. sp. lycopersica toxin (AAL) in cultured mammalian cells.* Mycopathologia 116: 97-104.
5. Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T., and Merrill, A.H.,Jr. 1991. *Inhibition of sphingolipid biosynthesis by fumonisins: Implications for diseases associated with Fusarium moniliforme.* J. Biol. Chem. 266: 14486-14490.
6. Wu, J., Spiegel, S., and Sturgill, T.W. 1995. *Sphingosine-1-phosphate rapidly activates the MAP kinase pathway by a G-protein dependent mechanism.* J. Biol. Chem. 270: 11484-11488.