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Mitogenic and Cytotoxic Effect of pure Fumonisin B_1, a carcinogen, in Sprague-Dawley Rats.

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ABSTRACT : **Fumonisin B_1 is hepatotoxic in all species, but liver carcinogenic and nephrotoxic in rat. Our objective was to investigate the effects of multiple iv dose of FB_1. Male Sprague-Dawley rats were injected intravenously (iv) with FB_1 at 1 mg/kg singly (T1), or daily for 2 (T2) or 3 (T3). T1 rats did not show any cytotoxicity in both liver and kidney. However, the most dramatic change occurred in this group was mitotic figures in liver, which increased 5.5-fold to that of control. Hepatotoxic effects were shown in T3, based on histopathology and serum chemistry. A few scattered single cell deaths occurred primarily in the centrilobular area of the liver in T2. Similar but more lesions in liver and a small number of degenerating cells with hypereosinophilic cytoplasm in outer stripe of medulla of kideny were found in T3 rats. Serum chemical profiles included liver enzymes increased, in which cholesterol was very sensitive. This study suggests that multiple exposure of low dose FB_1 cause cytotoxic in the liver earlier time point than kideny. FB_1 also stimulates mitosis in liver that may be associated with carcinogenesis.**

Key words : fumonisin B_1, serum cholesterol, cytotoxic, mitogenic

INTRODUCTION

The fumonisins, secondary metabolites of several species of *Fusaria,* especially *Fusarium moniliforme* and *F. proliferatum* are commonly found in contaminated corn and other food grains throughout the world. Fumonisin B_1 (FB_1) had been the most widely studied of the fumonisins and its presence in animal feeds has been associated with equine leukoencephalomalacia and pulmonary edema in swine(Marasas *et al.*, 1988a; Haschek *et al.*, 1992). The liver is affected in all species examined, and other target organs appear to be more species specific.

Gelderblom *et al.* (1988) reported FB_1 was hepatocarcinogenic to rats and it has been epidemiologically associated with human esophageal cancer in areas of southern Africa and China (Marasas *et al.*, 1988b; Rheeder *et al.*, 1992). Seemingly, fumonisins have potentially important human health concerns.

The fumonisins are structurally similar to sphingosine, the major long-chain base backbone of cellular sphingolipids, and block *de novo* sphingolipid biosynthesis by inhibiting sphinganine N-acyltransferase which leads to intracellular accumulation of sphinganine. Wang and colleagues (Wang *et al.*, 1991) hypothesized that disruption of sphingolipid biosynthesis is a likely mechanism of fumonisin toxicity. Recently Schroeder *et al.* (1994) showed that FB_1 induced mitogenesis via accumulation of sphingoid bases rather inhibition of complex sphingolipid biosynthesis.

The previous study showed that single iv dose (1.25 mg/kg BW) of FB_1 induced progressive injury to the kidney; cell proliferation in the liver and esophagus occurred in the absence of observable tissue injury but was associated with apoptosis in the kidney (Lim *et al.*, 1995). In a pilot study, female rats (8 months old) injected with FB_1 iv, daily for 1, 2, or 6 at 1mg FB_1/kg showed on histological changes in kidney of any rats, which may due to fact that the female rat is more resistant to FB_1-induced disruption of kidney sphingolipid metabolism

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than the male (Riley *et al.*, 1994). However, liver showed increased mitotic figure in the manner of injection frequency-dependent. This pilot study give a clue that liver may be more sensitive than kidney to multiple exposure of FB_1.

Thus, preliminary study was designed to determine the morphologic change in target organs following the multiple injection of low dose of FB_1.

Materials & Methods

Ten male Spraque-Dawley rats (150-200g) were purchased from Harlan Industries (Indianapolis, IN) and housed in polypropylene cages. Rats were maintained in a 12 hr light-dark cycle, and had free access to food and tap water. The rats were allowed to acclimate for 1 week prior to study. Fumonisin B_1 (purified to >95%, Dr RF Vesonder *et al.*, 1990) was dissolved in sterile phosphate buffered saline (PBS) before use.

The rats were divided into 4 groups. Rats were injected with FB_1 iv for 1 (T1, n=2), or 2 (T2, n=2) or 3 day (T3, n=3) consecutively at 1 mg/kg and euthanized CO gas on day 2, 3 and 5 respectively. This dose was based on previous study (Voss *et al*, 1993). Control rats (n=3) received PBS via the same route and euthanized on day 5.

