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Differential Effects of Fumonisin B_1 on Cell Death in Cultured Cells: the Significance of the Elevated Sphinganine

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Fumonisins are specific inhibitors of ceramide synthase in sphingolipid metabolism. An alteration in sphingolipid metabolism as a result of fumonisin exposure is related to cell death (Yoo *et al.*, 1992). The objective of this study was to investigate whether elevated free sphinganine levels are related to the sensitivity of cultured cells to fumonisin exposure. Fumonisin B_1 elevated the intracellular free sphinganine concentraions in both LLC-PK_1 and Chinese hamster ovary (CHO) cells. However, CHO cells are resistant to fumonisin cytotoxicity at 50 μM, while LLC-PK_1 cells are sensitive at concentrations greater than 35 μM. The intracellular concentration of free sphinganine in LLC-PK_1 cells treated at 50 μM fumonisin B_1 for 72 h was approximately 1450 pmol/mg protein relative to the 37 pmol observed in the control culture. Under the same conditions, the population of apoptotic cells in the 50 μM fumonisin B_1-treated culture was approximately 37% of the total compared to 12% in the control. The caspase III-like activity after 72 h in the 50 μM fumonisin B_1-exposed culture increased to approximately 50 pmol/mg protein/hr compared to 6 pmol/mg protein/hr in the control. L-cycloserine, a serine palmitoyltransferase inhibitor, reduced the fumonisin B_1-stimulated caspase III-like activity down to the control level. Under the same culture conditions, the intracellular concentration of free sphinganine after L-cycloserine plus fumonisin B_1 treatment was 140 pmol/mg protein compared to 1450 pmol/mg protein in fumonisin B_1 alone. The intracellular concentration of free sphinganine in CHO cells treated with 50 μM fumonisin B_1 for 72 h was approximately 460 pmol/mg protein, indicating that the mass amount of elevated free sphinganine in the CHO cells was about 32% of that in LLC-PK_1 cells. Adding exogenous sphinganine to the CHO cells along with 50 μM fumonisin B_1 treatment for 72 h caused both necrosis and apoptosis. In conclusion, the elevated endogenous sphinganine acts as a contributing factor to the fumonisin-induced cell death.

Key words: LLC-PK_1 cells, CHO cells, Fumonisin B_1, Sphinganine, L-Cycloserine, Necrosis, Apoptosis

INTRODUCTION

Fumonisins are a family of mycotoxins produced by *Fusarium moniliforme* (Sheldon) and *F. proliferatum*, and are contaminants of corn and related products. Fumonisins are involved in several animal diseases and suspected to cause human esophageal cancer (Nair *et al.*, 1998). The animal diseases associated with fumonisins are equine leukoencephalomalacia (ELEM), porcine pulmonary edema (PPE) syndrome, and liver cancer, hepatotoxicity and nephrotoxicity in rats. Epidemiological studies have shown that the occurrence of human esophageal cancer is strongly related to fumonisin contamination in corn and corn-related products (Turner *et al.*, 1999).

The chemical structure of fumonisins is similar to the sphingolipid backbone except that fumonisins have two tricarballylic acids and are water-soluble (Fig. 1) Ceramide synthase is an enzyme responsible for the acylation of sphinganine in the *de novo* synthesis of sphingolipids and the reacylation of sphingosine in sphingolipid turnover (Fig. 2). Fumonisins are specific ceramide synthase inhibitors. Fumonisin was known to elevate the level of free sphingoid bases, primarily free sphinganine, in LLC-PK_1 cells (Yoo *et al.*, 1992), ponies (Wang *et al.*, 1992), pigs (Riley *et al.*, 1993) and other animals. Sphingolipids can be found in all eukaryotic cells, where they are particularly abundant in plasma and related cell membranes,

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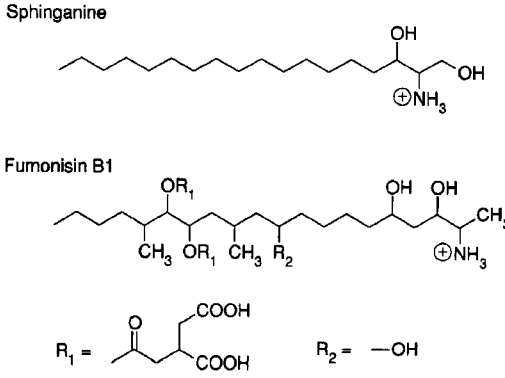

Fig. 1. Structures of fumonisin B_1 (MW 721.8) and sphinganine (MW 301.5). Fumonisin B_1 is the most abundant in corn-based feeds and foods contaminated with *Fusarium moniliforme*. Sphingosine is the same as sphinganine except for a single double bond between C4 and C5. In mammalian cells, the most common sphingoid base is D-erythro-sphingosine.

