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Early Ultrastructural Changes of Apoptosis Induced by Fumonisin B_1 in Rat Liver

Woo-Sung Moon[1], Joo-Heon Kim[2], Myoung-Jae Kang[1], and Dong-Geun Lee[1]

Abstract

A time sequential study was performed to investigate the histological and ultrastructural findings of fumonisin B_1-induced apoptosis in the male Sprague-Dawley rat liver. Six hours after administration of FB_1, marked morphologic changes of hepatocytes included the appearance of small vacuoles along the margin of cell membrane. Twelve hours after injection of FB_1, acidophilic degeneration of cells occurred, but no fragmented nucleus was evident around the centrilobular area, with few apoptotic cells. By electron microscope, the degenerated acidophilic cells revealed following changes: characteristic formation of cytoplasmic vacuoles, condensed cytoplasm, detachment from neighboring cells, and as well as margination of nuclear chromatin and swollen mitochondria with amorphous matrical deposit. The number of apoptotic cells or bodies was further enhanced at 24 hours in the vicinity of dense acidophilic cells, resulting in a marked increase over the values of control rats. Serum analysis revealed the elevation of cholesterol levels from the beginning to the end of this experiment. Morphologic data and serum findings in this study support the theory that FB_1-induced alteration of membrane lipid constituents of the hepatocytes are likely to be early key events in explaining the FB_1 apoptotic effect.

Key Words: Fumonisin, hepatocyte, apoptosis

INTRODUCTION

Fumonisins are a group of naturally occurring, structurally related compounds produced by *Fusarium moniliforme*, one of the most commonly occurring fungi on agricultural commodities, including corn growth throughout the world.[1-3] Several natural and experimental animal diseases are associated with *Fusarium moniliforme* or high levels of fumonisins: equine leukoencephalomalacia, porcine pulmonary edema syndrome, nephrotoxicity, hepatotoxicity, and carcinogenecity in rats.[4-6] The principal pathologic change of the liver in rats treated with fumonisin B_1 (FB_1) in short-term toxicity tests was progressive toxic hepatitis characterized by hepatocellular necrosis, bile duct proliferation and fibrosis.[7] However, there have been few morphologic studies that focused on the relations between FB_1 and apoptosis.

FB_1 is structurally similar to sphingosine and can inhibit the activity of ceramide synthase which leads to a change in the sphinganine:sphingosine ratio in rat hepatocytes.[8,9] This may be the mechanism of some of the toxic effects of FB_1 because sphingolipids regulate cell growth, differentiation and transformation.[8-10] Recent progress in defining the signal transduction mechanisms regulating apoptosis stems from recognition of the role of the sphingolipid, ceramide, as a novel lipid second messenger mediating apoptosis.[11]

The purpose of this study was to investigate the ultrastructural appearance of liver cell apoptosis caused by the injection of FB_1 into rats, putting emphasis on cell membrane change with respect to the possible relationship between the toxic effect of FB_1 and the sphingomyelin cycle. We examined rat liver tissue treated with FB_1 by using light microscopy, electron microscopy, and the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) assay to identify apoptotic cells among injured hepatocytes at the level of a single

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[1]Department of Pathology, Chonbuk National University, Medical School, Institute for Medical Science, Chonju, [2]Department of Pathology, Eulji University School of Medicine, Taejeon, Korea
Address reprint request to Dr. W. S. Moon, Department of Pathology, Chonbuk National University Medical School, Chonbuk 560-182, Korea. Tel: 82-652-270-3086, Fax: 82-652-270-3135
E-mail: mws@moak.chonbuk.ac.kr

cell in situ.

MATERIALS AND METHODS

Animals and toxin

Three-week-old male Sprague-Dawley rats were purchased from the Korea Research Institute of Chemical Technology and reared until the body weights reached 170 to 200 g in our laboratory. They were housed with a 12 hr light-dark cycle, and were supplied with pelleted food and *ad libitum* water. The rats were allowed to acclimate for 1 week prior to the study. The purified fumonisin B_1 (Sigma, St. Louis, MO, USA) was dissolved in phosphate buffered saline (PBS) prior to use.

Experimental design

The rats were injected with 1.25 mg FB_1/kg/day in PBS intravenously. Rats were administered with two doses of either FB_1 or PBS and were sacrificed 3, 6, 12, 24, and 48 hours after injection. Twenty-two rats were divided into 6 groups, which consisted of 7 controls and 3 treated rats in each group. This dose was based on a previous report.[12] Ether was used to induce anesthesia for collection of blood from the abdominal vena cava, as well as for sacrifice.

Serum biochemistry

Biochemical assays of serum samples consisted of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total cholesterol using an auto-analyzer (Sotchem SP-4410, Daiichi Kagaku Co., Kyoto, Japan) and commercial reagent kit purchased from Sigma Chemical Co.