Body weights were meaured before euthanasia. In control and T3 rats, bloods were collected from the abdominal vena cava during euthanasia. Serum samples were analyzed using an autoanalyzer (Hitachi 704) for the following parameters: alkaline phosphatase (ALP), alanine aminotranferase (ALT), aspartate aminotranferase (AST), gamma-glutamyl (GGT), creatinine, serum urea nitrogen (SUN), total bilirubin, total protein, and cholesterol. At necropsy, liver and kidney were removed, weighed and preserved in 10% neutral buffered formalin, then processed routinely, sectioned at 4 microns, stained with hematoxylin and eosin, examined by light microscopy.

Results

No clinical signs were observed during the experimental period and all rats survived to the end of the experiment. Relative weights of liver and kidney in treated groups were significantly decreased in T3 rats (relative weight in liver: 4.13±0.08 for T3 vs. 5.03±0.27 for control; relative weight in kidney: 0.74±0.03 for T3 vs. 0.82±0.04 for control).

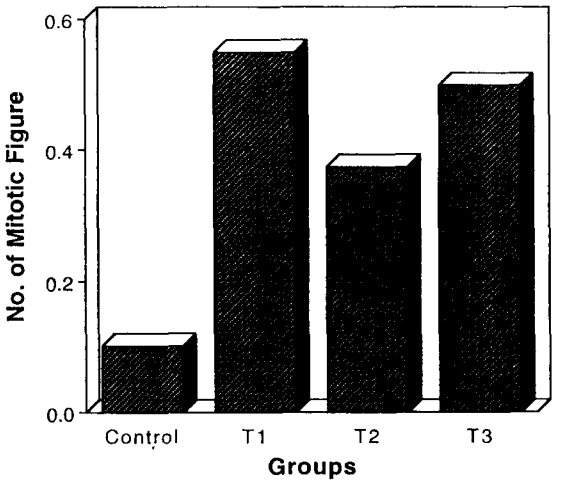

Fig. 1. The effect of a single (T1), or multiple injection (T2 or T3; daily for 2 or 3 days) of pure FB_1 at 1 mg/kg on hepatocyte mitosis in Sprague-Dawley rats. Numbers were determined by counting the mitotic figures in left lateral lobe in 20 random fields (X200).

In T3 rats, the activities of liver-associated enzymes, ALP, ALT, AST, as well as the levels of bilirubin were elevated. Similarly, creatinine, SUN, and total protein were increased slightly (data not shown). The marked increase was observed in cholesterol (158±41 mg/dl for T3 vs. 84.5±1 for control).

Microscopically, the most prominent change was mitotic figures in T1 liver, even no morphological injury was not seen. The number of mitotic figures was increased in 24 hr after dosing, to 5.5-fold that of control. In T2 rats, the number of mitosis seemed to decreased transiently, then increased in T3 rats (Fig. 1).

Hepatotoxic effect was observed in T3. The lesions were scattered occasional single cell necrosis around the central vein, characterized by pyknotic nuclei, hyaline droplets, and mitotic figures. The hepatic lesions were primarily centrilobular (Fig. 2). Renal lesions were localized to the tubules in the outer stripe of medulla, in which a few degenerating cells with hypereosinophilic cytoplasm was seen in T3 (Fig. 3). However, any renal lesion was not seen in T1 and T2 microscopically.

Single cell death in both organs regarded as apoptosis was seen occasionally, which characterized by shrinkage with condensation of cytoplasm, and fragmentation or margination of compacted nuclear chromatin.