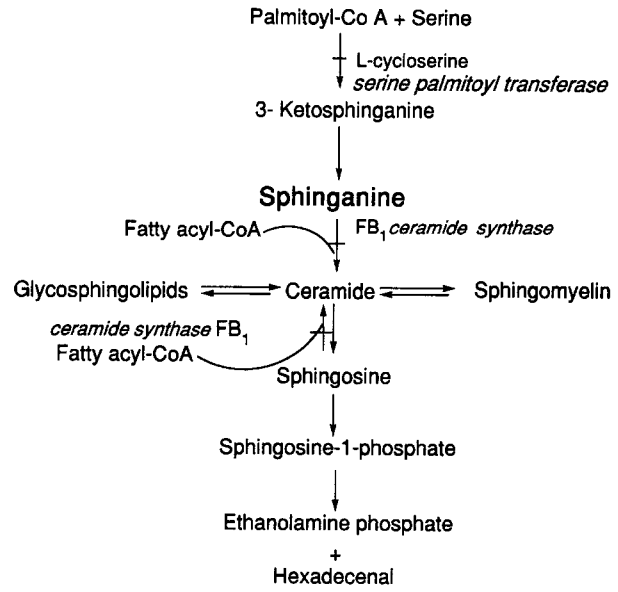

Fig. 2. The pathway of sphingolipid metabolism, and inhibition sites by fumonisin B_1 and L-cycloserine. Fumonisin B_1 is a potent inhibitor of ceramide synthase (Wang *et al.*, 1991), the enzyme that is responsible for acylation of sphinganine in the *de novo* synthesis of sphingolipids and the reacylation of sphingosine that is formed upon sphingolipid turnover.

such as Golgi membranes and lysosomes. In mammalian cells, common sphingoid bases are D-*erythro*-sphingosine and sphinganine, and complex sphingolipids are ceramide, sphingomyelin and other glycosphingolipids such as cerebrosides and gangliosides.

In Sprague-Dawley rats fed pure fumonisin B_1 for 4 weeks, the free sphinganine and free sphingosine levels were elevated and the complex sphingolipids levels decreased in the liver, kidney, serum and urine (Riley *et al* 1994a). The severity of ultrastructural lesions in the kidney and liver were correlated with free sphinganine and free sphingosine elevation. LLC-PK_1 pig kidney epithelial cells showed that fumonisin B_1 inhibited cell proliferation and increased cell death (Yoo *et al.*, 1992) Moreover, elevated free sphingoid bases and depleted complex sphingolipids were contributing factors in fumonisin-induced cell death (Yoo *et al.*, 1996a).

To understand the different sensitivity of cultured cells to fumonisin exposure, cell death following fumonisin B_1 exposure in LLC-PK_1 cells and CHO cells was quantified, the intracellular concentration of free sphinganine was modulated, and the mechanistic relationship between the elevated free sphinganine and increased cell death was determined.

EXPERIMENTAL PROCEDURES

Reagents

Fumonisin B_1 was purchased from Biomol Research Laboratory, Inc. (Plymouth, PA). Sphinganine (DL-*erythro*-dihydrosphingosine), sphingosine (D-sphingosine), L-cycloserine, 2-mercaptoethanol, β-NADH, sodium pyruvate, sodium-*N*-lauroylsarcosinate, PIPES, EGTA, cytochalasin B, PMSF, leupeptin, pepstatin A, antipain, chymopapain, HEPES, CHAPS, dithiothreitol, ovalbumin and RNase A were all purchased from the Sigma Chemical Co. (St. Louis, MO). C_{20}-sphinganine was purchased from Matreya, Inc. (Pleasant Gap, PA), and O-phthaldialdehyde (OPA) from Nakarai Chemicals. Ltd. (Kyoto, Japan). Dulbecco's modified Eagle medium/Ham's F-12 (DMEM/F-12), fetal bovine medium (FBS), penicillin-streptomycin (10,000 units/ml penicillin G sodium and 10,000 μg/ml streptomycin sulfate in 0.85% saline), Dulbecco's phosphate buffered saline (PBS), trypsin-EDTA (0.25% trypsin, 1 mM EDTA-4Na) and Triton-X 100 were obtained from Gibco BRL (Grand Island, NY). The BCA protein assay kit was purchased from Pierce (Rockford, IL), and Ac-DEVD-MCA from the Peptide Institute, Inc. (Osaka, Japan).

