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Disruption of Sphingolipid Metabolism as a Potential Mechanism of Fumonisin Inhibition of Cell Growth in LLC-PK$_1$ Cells

Hwan-Soo Yoo[1] and Yeo-Pyo Yun[2]

[1]*Pulmonary-Critical Care Medicine Branch, NHLBI, National Institutes of Health, Bethesda, Maryland 20892, U.S.A.*
[2]*College of Pharmacy, Chungbuk National University, Cheong-Ju, 360-763 Korea*

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ABSTRACT : *Fumonisins are a family of mycotoxins produced by the fungus Fusarium moniliforme which is a common contaminant in corn. Fumonisins are potent inhibitors of sphingosine and sphinganine N-acyltransferase (ceramide synthase), key enzymes in sphingolipid metabolism. The purpose of this study was to provide the evidence that the elevated levels of free sphingoid bases (primarily sphinganine) and depletion of complex sphingolipids were closely related to the inhibition of cell growth in LLC-PK$_1$ cells exposed to fumonisin B$_1$ ($\leq$ 35 μM). Concentrations of fumonisin B$_1$ between 10 and 35 μM were known to inhibit cell growth without cytotoxicity in LLC-PK$_1$ cells (Yoo et al. Toxicol. Appl. Pharmacol. 114, 9-15, 1992). Cells exposed to 35 μM fumonisin B$_1$ for 48 and 72 hr developed a fibroblast-like (elongated and spindle-shaped) appearance and were less confluent than normal cells. At between 24 and 48 hr after exposure to fumonisin B$_1$ cells were beginning to show the inhibition of cell growth and at 72 hr the number of viable cells in fumonisin-treated cultures was about 50% of concurrent control cultures. During the 24 hr lag period preceding inhibition of cell growth, the free sphinganine levels in cells exposed to 35μM fumonisin B$_1$ were highly elevated (approximately 230 fold higher than normal cells). The elevated levels of free sphinganine were 435$\pm$14 pmoles/10^6 cells at 48 hr and approximately 333$\pm$11 pmoles/10^6 cells in cells exposed to 35 μM fumonisin B$_1$ at 72 hr, while the levels of free sphinganine in normal cells were less than 2 pmoles/10^6 cells. Under the same condition, depletion of intracellular complex sphingolipids as a consequence of fumonisin inhibition of de novo sphingolipid biosynthesis and turnover pathway was appeared. Content of free sphingoid bases in dividing cells was more elevated than in confluent cells at 24~48 hr after cells were exposed to 20 μM fumonisin B$_1$. The dividing cells were showing the inhibition of cell growth at 48~72 hr and 20 μM fumonisin B$_1$. The results of this study support the hypothesis that the inhibition of cell growth is very well related to the disruption of sphingolipid metabolism in LLC-PK$_1$ cells.*

Key Words : *Fumonisin B$_1$, LLC-PK$_1$ cell, Sphingolipids, Sphinganine*

I. INTRODUCTION

Fumonisins are a group of naturally occurring, structurally related compounds produced by *F. moniliforme*, one of the most commonly occurring fungi in corn (for review see Nelson *et al.*, 1993). There are currently several diseases associated with fumonisins: equine leukoencephalomalacia, porcine pulmonary edema syndrome, and liver cancer, hepatotoxicity and nephrotoxicity in rats. In addition, *F. moniliforme* culture materials have been shown to induce atherosclerosis in non-human primates subsequent to chronic liver toxicity (Fincham *et al.*, 1992).

Fumonisins and the structurally related AAL-toxins produced by *Alternaria alternata* f. sp. *lycopersici* are specific inhibitors of sphingosine and sphinganine *N*-acyltransferase (ceramide synthase); key enzymes in *de novo* sphingolipid biosynthesis and turnover (Wang *et al.*, 1991; Merrill *et al.*, 1993). Inhibition of ceramide synthase results in (i) inhibition of *de novo* sphingosine biosynthesis, (ii) an increased intracellular concentration of free sphinganine, and sometimes free sphingosine, (iii) depletion of complex sphingolipids, (iv) an increase in sphingosine and sphinganine degradation products and (v) an increase in lipid products derived from the increase in sphingoid base degradation products (for review see Merrill *et al.*, 1994).

A previous study with LLC-PK$_1$ cells showed that fumonisin B$_1$ was cytostatic (inhibition of cell growth) at 10-35 μM, and was both cytostatic and

cytotoxic at > 35 μM (Yoo *et al.*, 1992). Inhibition of *de novo* sphingosine biosynthesis as an early event preceded the inhibition of cell growth and occurred long before the cell death in LLC-PK_1 cells exposed to fumonisin B_1 (Yoo *et al.*, 1992). The dose response for the decrease in *de novo* sphingosine biosynthesis at 7 hr closely paralleled the dose response for the effects on cell growth and cell death at 3~5 days. Fumonisin-induced disruption of sphingolipid metabolism was hypothesized to be the cause of the inhibition of cell growth and cytotoxicity in LLC-PK_1 cells.