Pathology and organ weight

Animals were sacrificed with ether and necropsied. Body and liver weights were recorded and the relative weights of liver (liver to body weight ratio) were calculated. Slices of the left lateral lobe and median lobe in liver were collected. Tissue samples were fixed in 10% neutral buffered formalin, then processed routinely, sectioned at 4−5 micrometers of thickness, and stained with hematoxylin-eosin (HE), and examined by light microscopy.

TUNEL assay

Tissue sections 4 μm thick were collected on positively charged slides. The nuclear DNA fragmentation of apoptotic cells was labeled in situ using TUNEL method[13,14] as follows. The sections were first deparaffinized and treated for 15 minutes with 20 μg/ml proteinase K (Boehringer Mannheim, Mannheim, Germany) in 0.1 mol/L Tris-HCl buffer (pH 7.4). They were then incubated with 0.3 U/μl terminal deoxynucleotidyl transferase (Life Technologies, Inc., Grand Island, NY, USA) and 0.04 nmol/μl biotinylated dUTP (Boehringer Mannheim, Mannheim, Germany) in terminal deoxynucleodyl transferase buffer (Life Technologies, Inc., Grand Island, NY, USA) at 37°C for 60 minutes. The sections were incubated for 10 minutes with 2% bovine serum albumin followed by 30 minutes in peroxidase-conjugated streptavidin (DAKO, Carpinteria, CA, USA) diluted 1 : 300 with PBS. Peroxidase activity in the sections was visualized by adding 0.025% 3,3-diaminobenzidine in 0.05 mol/L Tris-HCl (pH 7.4) solution containing 0.01% H_2O_2 for 5 minutes, and then counterstained with Harris' hematoxylin. The negative controls included sections treated with the TdT buffer solution, omitting TdT enzyme.

Electron microscopy

The liver (1 mm^3) samples taken from each control and treated animal were fixed in 2.5% glutaraldehyde in 0.05 mol/L carcodylate buffer (pH 7.4) containing 1% sucrose. Blocks from the fixed tissue were immersed in 0.1 mol/L phosphate buffered (pH 7.4) 1% osmium tetroxide for 2 hours and then dehydrated and embedded in Epon 812. Semithin sections were stained with toluidine blue for evaluation by light microscopy. Thin sections were doubly stained with uranyl acetate and lead citrate for examination by electron microscopy (JEOL JEM EXII).

RESULTS

Relative liver weight change

To correct liver weights for any differences in body

Table 1. Effect of Intravenous Administration of FB_1 on Liver Weight

Group	Body weight (gm) (mean±SD)	Liver weight (gm) (mean±SD)	Relative liver weight to body weight (mean±SD)	Relative % to control
Control	180.8±8.1	10.1±0.3	5.3±0.4	100
3 hours*	181.6±1.4	10.2±0.2	5.6±0.1	105.2
6 hours	169.7±6.9	8.9±0.1	5.2±0.1	98.1
12 hours	180.8±17.9	9.0±0.9	4.9±0.5	93.1
24 hours	185.3±1.0	10.2±0.1	5.4±0.5	102.6
48 hours	186.1±3.2	9.7±0.4	5.2±0.3	98.9

* Hours after two treatments of FB_1 (1.25 mg/kg/day).

weight between animals, the percentage of liver weight to body weight was calculated for each group and 100% was set for controls at each time point. Liver weights gradually decreased until 12 hours post-dosing, but were elevated at 24 hours to 102.6%, and decreased again at 48 hours (Table 1). These results suggested that active compensatory regeneration to liver injury occurred at 24 hours, and this was supported histologically by a large number of mitosis. However, these results were not significant compared to the control group.

Histologic data

In the livers of rats sacrificed at 3 hours after FB_1 administration, there was a moderate degree of lobular disarray with mild increment of sinusoidal macrophages. Ballooning of the hepatocytes with the appearance of pale, vacuolar cytoplasm was first observed around zone III. There was no evidence of apparent apoptotic cells or inflammatory reactions. A few mitotic cells were found. Six hours after treatment, the hepatocytes of zone III of the acinus showed denser, more granular, and eosinophilic cytoplasms. The hepatocytes of the midzone revealed vacuolar change. Very little evidence of apoptosis was found. Examination of the liver at 12 hours after treatment disclosed scattered cells acidophilic degeneration, located primarily in the centrilobular area (Fig. 1A). Upon careful examination, a few scattered apoptotic cells could be detected (Fig. 1B). Twenty-four hours after administration, 2−3 apoptotic cells per high power field were observed around the central vein. Scattered hepatocytes underwent cytoplasmic shrinkage, nuclear condensation and detachment from neighboring cells. Subsequently, condensed cells showed bleb formation followed by fragmentation, giving rise to eosinophilic roundish apoptotic bodies (ABs). Interestingly, ABs were frequently found in proximity to the cells with acidophilic degeneration (Fig. 1C). ABs containing nuclear remnants were seen either in sinusoid or within cytoplasm of intact hepatocytes. The number of sinusoidal macrophages had markedly increased. The distribution of apoptotic cells coincided with that of the phagocytes, which occasionally phagocytosed ABs. A large number of hepatocytes in mitosis were observed in zone III, suggesting that active regeneration occurred by a compensatory mechanism (Fig. 1D). No lytic necrosis or inflammatory reaction could be observed. Examination of liver tissues at 48 hours after FB_1 treatment revealed preserved architectures with decreased ABs and mild mononuclear cell infiltration around the centrilobular zone.