Cell culture

The LLC-PK_1 cells (CRL 1392, passage 197) were obtained from the American Type Culture Collection (Rockville, MD) and the CHO cells from Korea Research Institute of Bioscience and Biotechnology (Dae-jeon, Korea). The cells were grown and maintained in either 25- or 75 cm^2 culture flasks containing DMEM/Ham's F12 (1:1) with 5% FBS at 37°C in a humidified 5% CO_2 atmosphere. In all experiments, the cells were seeded at a density of 50,000 cells per well in each well of a 6-well plate (10 cm^2/well) The cells were allowed to attach and grow for 24 h. They were then treated with the given reagents (fumonisin B_1, L-cycloserine, and caspase-III inhibitor) for a further 72 h. The cultured cells were harvested by centrifugation following trypsin-EDTA treat

ment. The cell pellets were stored for further experiments.

Lactate dehydrogenase assay

The cytotoxicity was determined by measuring the released activity of lactate dehydrogenase (LDH) from the total LDH activity. The culture medium was transferred to a glass tube to determine the released LDH activity. The total LDH was released from the cells by the addition of 0.1% Triton X-100 followed by brief incubation at 37°C. Stock solutions of sodium pyruvate (1%, w/v) and β-NADH (0.75%, w/v) were added to both the medium and cell lysate. Subsequently, the LDH activity was measured using a Jasco V-530 UV-vis spectrophotometer (Tokyo, Japan), and the released LDH activity was expressed as a percentage of the total cellular LDH activity.

Sphingoid bases extraction and analysis

The intracellular sphinganine and sphingosine concentrations in base- and acid-treated cell extracts were determined by high performance liquid chromatography (HPLC) utilizing a modification (Yoo *et al.*, 1996b) of the extraction methods described by Riley *et al.* (1994b), Merrill *et al.* (1988), and Gaver and Sweeley (1965). Briefly, the culture medium including floating cells was transferred to 1.5 ml polypropylene tubes and centrifuged to save pellets. The cells attached to the culture plates following trypsin-EDTA treatment were collected by brief centrifugation. The cell pellets were dissolved in a sodium *N*-lauroylsarcosinate solution. The lysates were then subjected to base hydrolysis to determine the free sphingoid bases, and acid hydrolysis to determine the total sphingolipids, and protein levels. The sphingoid bases were quantified based on the recovery of a C_{20}-sphinganine internal standard. The intracellular amounts of sphingolipids from the lipid extracts were analyzed by HPLC with fluorescence detection. The concentration of complex sphingolipids was calculated by subtracting the concentration of free sphingoid bases (base-hydrolyzed extracts) from the total sphingolipid concentration (acid-hydrolyzed extracts).

Detection of fragmented DNA by agarose gel electrophoresis

The LLC-PK1 cells were treated as indicated, harvested by Trypsin-EDTA, and the cell pellets were then rinsed with PBS. The pellets were incubated with 700 μl lysis buffer (50 mM Tris-HCl, 10 mM EDTA, 0.5% sodium-*N*-lauroylsarcosinate, pH 8.0) and 10 μl RNase (20 mg/ml) at 37°C for 1 h. The lysate was further incubated by the addition of Proteinase K (Boehringer, Mannheim, Germany) for another hour. The DNA was precipitated with a 50 μl 3 M sodium acetate solution (pH 6.0) and 500 μl ice-cold isopropyl alcohol at -20°C for 2 h. The DNA was pelletized by centrifugation at 3,000xg for 30 min at 4°C then washed with 1 ml of 70% ice-cold ethanol, and dried under vacuum. The DNA samples dissolved in the TE buffer were loaded and run on 2% agarose gel with a TAE running buffer solution (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). The DNA bands were visualized with ethidium bromide, and the gel was photographed under UV light.

Flowcytometric analysis

The apoptotic cell population of the cultured cells was determined using a Becton Dickinson FACScan (San Jose, CA).The cells were treated and incubated with the indicated agents, and harvested with trypsin-EDTA. The cell pellets were fixed overnight with 70% ice-cold ethanol and washed twice with PBS. The cells were stained with a propidium iodide solution for 1 h and analyzed with flowcytometry. The percentage of apoptotic cell population was then estimated.