The fumonisin inhibition of *de novo* sphingosine biosynthesis in LLC-PK_1 cells was accompanied by a large elevation of intracellular free sphingoid bases (Yoo *et al.*, 1992). Greater than 90% of the elevated level of free sphingoid bases was due to an increase in free sphinganine. Similar increase in free sphinganine have been reported in the ponies, pigs, rats and other animals fed diets containing fumonisins (Wang *et al.*, 1992; Riley *et al.*, 1993; Riley *et al.*, 1994). The fumonisin-induced increase in intracellular free sphinganine concentration offers a reasonable mechanism for the observed effects on cell growth and increased cell death in LLC-PK_1 cells. Elevation of intracellular free sphingoid base concentration by exogenous addition of sphingoid bases to animal cells has been shown to inhibit cell growth and be toxic to cells (Merrill, 1991; Spiegel *et al.*, 1993; Merrill *et al.*, 1994).

The purpose of this study was to provide the evidence that elevated levels of free sphingoid bases (primarily sphinganine) and depletion of complex sphingolipids were closely related to the inhibition of cell growth in LLC-PK_1 cells exposed to fumonisin B_1 (< 35 μM). This study was to support the conclusion of earlier published study (Yoo *et al.*, 1992) and link to the future mechanism studies of fumonisin-induced cellular effects (cell death and inhibition of cell growth). The specific objectives of the present work were to determine: (i) the fumonisin inhibition of cell growth, (ii) the fumonisin disruption of sphingolipid metabolism (elevation of free sphingoid bases and depletion of complex sphingolipids) at 35 μM concentration of fumonisin B_1, and (iii) the comparison of free sphinganine elevations between dividing cells and confluent LLC-PK_1 cells exposed to 20 μM fumonisin B_1.

II. MATERIALS AND METHODS

1. Fumonisin and other reagents

Fumonisin B_1 was purchased from the Division of Food Sciences and Technology, CSIR (Pretoria, South Africa). The purity of the South African fumonisin B_1 was estimated to be greater than 90% based on a combination of HPLC and GC-MS (Plattner *et al.*, 1990; Wilson *et al.*, 1990). C_{20}-sphinganine was prepared synthetically (Nimkar *et al.*, 1988). Dulbecco's modified Eagle medium (DMEM), Ham's F12, fetal calf serum (FCS), Dulbecco's phosphate buffered saline (PBS), Hank's balanced salt solution (HBSS, 10X) and trypsin (3116.47 units/mg) were obtained from Gibco BRL, ethylenediamine tetraacetic acid (EDTA, tetrasodium dihydrate) from Calbicohem-Behring Corp. (La Jolla, CA), and 0.4% trypan blue solution from Sigma (St. Louis, MO).

2. Cells

Renal epithelial cells (LLC-PK_1, CRL 1392, passage 1972) were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown and maintained as previously described (Riley *et al.*, 1987). For all experiments with LLC-PK_1 cells, the cells were subcultured at approximately 3000 viable cells/cm^2 in 25-cm^2 flasks containing DMEM/Ham's F12 (1:1) with 5% fetal calf serum at 37°C and 5% CO_2. Cells were allowed to attach and grow for 24 hr prior to addition of fumonisin B_1.

3. Proliferation assay

The effect of fumonisin B_1 on cell growth was determined by counting the viable cells. Culture medium was aspirated, cells were rinsed with 1X HBSS, and 500 μl of 0.5% trypsin-EDTA added (volumes for 25-cm^2 flask), and cells incubated for 20-30 min in a 37°C, CO_2 incubator to detach the cells from the bottom of the flask. Cells were suspended in 5 ml of PBS, 100 μl of cell suspension transfered to the 3 ml of glass tube and 10 μl of 0.4% trypan blue added. Cell number was determined by counting viable (trypan blue-excluding) cells using a hema-

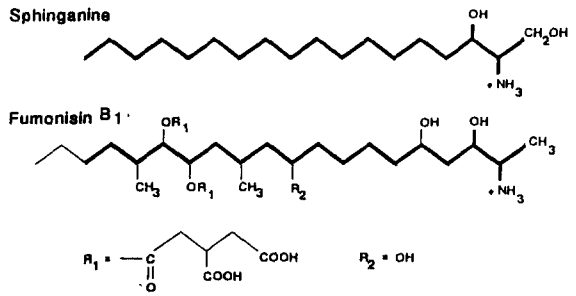

Fig. 1. Structures of fumonisin B_1 and sphinganine. Sphingosine is the same as sphinganine except for one double bond between the C4 and C5.