Serum biochemistry

Activities of liver associated enzymes, ALT and AST, as well as total cholesterol value in the sera from control and treated groups, were significantly different, indicating hepatocellular injury associated with FB_1. Serum ALT and AST from the treated groups decreased in rats after 3 hours of initial increase, and then markedly increased at 24 hours. Serum cholesterol of the treated group elevated from 3 hours to 48 hours after treatment, suggesting that cholesterol metabolism was steadily affected even though histologic findings revealed no apparent apoptotic cells in the liver (Table 2).

TUNEL assay

Few TUNEL positive cells were observed in the liver of rats after 6 hours of treatment. By 12 hours

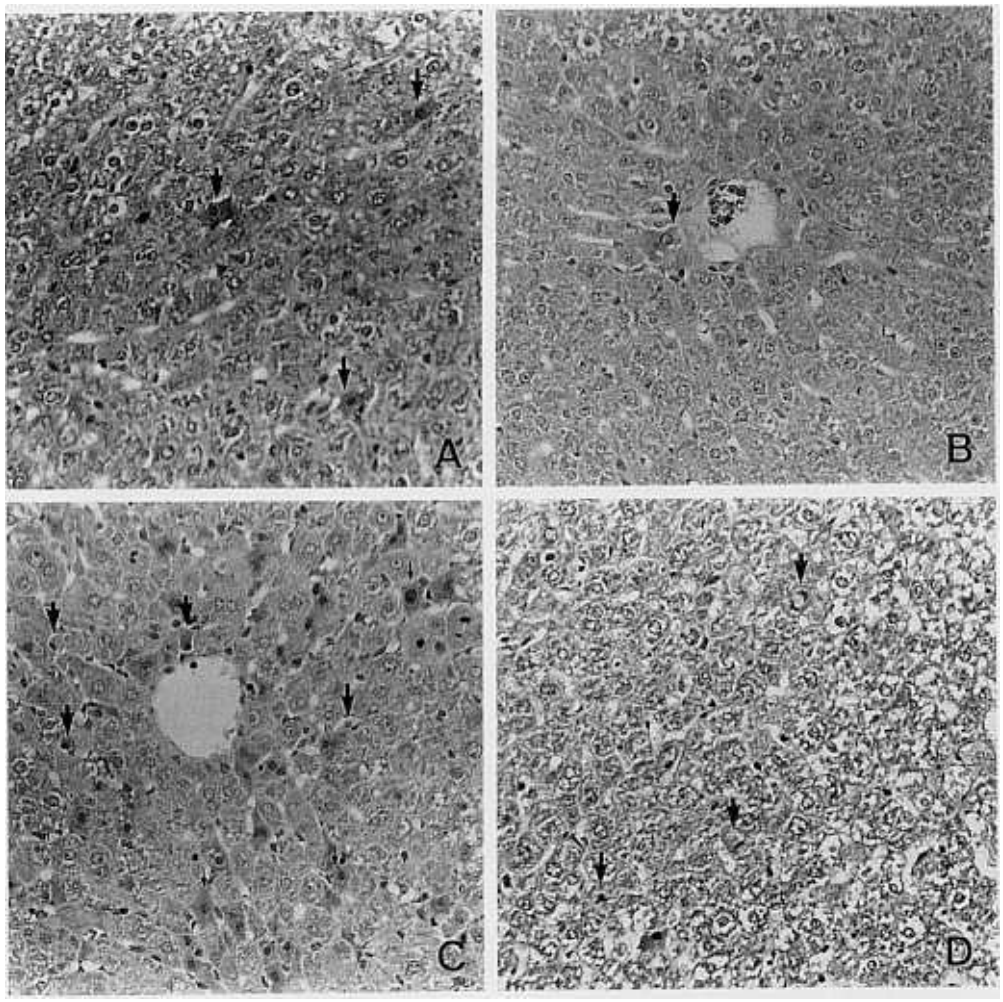

Fig. 1. Histologic findings of rat liver after two treatments (1.25 mg/kg/day) of fumonisin B_1. A) A few scattered shrunken hepatocytes (arrow) showed condensation of the cytoplasm and nuclear pyknosis, 12 hours after administration of FB_1 (HE, ×200). B) Apoptotic cells around the central vein. Note apoptotic hepatocyte with characteristic crescenteric chromatin clumps in nucleus (arrow), 12 hours after the administration of FB_1 (HE, ×200). C) Frequent apoptotic hepatocytes (large arrow) in the proximity of the cells with shrunken hepatocytes (small arrow), 24 hours after the administration of FB_1 (HE, ×200). D) A large number of mitotic hepatocytes, suggesting active regeneration, 24 hours after the administration of FB_1 (HE, ×200).