Caspase III-like activity assay

Apoptosis was determined by measuring the caspase III-like activity. The cell pellets were resuspended in an extraction buffer (50 mM PIPES-NaOH pH 7.0, 50 mM KCl, 5 mM EGTA, 2 mM $MgCl_2$, 1 mM DTT, 20 μM cytochalasin B, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 50 μg/ml antipain, 10 μg/ml chymopapain). The cell suspension was lysed by five freeze-thaw cycles in liquid nitrogen. For the Ac-DEVD-MCA cleaving activity assay, the cell lysate was mixed with an ICE buffer (100 mM HEPES-KOH pH 7.5, 10% sucrose, 10 mM DTT and 0.1 mg/ml ovalbumin) containing Ac-DEVD-MCA, a caspase substrate.The enzyme reactions were performed in 96-well plates at 30°C for 1 h. The free AMC released was then measured at 320 nm (excitation) and 444 nm (emission) in Molecular Devices fluorescence reader (Sunnyvale, CA). The enzyme activity was calculated from the AMC standard curve.

BCA protein assay

The total cellular protein content was determined (Smith *et al.*, 1985) in order to both normalize the results and estimate cell growth. The cell lysate from the solubilized pellet was mixed with the BCA reagent and incubated for 30 min. The protein content was quantitated with a Molecular Devices ELISA reader (Sunnyvale, CA) at 562 nm based on the standard curve.

Statistics

All values were expressed as a mean ± the standard deviation. Differences between treatments were analyzed statistically by an unpaired Student's *t*-test. A p value <0.05 or <0.01 was considered statistically significant.

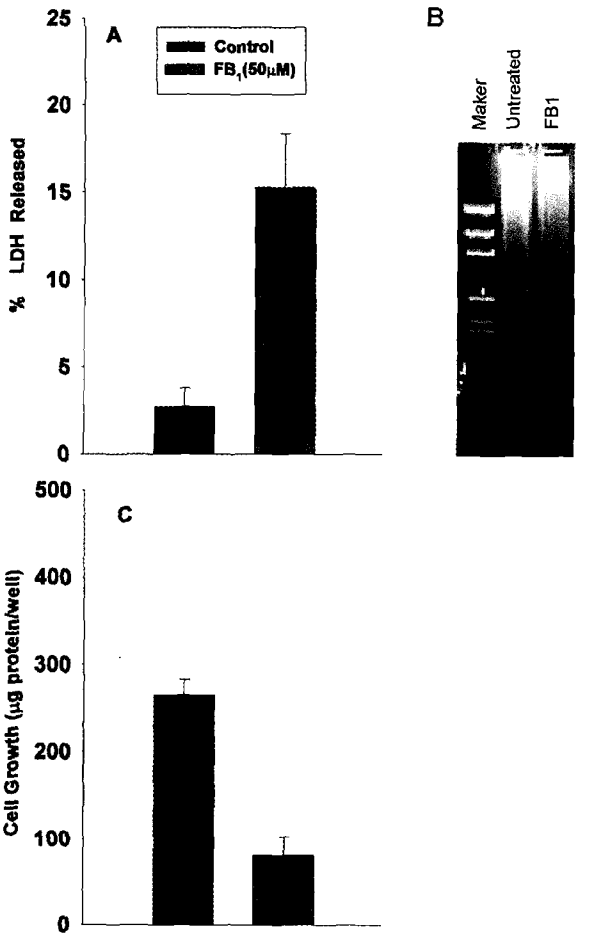

Fig. 3. Comparison of LDH release (A), DNA fragmentation (B) and protein content (C) in control and 50 μM fumonisin B_1 (FB_1)-treated LLC-PK_1 cells. The cells were seeded at a density of 5×10^4 cells in 10 cm^2 well and grown for 24 h. The old medium was changed with a fresh one and the cells were incubated with fumonisin B_1 for a further 72 h. The cultured cells were assayed for released LDH activity, analyzed for DNA fragmentation and measured for protein concentration. The data is represented as a means ± SD from at least three independent experiments performed in triplicates.