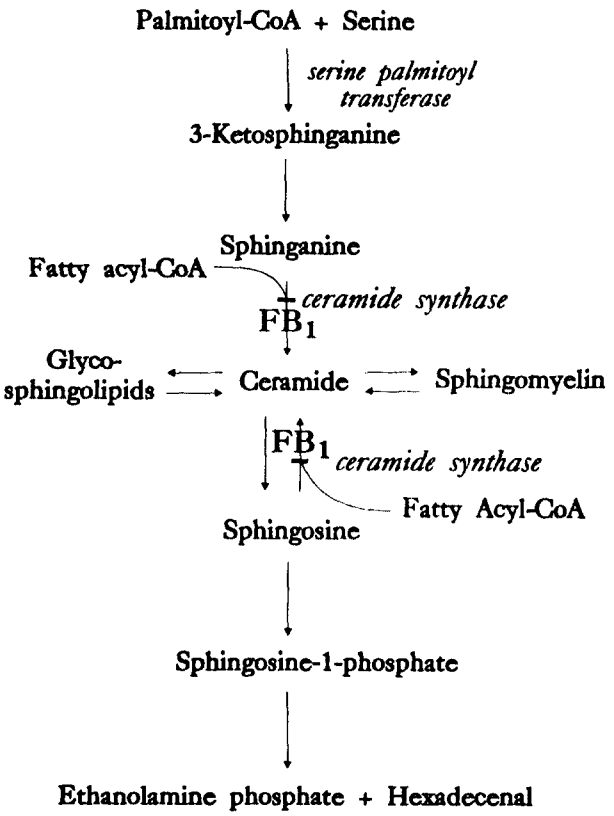

Fig. 2. Flow chart showing the pathways (Merrill and Jones, 1990) for *de novo* sphingolipid biosynthesis, turnover of complex sphingolipids and breakdown of sphingosine (sphinganine). *De novo* sphingolipid biosynthesis occurs in the endoplasmic reticulum. Glycosphingolipids are formed via glycosylation in the golgi apparatus and sphingomyelin is synthesized in the golgi apparatus, the plasma membrane and other organelles. Turnover of complex sphingolipids occurs in the plasma membrane, lysosomes, and endosomes. The steps inhibited by fumonisin B_1 (FB_1) are shown; ceramide synthase (sphingosine and sphinganine *N*-acyltransferase.

tocytometer under the microscope.

4. Free sphingosine (S_o) and sphinganine (S_a) content of cells

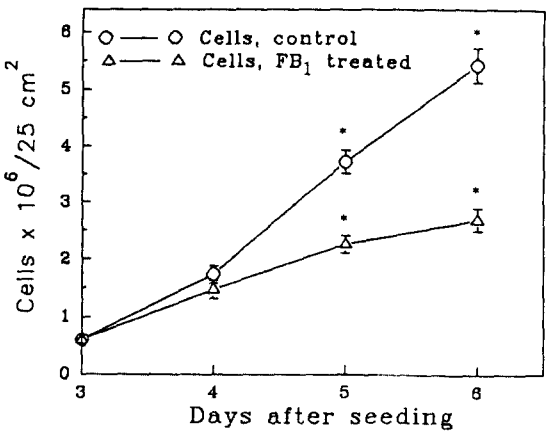

Fig. 3. Fumonisin inhibition of cell growth as measured by the viable cell numbers in 25-cm^2 culture flasks. LLC-PK_1 cells were seeded, allowed to grow for 3 days, medium exchanged, cells dosed with 35 μM fumonisin B_1 and incubated 72 hr. Values are the means (n=3) ± SD of three culture flasks. Asterisks indicate values for fumonisin B_1-treated cultures which are significantly different ($p < 0.05$) from values for control within time groups.

The relative amounts of sphinganine and sphingosine in base-treated cell extracts were determined by HPLC as previously described (Merrill *et al.*, 1988) with C_{20}-sphinganine as an internal standard.

5. Total sphingolipid content of cells

Total sphingolipids were determined by acid-hydrolyzing chloroform-methanol extracts of cells and HPLC analysis as described previously (Merrill *et al.*, 1988). Acid hydrolysis releases all sphingosine and sphinganine contained within complex sphingolipids (ceramides, sphingomyelin, glycosphingolipids, etc.). The sphingosine and sphinganine released after acid hydrolysis are then corrected for free sphingosine and free sphinganine, as determined from base-hydrolyzed samples, to give the complex sphingolipid content (total minus free).