Table 2. Effect of Intravenous Administration of FB_1 on Serum Chemistry

Group	Cholesterol (mg/dl)	ALT (IU/L)	AST (IU/L)
Control	75.5±16.2	69.8±18.6	122.4±17.9
3 hours*	98.3±8.1	86.6±26.1	208.0±21.5
6 hours	101.3±9.0	61.3±28.7	199.6±55.7
12 hours	100.3±14.1	41.3±2.1	147.0±8.8
24 hours	140.0±12.3	92.0±13.5	205.6±15.0
48 hours	123.0±8.7	75.6±9.0	184.3±16.0

ALT, alanine aminotransferase; AST, aspartate aminotransferase.
* Hours after two treatments of FB_1 (1.25 mg/kg/day).

after administration, the TUNEL-positive cells were distributed in foci over the centrilobular area of the lobule. Intense staining was observed in nuclei and nuclear fragments with the morphological characteristics of apoptosis (Fig. 2). Some ballooned cells exhibited weak and nonspecific cytoplasmic staining patterns. The distribution of apoptotic cells and Abs coinided relatively well with that of proliferated macrophages. The number of TUNEL-positive apoptotic cells increased and peaked at 24 hours and then decreased rapidly by 48 hours after FB_1 administration.

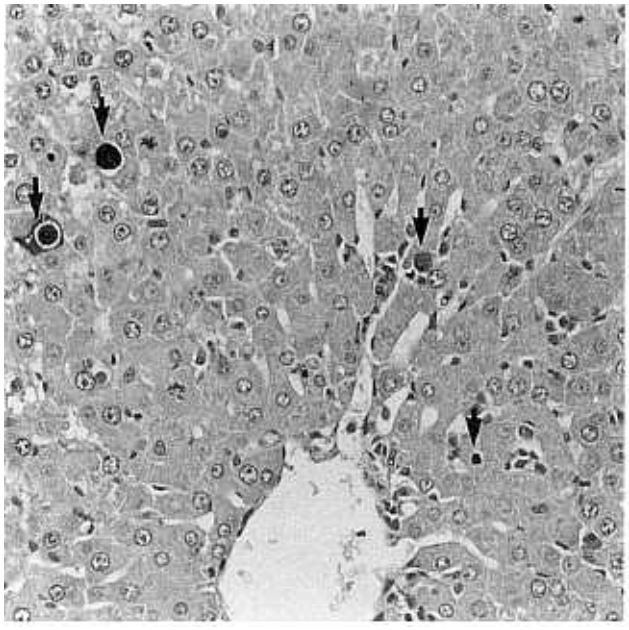

Fig. 2. TUNEL-positive apoptotic hepatocytes and apoptotic bodies, 24 hours after the administration of FB_1 (TUNEL, ×200).

Ultrastructural findings

Ultrastructural study confirmed the aforementioned histological findings. Examination of the liver at 3 hours and 6 hours after treatment disclosed the presence of striking differences between hepatocytes in different zones of the acinus. The liver cells located in periphery of the acinus disclosed empty cytoplasm with a paucity of intracytoplasmic organelles, and peripheral electron-dense, amorphous materials. Very often, distinct hepatocytes were found that revealed numerous small-sized vacuoles along the cytoplasmic membrane with relative preservation of cytoplasmic organelles (Fig. 3). Hepatocytes in zone I of the acinus were relatively well preserved compared to those in zone III. At 12 hours after treatment, variability in the electron density of the cytoplasm was observed among the cells. Some hepatocytes exhibited condensed cytoplasms, while others showed clear cytoplasms containing few mitochondria and hypertrophic smooth endoplasmic reticulum. At this