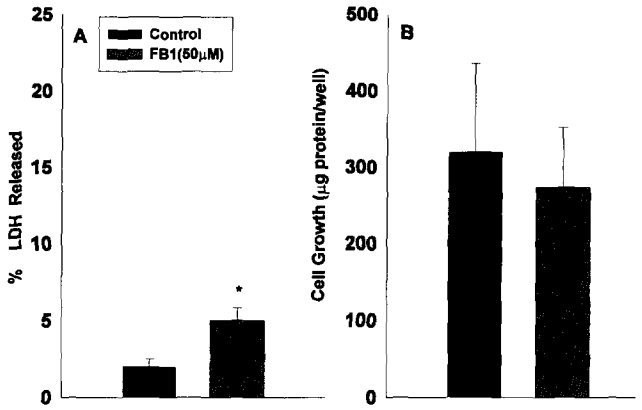

Fig. 4. Comparison of LDH release (A) and protein content (B) in the control and 50 μM fumonisin B_1 (FB_1)-treated CHO cells. The cells were seeded at a density of 5×10^4 cells in 10 cm^2 per well and grown for 24 h. The old medium was changed with a fresh one and the cells were incubated with fumonisin B_1 for a further 72 h The cultured cells were assayed for released LDH activity and protein concentration. The data is represented as a mean ± SD from at least three independent experiments performed in triplicates. Asterisk (*) indicates the means which are significantly different ($p<0.01$) from the control.

RESULTS

The LLC-PK_1 cells are sensitive and CHO cells resistant to fumonisin B_1.

Visual observation of the LLC-PK_1 cells treated with 50 μM fumonisin B_1 for 72 h confirmed that there were a few attached cells and many floating cells, and even the attached cells appeared fibroblast-like (Yoo *et al.*, 1992). 50 μM fumonisin B_1 significantly increased the percentage of LDH release after 72 h, which is indicative of cytotoxicity, while after 24 h and 48 h, the cells treated with fumonisin B_1 did not undergo cell death (Yoo *et al.*, 1996a). The total released LDH activity in the fumonisin B_1-treated culture for 72 h was about 14% higher than in the control (Fig. 3A). 50 μM fumonisin B_1 treatment for 72 h significantly decreased the protein level from the attached cells by approximately 60% compared to control (Fig. 3C). This indicates that fumonisin B_1 inhibited cell growth. Under the same culture conditions, the CHO cells were slightly cytotoxic to fumonisin B_1 (Fig. 4A). However, cell growth was not inhibited by fumonisin B_1 (Fig. 4B).

Fumonisin B_1 induced apoptosis in LLC-PK_1 cells, but not in CHO cells.

The LLC-PK_1 cells exhibited apoptosis after exposure to 50 μM fumonisin B_1 for 72 h. Following fumonisin B_1 exposure, the LLC-PK_1 cells showed the typical DNA ladder on the agarose gel electrophoresis, which is one of the apoptotic biomarkers (Fig. 3B), and included an apoptotic cell population approximately 37% of the total cells (Fig. 5A). The caspase-III-like activity, a signaling component in the apoptotic pathway, was also higher in fumonisin B_1-treated LLC-PK_1 cells. The caspase-III-like activity in the cells treated with 50 μM fumonisin B_1 for 72 h was approximately 50 pmol/mg protein/hr, while it was approximately 6pmol/mg protein/hr in the control culture (Fig. 5B). The fumonisin B_1-induced activation of the caspase III-like enzyme was completely inhibited (Fig. 5B) and the population of apoptotic cells from FACS analysis was partially reduced by Ac-DMQD-CHO, a caspase inhibitor (Fig. 5A) in the LLC-PK_1 cell. This suggests that a caspase activation-independent pathway may be involved in fumonisin B_1-induced apoptosis in

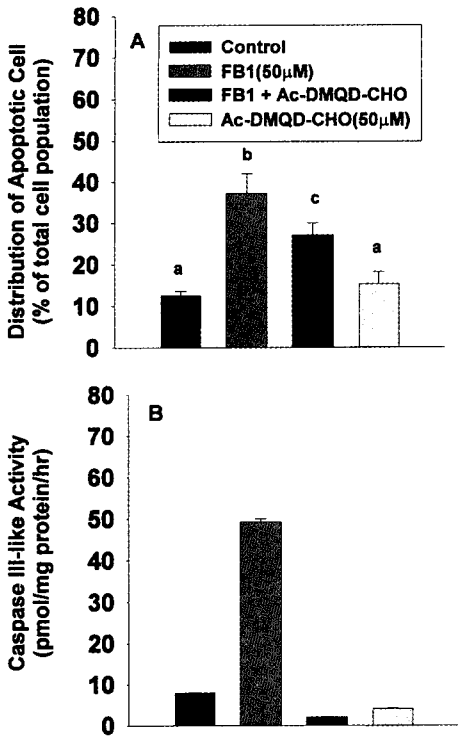

Fig. 5. Apoptotic cell population (A) and elevation of caspase III-like activity (B) induced by fumonisin B_1 (FB_1) in LLC-PK_1 cells. The cells were incubated with 50 μM fumonisin B_1 and 50 μM Ac-DMQD-CHO, caspase inhibitor, for 72 h. The apoptotic cell population was analysed by flowcytometry and caspase III-like activity was measured by fluorescence reader. The data is represented as a mean ± SD from at least three independent experiments performed in triplicates. Different letters indicate means which are significantly different ($p<0.01$) from each other.