6. Statistics

Unless indicated, all values were expressed as means plus or minus the standard deviation (SD). Difference between treatments were analyzed statistically by analysis of variance using the general linear models procedure (SAS Institute, Inc., 1985). Where indicated, data were fitted to linear (first-order) regression models, and the best fit was decided on the basis of a comparison of the *t* statistics for

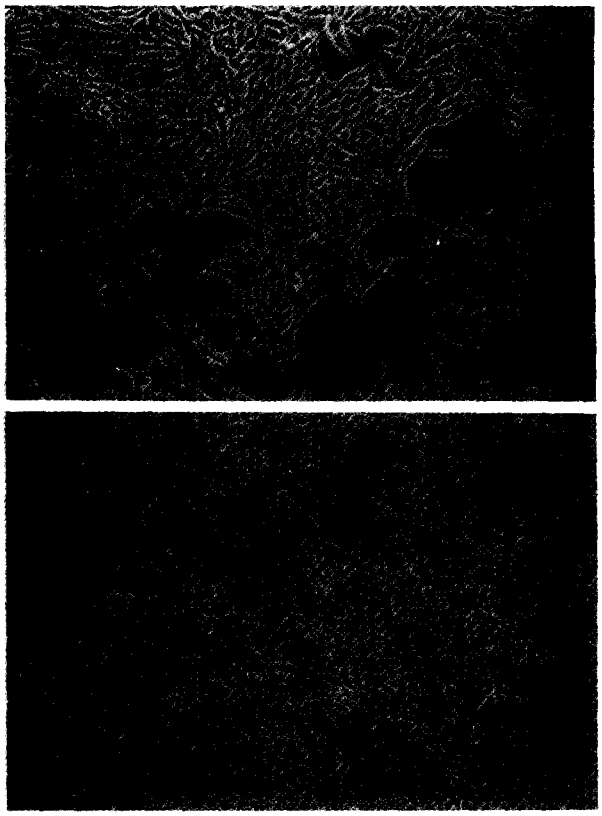

Fig. 4. Comparison of cell morphology between control (above) and 35 μM fumonisin B_1-treated (below) cultures for 72 hr (6 day-old).

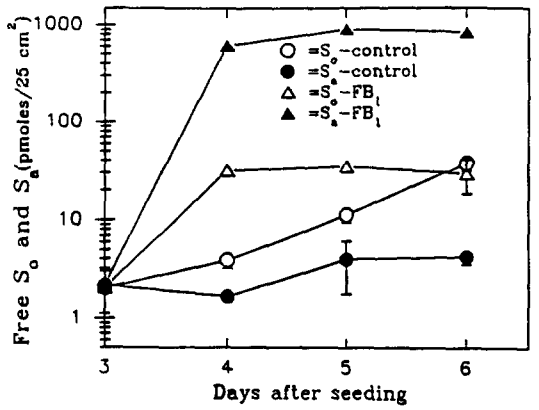

Fig. 5. Fumonisin-induced elevation of free sphingoid bases in 35 μM fumonisin B_1-treated cultures.

Table 1. Free sphingoid base and complex sphingolipid concentrations in normal and 35 μM fumonisin B_1-treated cultures

		Free (pmol/10^6 cells)		Complex (nmol/10^6 cells)	
FB_1 (μM)		S_o	S_a	S_o	S_a
0	24 hr	2±1	1±1	1.2±0.1	0.1±0
	48	1±1	1±1	1.4±0.1	0.3±0.1
	72	6±3	1±1	1.9±0.2	0.2±0
35	24 hr	20±4*	400±10*	0.9±0.1*	0.1±0
	48	11±3*	435±14*	0.5±0.1*	0.1±0*
	72	7±2	333±11*	0.4±0.1*	0.1±0*

These data were based on values obtained as described in the legend to Figures 3,4 and 5, and expressed as either pmol or nmol per million cells. Values are the means (n=3) ±SD. Asterisks indicate values for the 35 μM fumonisin B_1 which are significantly different ($p<0.05$) from the 0 μM fumonisin B_1 values.

the model parameters. Differences with probabilities of < 0.05 were defined as statistically significant.