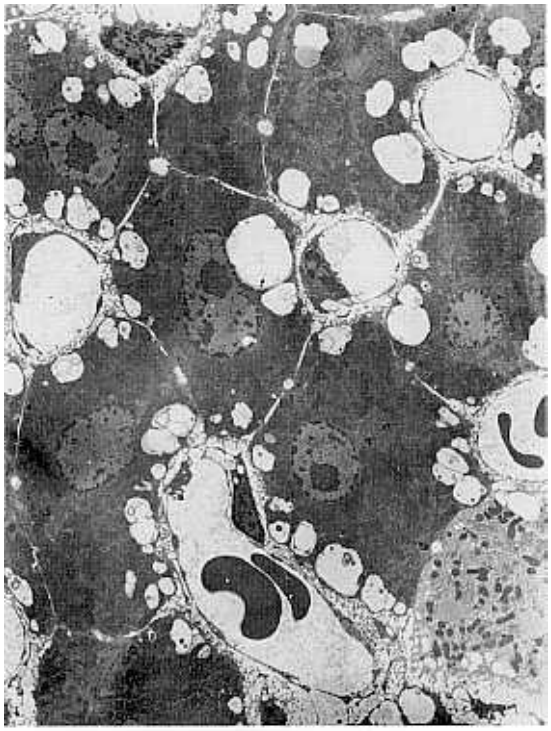

Fig. 3. Electron microscopic findings of rat liver. Note the formation of cytoplasmic vacuoles along the cell membrane, 6 hours after the administration of FB_1 (Uranyl acetate and lead citrate, ×2500).

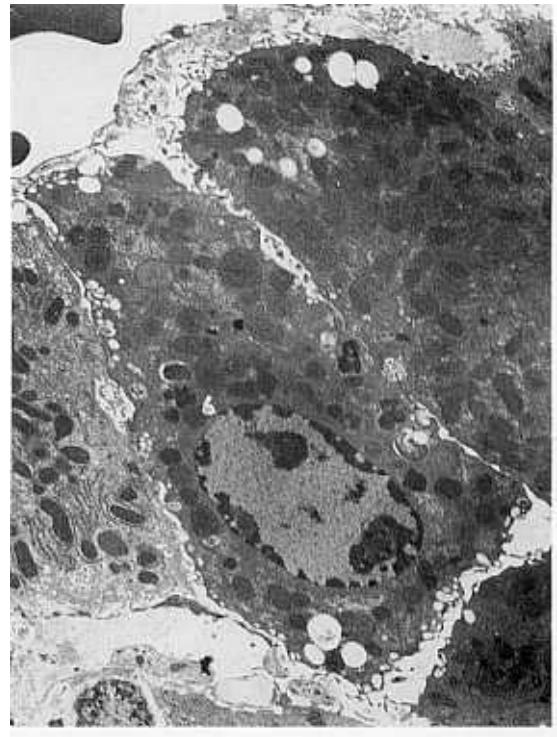

Fig. 4. Electron microscopic findings of rat liver. Hepatocytes showing condensation of cytoplasm, margination of nuclear chromatin with cytoplasmic vacuoles were detaching from neighbouring cells, 12 hours after the administration of FB_1 (Uranyl acetate and lead citrate, ×2500).

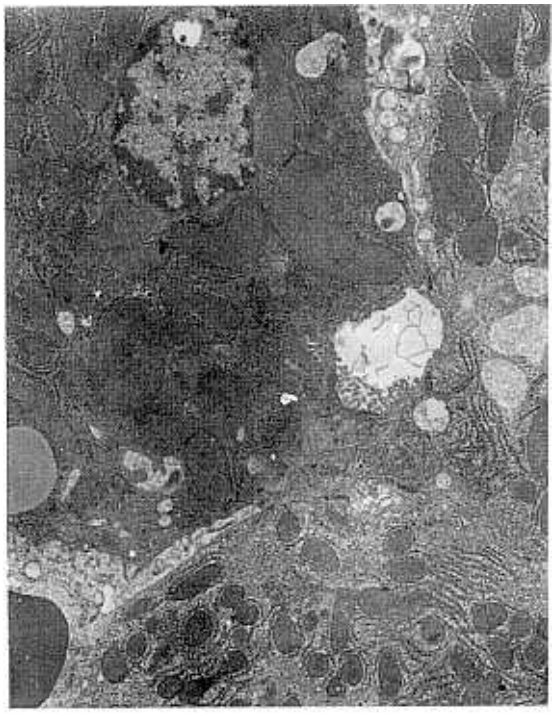

Fig. 5. Electron microscopic findings of rat liver. Hepatocyte showing swollen mitochondria with dense matrical deposit and disruption of membrane, 12 hours after the administration of FB_1 (Uranyl acetate and lead citrate, ×4000).