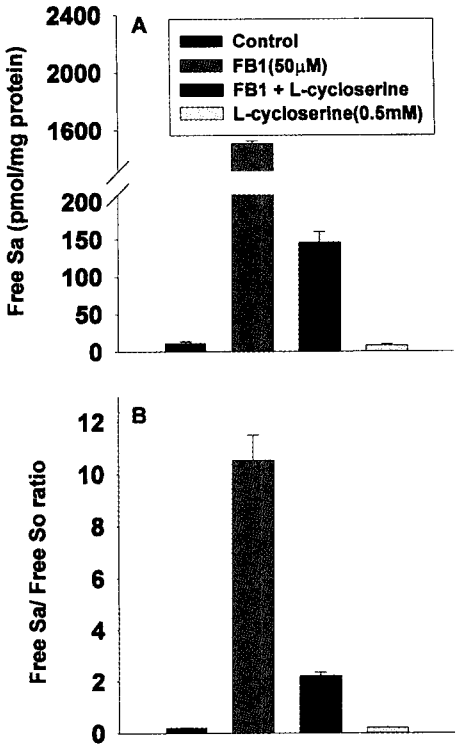

Fig. 6. The reversal of the fumonisin-induced increase in free sphinganine concentration (A) and sphinganine/sphingosine (Sa/So) ratio (B) by L-cycloserine in LLC-PK_1 cells. The cells were incubated with 50 μM fumonisin B_1 (FB_1) and 0.5 mM L-cycloserine for 72 h. The harvested cells were analysed by HPLC. The data is represented as a mean ± SD from at least three independent experiments performed in triplicate.

LLC-PK_1 cells. The CHO cells treated with 50 μM fumonisin B_1 for 72 h showed a caspase activity of 1-2 pmol/mg protein/hr compared to 6-7 pmol/mg protein/hr in the control, indicating a suppression of enzyme activity in the presence of fumonisin B_1 (Fig. 8B).

Fumonisin B_1 elevated endogenous free sphinganine differentially in LLC-PK_1 cells and CHO cells.

Free sphinganine is one of intermediates in *de novo* sphingolipid biosynthesis, and is actively involved in cell death and proliferation in mammalian cells. The intracellular concentration of free sphinganine in the LLC-PK_1 cells treated with 50 μM fumonisin B_1 for 72 h was 1450 pmol per mg protein (control level=37 pmol per mg), indicating huge elevation of free sphinganine in the LLC-PK_1 cells (Fig. 6A). The ratio of sphinganine/sphingosine can be a useful biomarker for fumonisin exposure in both animals and cultured cells. The ratio of sphinganine/sphingosine in fumonisin B_1-treated cultures at 50 μM for 72 h was approximately 10.0 (0.2 in the control), indicating that the sphinganine/sphingosine ratio is a sensitive biomarker for changes in fumonisin B_1-induced sphingolipid metabolism (Fig. 6B). The CHO cells showed that the intracellular concentration of free sphinganine following exposure to 50 μM fumonisin B_1 for 72 h was about 460 pmol per mg protein (Fig. 7A), and the ratio of free sphinganine/free sphingosine was approximately 10.0 (Fig. 7B). Although this ratio in the fumonisin B_1 treated cells were the same in both cell lines, the mass amount of elevated free sphinganine in the CHO cells was shown to be about 32% of the one in LLC-PK_1 cells.

Elevated intracellular free sphinganine is a key regulator of the fumonisin sensitivity between LLC-PK_1 cells and CHO cells.