III. RESULTS

1. Fumonisin B_1-induced inhibition of cell growth occurred in a time-dependent manner with a 24-hr lag period.

At 48 and 72 hr 35 μM fumonisin B_1 significantly decreased the rate of increase in cell number, while at 24 hr fumonisin B_1-exposed cells showed no difference from normal cells in growth kinetics (Fig. 3). The number of viable cells at 24 hr was approximately 1.5×10^6/25-cm^2 flask in both fumonisin B_1-treated and normal cultures. During the 24-hr lag period, cells exposed to 35 μM fumonisin B_1 appeared normal based on the epithelial morphology, growth kinetics (same slope in growth curve as normal cells) and formation of domes (fluid-filled blisters indicative of transepithelial movement of sodium). There was no detached and floating cells in fumonsin-treated culture during the first 24 hr period of fumonisin exposure and no difference in lactate dehydrogenase (LDH) release between normal and fumonisin-treated cultures (unpublished data) indicating no cytotoxicity. At 48 hr the growth curve in fumonisin B_1-exposed cells was beginning to deviate from the normal cell growth curve, lose the normal epithelial cell morphology and appear fibroblast-like (Fig. 4). The number of viable cells in normal culture was approximately 3.8×10^6/25 cm^2 flask, while in 35 μM fumonisin B_1-treated culture approximately 2.2×10^6/25 cm^2 flask (58% of the concurrent control). The rate of cell growth markedly slowed down in fumonisin-treated cultures at 48-72 hr, while the rate of cell growth in normal cultures at 48-72 hr was approximately same as at 0-48 hr. The inhibition of cell growth in fumonisin B_1-treated cultures at 72 hr was approximately 50% of

Table 2. Comparison of free sphingoid base content between dividing and confluent cells in 20 μM fumonisin B_1-treated cultures

	Free S_o+S_a (pmol/10^6 cells)	
	24 hr	48 hr
Confluent	77±12	99±15
Dividing	500±23*	1167±37*

$LLC\text{-}PK_1$ cells were seeded at different cell densities (2500 cells/cm^2 for dividing cells and 10000 cells/cm^2 for confluent cells) in 25-cm^2 culture flasks, allowed to grow for 3 days, medium exchanged, cells dosed with 20 μM fumonisin B_1 and incubated for another 24 and 48 hr. Values are the means (n=3)±SD. Asterisks indicate values for confluent cells which are significantly different ($p<0.05$) from values for dividing cells values within time groups.

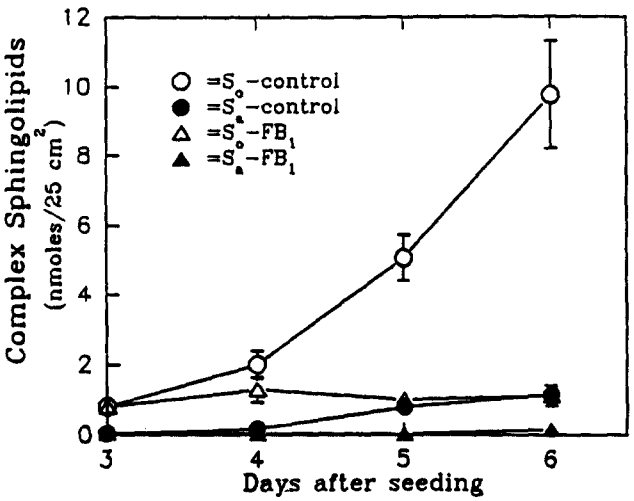

Fig. 6. Fumonisin-induced decrease in complex shingolipid content in 35 μM fumonisin B_1-treated cultures.

the concurrent control. Visual observation of cell morphology at 72 hr in both 35 μM fumonisin B_1-treated and normal cultures confirmed that there were fibroblast-like appearance and much less confluence of attached cells (approximately 50% of the concurrent control) in fumonisin B_1-treated cultures, while normal cultures showed normal epithelial cell morphology, many domes, 100% confluence and fewer floating cells (Fig. 4).

2. Elevation of free sphingoid bases was an early event in the fumonisin-induced sequential events.

Free sphingoid bases (primarily free sphinganine) were highly elevated (400±10 nmoles/10^6 cells) at 24 hr and maximal (435±14 nmoles/10^6 cells) at 48 hr, and free sphinganine accounted for over 90% of the increase (Fig. 5 and Table 1). The elevation of free sphinganine level as a consequence of inhibition of *de novo* sphingolipid biosynthesis preceded the inhibition of cell growth at 48 and 72 hr. The amount of free sphingosine resulting from the inhibition of sphingolipid turnover pathway was elevated at 24 hr and then came back to the normal level (7±2 pmoles/10^6 cells) at 72 hr (Fig. 5 and Table 1). Normal cells contained trace amounts of free sphingosine and free sphinganine, and free sphingosine had always higher amount than free sphinganine in normal cultures. At 72 hr the level of free sphinganine (333±11 pmoles/10^6 cells) in 35 μM fumonisin B_1-exposed culture was decreased, but still much higher than the level (1±1 pmoles/10^6 cells) in normal cell culture.

3. Cell division was a primary contributing factor for the elevation of free sphingoid bases which was closely related to the inhibition of cell growth.