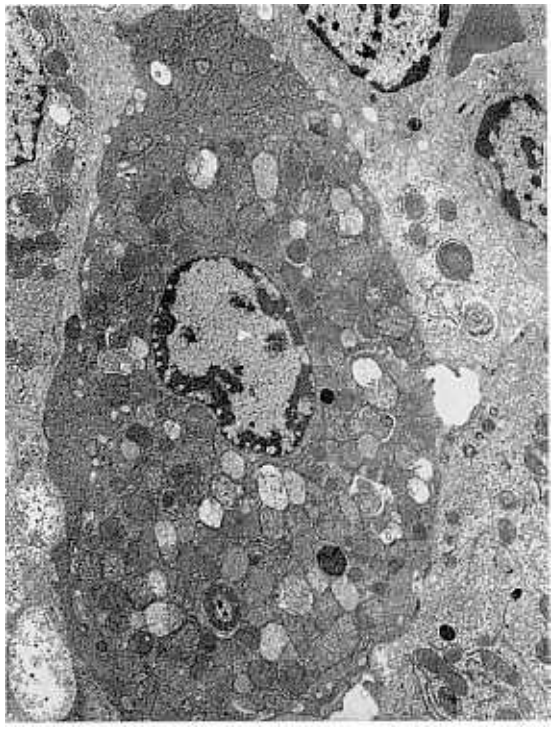

Fig. 6. Electron microscopic findings of rat liver. Apoptotic hepatocyte with condensed cytoplasm, crowed organelles, and characteristic condensed chromatin, 24 hours after the administration of FB_1 (Uranyl acetate and lead citrate, ×4000)

point, it was possible to observe the pre-apoptotic phase of hepatocytes: cytoplasmic condensation of scattered hepatocytes, formation of cytoplasmic vacuoles, and detachment from neighboring cells (Fig. 4). The nucleus was often swollen, or marginated and cytoplasm contained swollen mitochondria with amorphous matrical deposit (Fig. 5). Occasionally, a few ABs containing clumped chromatin were observed inside a sinusoid. At 24 hours after FB_1 treatment, apoptotic cells and ABs were frequently seen both in sinusoids, and within the cytoplasm of hepatocytes. Hepatocytes undergoing apoptosis were characterized by cell rounding, the formation of sharply-delineated, uniformly finely granular masses that became marginated against the nuclear membrane, condensation of cytoplasmic organelles, and the appearance of cell surface and bleb formation followed by cell fragmentation (Fig 6 and 7). Phagocytosed ABs showed different shapes and volume: some contained only clumped cytoplasmic organelles, while others showed fragments of condensed chromatin, some were very small while others were

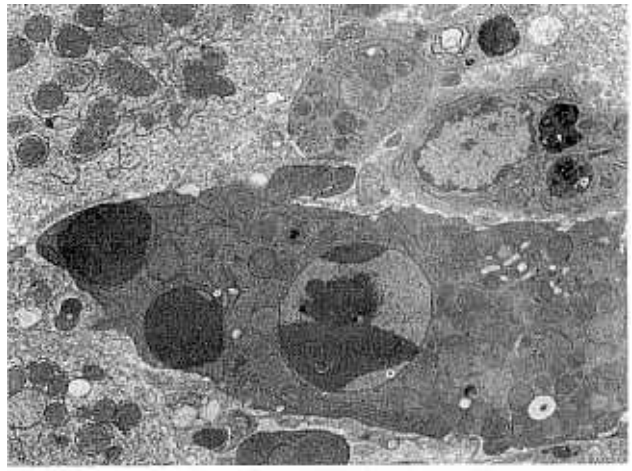

Fig. 7. Electron microscopic findings of rat liver. Advanced apoptosis, formation of blebs and detachment of multiple apoptotic bodies, 24 hours after the administration of FB_1 (Uranyl acetate and lead citrate, ×3000).

so large as to press the hepatocytic nucleus and modify its shape, and some globules showed apparently intact organelles while others showed evi-

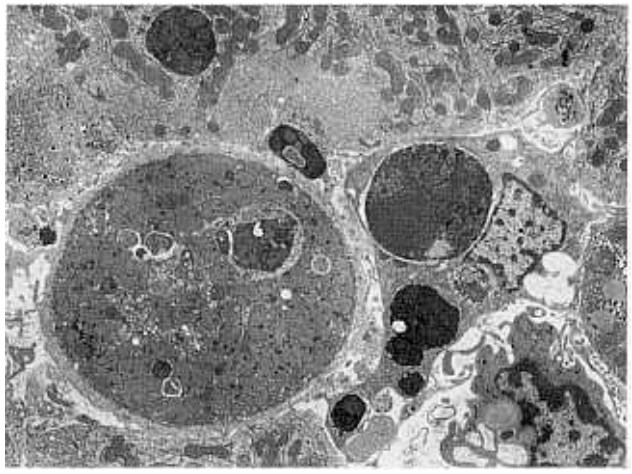

Fig. 8. Electron microscopic findings of rat liver. Various stages of apoptotic bodies lie in the sinusoidal lumen, within the cytoplasm of neighbouring hepatocytes, or sinusoidal macrophages, 24 hours after the administration of FB_1 (Uranyl acetate and lead citrate, × 1500).

dence of initial degradation (Fig. 8). At 48 hours, ABs decreased and recruits of mononuclear cells were found.