One strategy to modulate the level of intracellular sphingolipids begins with inhibiting serine palmitoyltransferase, the first enzyme in the *de novo* sphingolipid pathway. Treatment simultaneously with fumonisin B_1 (50 μM) and L-cycloserine, a serine palmitoyltransferase inhibitor, for 72 h in LLC-PK_1 cells reduced the released LDH activity to 4% from the 15% in the fumonisin B_1 alone-treated

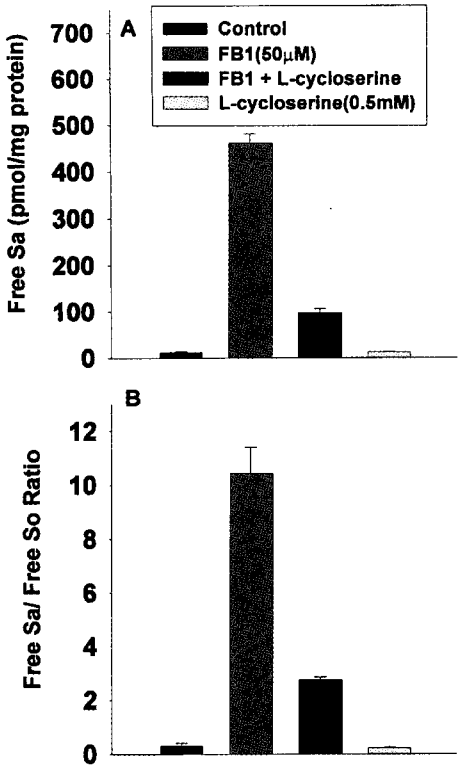

Fig. 7. The fumonisin-induced elevation of free sphinganine concentration (A) and sphinganine/sphingosine (Sa/So) ratio (B) in CHO cells. The cells were incubated with 50 μM fumonisin B_1 (FB_1) and 0.5 mM L-cycloserine for 72 h. The harvested cells were analysed by HPLC. The data is re presented as a mean ± SD from at least three independent experiments performed in triplicate.

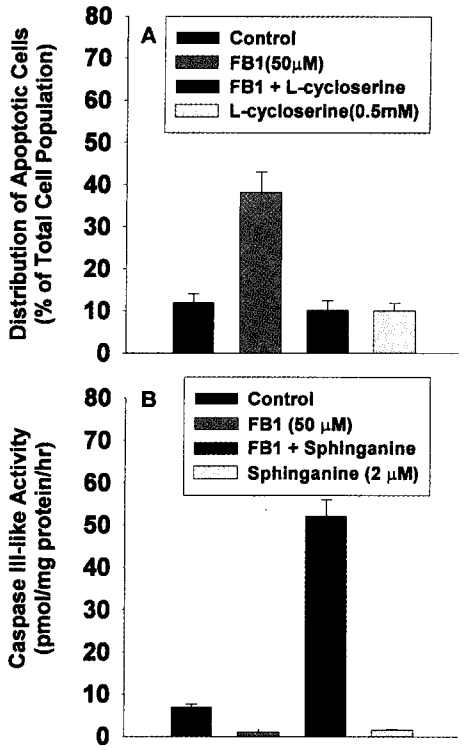

Fig. 8. The reversal of fumonisin-induced apoptosis by L-cycloserine in LLC-PK_1 cells (A) and the induction of caspase III-like enzyme activation by exogenously added sphinganine in CHO cells (B). The cells were incubated with 50 μM fumonisin B_1 (FB_1) and either 0.5 mM L-cycloserine or sphinganine for 72 h. The harvested cells were analysed by either flowcytometry or a fluorescence reader. The data is represented as a mean ± SD from at least three independent experiments performed in triplicate.

(data not shown), and decreased the population of apoptotic cells from 37% to 12% (Fig. 8A). Fumonisin B_1 induced-apoptosis was also completely inhibited by L-cycloserine in the LLC-PK_1 cells (Fig. 8A). Under the same condition, the concentration of intracellular free sphinganine in the L-cycloserine /fumonisin B_1-treated LLC-PK_1 cells was drastically reduced to approximately 140 pmol per mg protein from 1450 pmol in the 50 μM fumonisin B_1-treated cultures for 72 h (Fig. 6A). This indicates that free sphinganine elevation in the LLC-PK_1 cells may be closely related to fumonisin B_1-induced cell death (necrosis and apoptosis). When sphinganine at 2 μM concentration was added exogenously to the CHO cells along with 50 μM fumonisin B_1 treatment for 72 hr, caspase III-like activity increased to 50 pmol/mg protein/hr from 7 pmol/mg protein/hr in the control (Fig. 8B).