Dividing cells showed larger elevation of free sphinganine (approximately 77-99 pmoles/10^6 cells) than confluent cells (approximately 500-1167 pmoles/10^6 cells) in 20 μM fumonisin for 24~48 hr (Table 2). The elevation of free sphingoid bases was due to an increase in free sphinganine in dividing cell cultures treated with fumonisin B_1. In dividing $LLC\text{-}PK_1$ cells *de novo* sphingolipid biosynthesis was very active and inhibition of sphinganine *N*-acyltransferase in *de novo* sphingolipid biosynthesis resulted in large elevation of free sphinganine. At 48 hr and 20 μM fumonisin B_1 the slight inhibition (approximately 5~10%) of cell growth occurred and at 48 hr and 50 μM fumonisin B_1 35% inhibition of cell growth and slight cytotoxicity was shown in dividing $LLC\text{-}PK_1$ cells (unpublished data), while at 48 hr and 20 μM fumonisin B_1 confluent cells did not show any toxicity. Elevation of free sphinganine appeared to be a contributing factor leading to the fumonisin-induced inhibition of cell growth.

4. Depletion of total complex sphingolipids was related to the inhibition of cell growth in dividing cells.

The content of complex sphingolipids was pro-

portional to the increase in viable cell numbers in normal cell cultures (Fig. 3 and Table 1), while complex sphingolipid biosynthesis was inhibited at 24 hr and completely shut down at 48 and 72 hr in 35 μM fumonisin B_1 (Fig. 6 and Table 1). In normal LLC-PK_1 cells complex sphingolipids containing sphingosine backbone were composed of over 90% of the total, and dihydroceramide, the only complex sphingolipid containing sphinganine backbone, made up the remainder. In fumonisin-treated cultures, the content of complex sphingolipids was about 75% at 24 hr, 35% at 48 hr, and 24% of the concurrent control cultures at 72 hr. In fumonisin-treated cultures depletion of complex sphingolipids as well as elevation of free sphingoid bases appeared to be a contributing factor for the inhibition of cell growth (Fig. 6 and Table 1). Depletion of complex sphingolipids was preceded by elevation of free sphingoid bases and followed by the inhibition of cell growth and cytotoxicity (unpublished data). Fumonisin-induced molecular and cellular events occurred in a sequential and time-dependent manner in LLC-PK_1 cells.

IV. DISCUSSION

The inhibition of cell growth in the presence of fumonisin B_1 occurred between 10 and 35 μM during the dividing stage of LLC-PK_1 cells (Yoo *et al.*, 1992). Other dividing cell lines are also sensitive to fumonisins. Dog kidney distal tubule (MDCK) and rat hepatoma (H4TG) cell lines are highly sensitive to fumonisins B_1 and B_2 (Shier *et al.*, 1991). From the studies of fumonisin toxicity using these cell lines the active cell division appears to be the requirement for the fumonisin-induced cellular effects (the inhibition of cell growth and cytotoxicity). In dividing LLC-PK_1 cells there was at least a 24-hr lag period for the inhibition of cell growth and cytotoxicity. The 24-hr lag period of fumonisin inhibition of cell growth and cytotoxicity may be related to the cell division of LLC-PK_1 cells because the doubling time for this cell line is approximately 24 hr (Riley *et al.*, 1995). The morphological change of LLC-PK_1 cells from epithelial type to fibroblast-like appearance in fumonisin-treated cultures may indicate the loss of cell-cell contact. However, the confluent LLC-PK_1 cells did not show any cytotoxicity when exposed to fumonisin B_1 up to 1 mM for 29 hr (Yoo *et al.*, 1992).

Fumonisin B_1 inhibition of ceramide synthase in sphingolipid metabolism resulted in both elevation of free sphingoid bases and depletion of complex sphingolipids in LLC-PK_1 cells. Elevation of free sphingoid bases (Primarily free sphinganine) was an early event leading to other fumonisin-induced sequential events, and even at 6 hr the significant elevation of free sphingoid bases was shown without any signs of cytotoxicity and inhibition of cell growth (Yoo *et al.*, 1992). Therefore, elevated level of free sphingoid bases can be used as an early and sensitive biomarker for the fumonisin exposure in animals and human. Depletion of complex sphingolipids occurred following the elevation of free sphingoid bases and preceded the inhibition of cell growth and cytotoxicity. Fumonisin-inhibition of both *de novo* sphingolipid biosynthesis and turnover pathway resulted in the dilution of complex sphingolipid pool in dividing cells. Depletion of complex sphingolipids appears to be directly related to the cell division.