DISCUSSION

It is evident from the present study that the administration of mycotoxin FB_1 can induce cell death in rat liver. The type of cell death observed after FB_1 treatment was apoptosis. No evidence of cell necrosis could be observed at any time in the study.

Morphological data from the present study showed that the earliest changes of hepatocytes affected by FB_1 were the appearance of numerous small cytoplasmic vacuoles. Microscopically, hepatocytes exhibiting dense eosinophilic, branched cytoplasm with nuclear condensation were observed 12 hours after treatment. These pre-apoptotic cells were characterized by electron microscope as follows: formation of cytoplasmic vacuoles, swollen mitochondria, dense cytoplasmic ground substances, and detachment from neighboring cells without remarkable nuclear change. These pre-apoptotic changes occurred 12 hours after FB_1 administration. Pre-apoptotic cells may progress to characteristic apoptotic cells showing advanced condensation of cytoplasmic organelles, irregularity of cell surface with formation of blebs, crescentic margination and fragmentation of nucleus, and final detachment of multiple ABs. Previous studies have suggested that stellate ABs and round ABs represented two morphologic forms of apoptosis.[15] Previously mentioned stellate ABs could be regarded in the same light category as the pre-apoptotic cells described above. The cytoplasm and nuclear degeneration are both less severe in stellate ABs, suggesting that stellate ABs can evolve into round ABs. In this study, similarities between the stellate-shaped cells and apoptotic cells were as follows: 1) both had cytoplasmic and nuclear shrinkage; 2) both forms were topographically associated in areas of occurrence; and 3) both occurred time sequentially. Diens et al., in a study of non A-non B hepatitis, indicated that eosinophilic granular cytoplasmic change progressed to stellate-shaped acidophilic cells and then to classic acidophilic bodies.[16] Several observations,[15,16] as well as this study, supported the possibility that cells showing cytoplasmic vacuoles, dense cytoplasm, and simple cytoplasmic borders with no evidence of advanced nuclear changes, were precursors of apoptotic cells.

The hepatic tissues analyzed up to 12 hours after FB_1 treatment disclosed few morphologically detectable apoptotic cells. With careful examination, a few ABs could be seen localized around zone III. A massive increase of ABs was noticed 24 hours after treatment. Electron microscopic analysis showed the presence of numerous ABs containing nuclear remnants in the extracellular space, as well as within the cytoplasm of intact hepatocytes, but apopotic cells with early nuclear changes were sparsely seen. These findings indicate that the kinetics of the early apoptotic process are extremely fast. In fact, the localization of ABs inside the cytoplasm of intact hepatocytes or macrophages is considered to be the last step in the apoptotic process, just before the degradation of globules.

Fumonisins are mycotoxins produced by *Fusarium moniliforme* and related fungi that are common contaminants of corn, sorghum and related grains throughout the world.[1-6,17] FB_1, the most abundant fumonisin, is nephrotoxic and hepatotoxic in many animals,[7,18-20] and it is hepatocarcinogenic in rats.[18] Epidemiologic studies have linked fumonisins to high incidences of human esophageal cancer in the Transkei region of southern Africa.[1,17] FB_1 alters cell morphology, cell-cell interactions, the behavior of cell surface proteins, protein kinases, metabolism of other

lipids, cell growth, regeneration, and viability.[21,22] Recently, the induction of apoptosis after FB_1 treatment has been demonstrated in cultured cells and animal tissues.[23-25] The mechanism of FB_1 toxicity is not fully understood. FB_1 resembles the basal backbone of sphingolipids, a class of membrane lipids that play an important role in signal transduction pathways, cell growth, differentiation, and cell death.[22] FB_1 and other members of the fumonisin family are potent and competitive inhibitors of ceramide synthase, an enzyme that catalyzes the acylation of sphingolipids and reutilization of sphingosine derived from sphingolipid turnover.[9,22,26] Fumonisin-induced depletion of complex sphingolipids and the accumulation of free sphingoid bases and their metabolites can, at least partially, account for the FB_1 effects on cell proliferation, differentiation, and increased cell death.[27-29]