DISCUSSION

The sensitivity to fumonisin in cultured cells varies. Several proliferating cell lines are sensitive to fumonisins. The rat hepatoma (H4TG), the dog kidney cell line (MDCK) and the pig kidney cell line (LLC-PK_1) are cytotoxic to fumonisins B_1 and B_2 (Shier *et al.*, 1991, Yoo *et al.*, 1992). Cytotoxicity in LLC-PK_1 cells was observed after 72 h exposure to fumonisin B_1 at concentrations >35 μM. The fibroblast-like morphology and the many floating cells observed after fumonisin B_1 exposure for at least 72 h were characteristics of fumonisin B_1 cytotoxicity in LLC-PK_1 cells. At 50 μM fumonisin B_1 exposure for 72 h, the LLC-PK_1 cells exhibited both necrosis and apoptosis in addition to cell growth inhibition (Figs. 3 and 5). However, the primary rat hepatocytes and the CHO cell line were resistant to fumonisins (Wang *et al.*, 1991; Norred *et al.*, 1991; Fig. 4). The CHO cells did not show any apoptotic cell death after 50 μM fumonisin B_1 treatment for 72 h and the background level of apoptosis was even suppressed by fumonisins (Fig. 8). The underlying mechanism of this differential sensitivity to fumonisins in two types of cells is not clearly understood.

Fumonisins are specific ceramide synthase inhibitors and alter the sphingolipid metabolism (Wang *et al.*, 1991). Free sphingoid bases, complex sphingolipids and their degradation products are known to play important roles in cellular functions. The disruption of the sphingolipid

metabolism has emerged as a potential toxic mechanism of fumonisins (Merrill *et al.*, 1997). The elevation of either free sphinganine, one of early events in fumonisin-altered sphingolipid metabolism, or sphinganine-1-phosphate may play a key role in the differential toxicity of fumonisins in cultured cells. In LLC-PK_1 cells, fumonisin B_1 began to increase the free sphinganine level during the early period of exposure and maintained the enormous level (Yoo *et al.*, 1992; Yoo *et al.*, 1996a; Fig. 6). In addition, fumonisin B_1 may also increase the sphinganine-1-phosphate concentration. However, the intracellular concentration of free sphingosine in fumonisin-treated LLC-PK_1 cells was lower than in untreated cells. Thus, free sphingosine and sphingosine-1-phosphate may not be involved in fumonisin-induced toxicity. Fumonisins also depleted the total complex sphingolipids including ceramides in LLC-PK_1 cells (Yoo *et al.*, 1996a), indicating that ceramides may not be related to fumonisin toxicity. Either L-cycloserine or ISP-1, a serine palmitoyltransferase inhibitor in *de novo* sphingolipid biosynthesis, is useful for modulating the endogenous free sphinganine levels in actively dividing cells exposed to fumonisins. L-cycloserine lowered the intracellular free sphinganine concentration in the fumonisin B_1-treated culture and decreased the level of both the necrotic and apoptotic cell deaths in LLC-PK_1 cells (Figs. 6 and 8), suggesting that either endogenous sphinganine elevation or its metabolites may be responsible. The best candidate for the sphinganine metabolite may be sphinganine-1-phosphate, which acts as either an agonist in blood-related cells or a signaling molecule in other mammalian cells (Hla *et al.*, 1999). The intracellular concentration of free sphinganine in CHO cells upon exposure to 50 μM fumonisin B_1 for 72 h was not as high as in the LLC-PK_1 cells (Figs. 6 and 7). The difference in elevated free sphinganine content between the LLC-PK_1 cells and CHO cells may indicate the range of free sphinganine concentrations that expresses fumonisin cytotoxicity. An addition of exogenous sphinganine to the CHO cell culture in the presence of fumonisin for 72 h reproduced the level both necrotic and apoptotic cell deaths (Fig. 8B). At exogenous sphinganine concentrations >5 μM, CHO cells showed both apoptosis and necrosis. However, exogenous sphinganine in the cultured cells continued to be metabolized and the intracellular sphinganine concentration decreased over the time period, while fumonisin inhibition of the sphingolipid metabolism increased the free sphinganine concentration with time.

In summary: (i) fumonisin is cytotoxic to LLC-PK_1 cells, but not to CHO cells, (ii) both apoptosis and necrosis occur simultaneously in LLC-PK_1 cells, (iii) the elevation of free sphinganine in LLC-PK_1 cells is much higher than in CHO cells, and (iv) the modulation of endogenous sphinganine concentrations with either L-cycloserine or exogenous free sphinganine indicates the contribution of sphinganine to fumonisin-induced cell death. In conclusion, either endogenous free sphinganine or its metabolite may act as a contributing factor in the fumonisin-induced cell death.

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