There is no doubt that free sphingoid bases and complex sphingolipids have important roles in regulating cell function, cell proliferation, differentiation, and growth (for review see Merrill, 1994). Sphingosine and other long-chain bases are known to be potent and reversible inhibitors of protein kinase C and inhibit cellular responses to protein kinase C activators, such as phorbol esters and diacylglycerol (Hannun *et al.*, 1986; Wilson *et al.*, 1986; Merrill *et al.*, 1986). Sphingosine and other sphingoid bases also inhibits Na^+/K^+ ATPase (Oishi *et al.*, 1990). Hanada *et al.* (1992) isolated a temperature-sensitive chinese hamster ovary (CHO) mutant cell with thermolabile serine palmitoyltransferase in *de novo* sphingolipid biosynthesis. When the mutant cells were cultivated at a non-permissive temperature, *de novo* sphingolipid biosynthesis stopped and the growth rate of the cells slowly decreased. Exogenous sphingomyelin restored the contents of complex sphingolipids to the normal levels even at the non-permissive temperature. Fumonisin-induced depletion of complex sphingolipids resulted in the inhibition of axonal growth in cultured hippocampal neurons (Harel and Futerman, 1993). Co-addition of a ceramide analog with fumon-

isin B_1 reversed the fumonisin inhibition of axonal growth. Thus, these studies with CHO mutant cell and hippocampal neuron indicate that complex sphingolipids are essential for the cell growth and necessary for the new membrane material of the growing axon.

Fumonisins clearly inhibit the cell growth and simultaneously alter the sphingolipid metabolism by inhibiting ceramide synthase in LLC-PK_1 cells. However, a mechanistic relationship between the alteration of sphigolipid metabolism and fumonisin-induced inhibition of cell growth has not been proven yet. The hypothesis of this study is that elevation of free sphingoid bases and depletion of complex sphingolipids are related to fumonisin inhibition of cell growth in cytostatic concentration of fumonisin B_1. The previous and present studies showed that disruption of sphingolipid metabolism was linked to the fumonisin-induced inhibition of cell growth and cytotoxicity in LLC-PK_1 cells. Considering the important roles of sphingolipids in biological systems, disruption of sphingolipid metabolism should be mechanistically related to the fumonisin inhibition of cell growth and cytotoxicity.

In summary, (i) fumonisin B_1 inhibited the growth of LLC-PK_1 cells at ≤ 35 μM, (ii) inhibition of cell growth occurred following the 24-hr lag period, during which cell morphology appeared to be normal, (iii) elevation of free sphingoid bases was shown to be highly elevated at 24 hr after exposure to fumonisin B_1 as an early event, (iv) depletion of complex sphingolipids occurred as an intermediate event between elevation of free sphingoid bases and inhibition of cell growth, (v) cell division was the prerequisite for the fumonisin-induced cellular events (cytostatic and cytotoxic effects), and (vi) dividing cells showed more sensitive response to the fumonisin B_1 for the elevation of free sphingoid bases than confluent cells.

REFERENCES

Fincham, J.E., Marasas, W.F.O., Taljaard, J.J.F., Kriek, N.P.J., Badenhorst, C.J., Gelderblom, W.C.A., Seier, J.V., Smuts, C.M., Faber, M., Weight, M.J., Slazus, W., Woodrof, C.W., van Wyk, M.J., Kruger, M., and Thiel , P.G. (1992): Atherogenic effects in non-human primate of *Fusarium moniliforme* cultures added to a carbohydrate diet, *Atherosclerosis*, **94**, 13-25.

Hanada, K., Nishijima, M., Kiso, M., Hasegawa, A., Fujita, S., ogawa, T., and Akamatsu, Y. (1992): Sphingolipids are essential for the growth of chinese hamster ovary cells. Restoration of the growth of a mutant defective in sphingolipid base biosynthesis with exogenous sphingolipids, *J. Biol. Chem.*, **267**, 23527-23533.

Hannun, Y.A., Loomis, C.R., Merrill, A.H., and Bell, R.M. (1986): Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding *in vitro* and in human platelets, *J. Biol. Chem.*, **261**, 12604-12609.

Harel, R., and Futerman, A.H. (1993): Inhibition of sphingolipid synthesis affects axonal outgrowth in cultured hippocampal neurons, *J. Biol. Chem.*, **268**, 14476-14481.

Merrill, A.H., Jr., and Jones, D.D. (1990): An update of the enzymology and regulation of sphingolmyelin metabolism, *Biochim. Biophys. Acta*, **1044**, 1-12.

Marrill, A. H., Jr. (1991): Cell regulation by sphingosine and more complex sphingolipids, *J. Bioenerg. Biomemb.*, **23**, 83-104.

Merrill, A.H., Jr., Liotta, D.C., and Riley, R.T. (1994): Sphingolipids as regulators of cellular growth, differentiation, and behavior *in Advances in Molecular Biology and cell Biology* (R.W. Gross Ed.), (JAI Press, Greenwich, CT); In press.

Merrill, A.H., Jr., Sereni, A.M., Stevens, V.L., Hannun, Y. A., Bell, R.M., and Kinkade, J.M., Jr. (1986): inhibition of phorbol ester-dependent differentiation of human promyelocytic leukemic (HL-60) cell sphinganine and other long-chain bases, *J. Biol. chem.*, **261**, 12610-12615.