In this respect, serum analysis data were impressive. There was fluctuation in hepatic enzyme levels according to the functional states of the liver. The reason for fluctuation in the liver enzyme level is unclear. We speculated that most injured hepatocytes undergo recovery in the course of time, but at 24 hours after treatment, severely injured hepatocytes progress to massive apoptosis. The morphologic findings that vacuolated hepatocytes decreased in number over time, and that the ultimate number of apoptotic cells was smaller compared to that of vacuolated cells, supported the above assumption. However, the cholesterol level remained elevated throughout the experimental period, which indicated that FB_1 could affect cholesterol metabolism from the beginning to the end of this experiment, despite the absence of apoptotic cell death. Early hepatic changes verified the above explanation by electron microscopic examination, and especially vacuolar change of the cell membrane was in agreement with the elevated total cholesterol level. Within days of giving ponies feed contaminated with FB_1, it is known to cause an increase in the amount of free sphinganine and a reduction in complex sphingolipids.[30] This finding suggests that consumption of fumonisin-contaminated feed disrupts sphingolipid metabolism and that changes in sphinganine and sphingosine seen before liver enzymes become noticeably elevated may be an early marker of exposure to fumonisins. Yoo et al. demonstrated that de novo sphingolipid biosynthesis in LLC-PK1 cells was significantly inhibited before the inhibition of cell proliferation or cytotoxicity was observed, suggesting that inhibition of de novo sphingolipid biosynthesis was an early event in the process leading to cytotoxicity.[10] The source of the increase in free sphingosine could be due to inhibition of the turnover pathway (sphingosine N-acyltransferase) without increased catabolism.[10]

The present morphologic data support previous functional studies that showed FB_1 induced changes in cellular membranes and sphingolipid biosynthesis. Altered membrane lipid constituents of the hepatocytes are likely to be the early key events that can explain the cytotoxic effects and altered growth responses induced by fumonisins in hepatocytes.[9,10,29,30] The exact mechanisms of FB_1 inducing apoptosis of hepatocytes are unclear. However, it is possible to postulate two mechanisms to explain the observations resulting from FB_1 treatment. First, depletion of critical sphingolipids by blockage of the de novo biosynthetic pathway could be predicted to affect cell function and survival because complex sphingolipids are involved in the regulation of cell surface receptors, ion pumps and other systems vital to cell survival. Second, the addition of exogenous free long-chain bases to cells has been shown to affect the diverse cell signaling system (inhibition of protein kinase C and phosphatidic acid phosphohydrolase, and activation of tyrosine kinase activity in the epidermal growth factor receptor).[11,21,22] Thus, the accumulation of endogenous sphinganine and sphingosine in the presence of fumonisins might lead to cell death or transformation via their effects on crucial signal transduction systems.

The mechanisms for recognizing and eliminating apoptotic cells in the liver need to be emphasized. Kupffer cells/macrophages, together with neighboring hepatocytes, fat storing cells, and endothelial cells, take part in clearing out apoptotic hepatocytes.[31] Because apoptotic cells were surrounded by Kupffer cells and macrophages, with an increase in number, it is suggested that the migration of such cells into the hepatic lobule is important for recognizing and phagocytosing ABs. It was found in this study that the number of apoptotic cells was highest in the regions enriched with macrophages.

FB_1 inhibits ceramide synthase, leading to accumulation of free sphingoid bases.[22,28,29] Despite its known biochemical actions, the mechanism of FB_1 toxicity is not fully understood. Cytokines are biologic response modifiers produced by macrophages, lym-

phocytes, and other cell types when activated by specific antigens or other stimuli. They are crucial in the development of immune responses, cell proliferation, differentiation, hematopoiesis, and inflammation.[32,33] The induction of apoptosis by macrophages is of interest, particularly considering recent reports that apoptotic occurrence is consistent with macrophage cytocidal activity,[34-37] and that aging neutrophils[38] and those cells from a regressing tissue[34-37] are engulfed prior to death of the tissue and subsequently acquire the morphology of typical apoptosis. Confirmed mediators of macrophage cytotoxicity currently include tumor necrosis factor α, nitric oxide, IL1 β, and reactive oxygen intermediates.[37,39-42] Pretreatment with the Kupffer cell inhibitor, gadolinium chloride, prevented cell death due to carbon tetrachloride.[43] A recent study indicated that FB_1 toxicity may involve secretion of TNF-α by TNF-α -producing macrophages without altering the production of interleukin-1 α or interferone-γ. This result suggests that TNF-α production may be a contributing factor to FB_1-induced apoptosis and other observed toxic effects in animals.[44] Sphingomyelin has emerged as a key participant in the signaling pathways of cytokines such as TNF-α, IL-1 β and IFN γ.[22] Regarding the close proximity of macrophages with apoptotic hepatocytes in the present morphologic data, a possible inter-relationship is suggested between macrophages and the induction or management of apoptosis. It remains to be determined whether fumonisins can disrupt sphingolipid biosynthesis and turnover, and as a consequence, alter lipid second messengers to induce changes in cytokine production of macrophages.

In summary, morphologic data and serum findings in this study support the theory that FB_1- induced changes of membrane lipid constituents of the hepatocytes are likely to be early key events in explaining FB_1 cytotoxic effects. Furthermore, this study suggests that the apoptotic effect of FB_1 on hepatocytes may serve as an experimental model to study the process of sphingolipid-mediated apoptosis, or to further characterize an unknown alternative pathway for generating death signals.

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