Merrill, A.H., Jr., van Echten, G., Wang, E., and Sandhoff, K. (1993): Fumonisin B_1 inhibits shingosine (sphinganine) *N*-acyltransferase and *de novo* sphingolipid biosynthesis in cultured neurons *in situ*, *J. Biol. chem.*, 268, 27299-27306.

Merrill, A.H., Jr., Wang, E., Mullins, R.E., Jamison, W.C., Nimkar, S., and Liotta, D.C. (1988): Quantitation of free sphingosine in liver by high performance liquid chromatography, *Anal. Biochem.*, **171**, 373-381.

Nelson, P.E., Desjardins, A.E., and Plattner, R.D. (1993): Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry, and significance, *Annu. Rev phytopathol.*, **31**, 233-252.

Nimkar, S., Menaldino, D., Merrill, A.H., Jr., and Liotta, D. (1988): A stereoselective synthesis of sphingosine, a protein kinase C inhibitor, *Tetrahedron Lett.*, **29**, 3037-3040.

Oishi, K., Zheng, B., and Kuo, J.F. (1990): Inhibition of Na, K-ATPase and sodium pump by protein kinase C regulators sphingosine, lysophosphatidylcholine, and oleic acid, *J. Biol. Chem.*, **265**, 70-75.

Plattner, R.D., Norred, W.P., Bacon, C.W., Voss, K.A.,

Peterson, R., Shakelford, D.D., and Weisleder, D.A. (1990): A method of detection of fumonisins in corn samples associated with field cases of equine leukoencephalomalacia, *Mycologia*, **82**, 698-70.

Riley, R.T., An, N.-H., Showker, J.L., Yoo, H.-S., Norred, W.P., chamberlain, W.J., Wang, E., Merrill, A.H., Jr., Motelin, G., Beasley, V.R., and Haschek, W.M. (1993): Alteration of tissue and serum sphinganine to sphingosine ratio: an early biomarker in pigs of exposure to fumonisin-containing feeds, *Toxicol. Appl. Pharmacol.*, **118**, 105-112.

Riley, R.T., Goeger, D.E., Showker, J.L., Cole, R.J., and Dorner, J. (1987): Age and growth related changes in cyclopiazoic acid-potentiated lipophilic cation accumulation by cultured cells and binding to freeze-thaw lysed cells, *J. Biochem. Toxicol.*, **2**, 251-264.

Riley, R.T., Hinton, D.M., Chamberlain, W.J., Bacon, C. W., Wang, E., Merrill, A.H., Jr., Voss, K.A. (1994): Dietary fumonisin B_1 induces disruption of sphingolipid metabolism in Sprague-Dawley rats: A new mechanism of nephrotoxicity, *J. Nutr.*, **124**, 594-603.

SAS Institute, Inc. (1987): SAS/STAT Guide for personal computers. cary, NC.

Shier, W.T., Abbas, H.K., and Mirocha, C.J. (1991): Toxicity of mycotoxins fumonisins B_1 and B_2 and *Alternaria alternata* f. sp. lycopersica toxin (AAL) in cultured mammalian cells, *Mycopathologia*, **116**, 97-104.

Spiegel, S., Olivera, A., and Carlson, R.D. (1993): The role of sphingosine in cell growth regulation and transmembrane signaling, *Adv. Lipid Res.*, **25**, 105-129.

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.

Wang, E., Ross, P.F., Wilson, T.M., and Riley, R.T. (1992): Alteration of serum sphingolipid upon exposure of ponies to feed containing fumonisins, mycotoxins produced by *Fusarium moniliforme*, *J. Nutr.*, **122**, 1706-1716.

Wilson, E., Olcott, M.C., Bell, R.M., Merrill, A.H., Jr., and Lambeth, J.D. (1986): Inhibition of the oxidative burst in human neutrophils by sphingoid long-chain bases, *J. Biol. Chem.*, **261**, 12616-12623.

Wilson, T.M., Ross, P.F., Rice, L.G., Osweiler, G.D., Nelson, H.A., Owens, D.L., Plattner, R., D., Reggiardo, C., Noon, T.H., and Pickrell, J.W. (1990): Fumonisin B_1 levels associated with an epizootic of equine leukoencephalomalacia, *J. Vet. Diag. Invest.*, **2**, 213-216.

Yoo. H.S., Norred, W.P., Wang, E., Merrill, A.H., Jr., and Riley, R.T. (1992): Fumonisin inhibition of *de novo* sphingolipid biosynthesis and cytotoxicity are correlated in LLC-PK_1 cells, *Toxicol. Appl. Pharmacol.*, **114**, 9-15.