Discussion

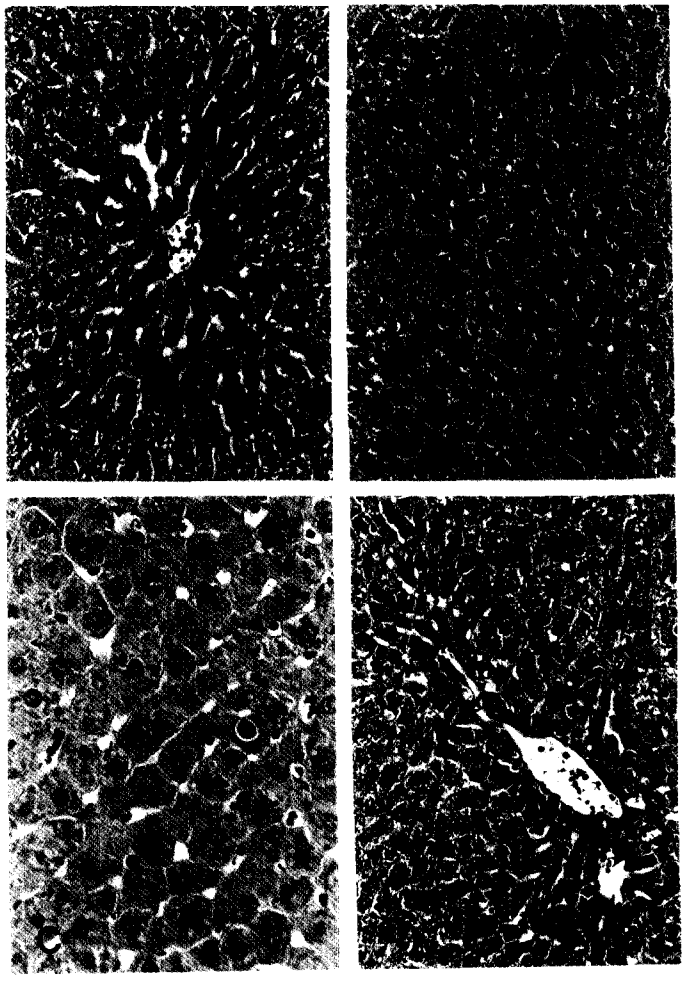

Fig. 2. Photomicrographs of liver from male Sprague Dawley rats injected 1 mg FB_1/kg. Hematoxylin and eosin, X450. A. Control. B. T1 rat showing mitosis (arrowheads); prophase, anaphase and telophase in counterclockwise direction from the top arrowhead. C. T2 rats showing apoptotic cell, characterized by shrinkage with condensation of cytoplasm, capping of nuclear chromatin and halo (arrow). X900. D. T3 rats showing mitotic figures, hyaline droplets (arrowheads) and degenerating cell (arrow).

The kidney and liver are target organs of FB_1 in the rat, and rabbit with kidney being the most senitive (Gumprecht *et al.*, 1995). In feeding study, the concentration of fumonisin B_1 that caused nephrotoxicity in SD rats was much less than that required to cause hepatotoxicity with regard to disruption of sphingolipid metabolism (Voss *et al.*, 1993, 1995). In their studies, rats given feed containing FB_1 at 15 ppm ingested 1.4 mg FB_1/kg/day and 9 ppm ingested 0.62 mg FB_1/kg/day.

The primary target organ of FB_1 is kidnay in rat injected a single iv with 1.25 mg FB_1/kg (Lim *et al.*, 1995). On the contrary, this multiple injection study with lower dose showed that liver was sensitive in the early time point, based on morphology. Cholesterol levels were remarkably elevated in T3 as reported previously in rats and mice (Voss *et al.*, 1993). Serum protein and serum urea nitrogen were increased, which indicated renal toxicity. The lesions of kidney in this study was similar but milder than those described previously in the rat (Lim *et al.*, 1995). The most prominent changes induced by FB_1 were mitotic figure in livers, although statistical significance was not achieved because of the small number of rats per group.

Even the carcinogenic mechanism of FB_1 is still not understood, several studies showed FB_1 is non-genotoxic (Norred *et al.*, 1992a) and a complete carcinogen (Gelderblom *et al.*, 1991), which cause the different stages of cancer development.

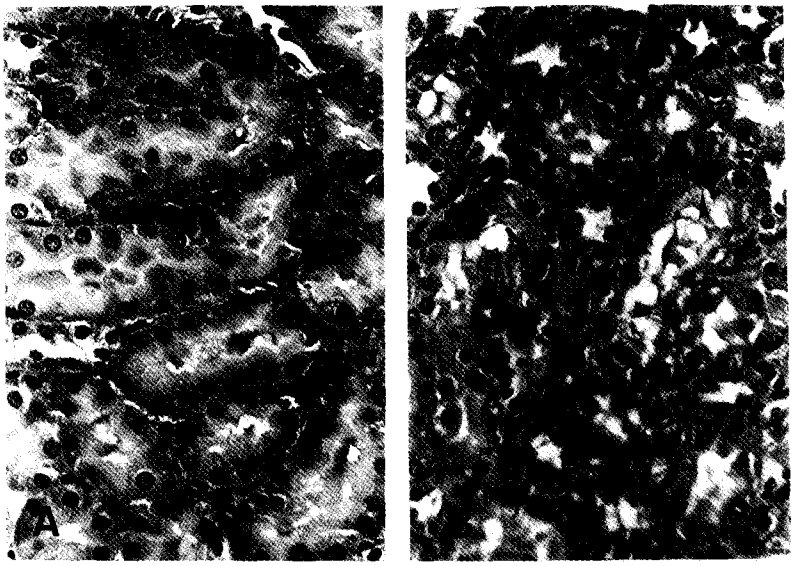

Fig. 3. Photomicrographs of kindey from male Sprague Dawley rats injected 1 mg FB_1/kg. Hematoxylin and eosin, X900. A. Control. B. T3 rats. The tubules in the outer stripe of medulla showed degenerating cells with hypereosinophilic cytoplasm (arrowheads) and apoptosis with margination of nuclear chromatin (arrow).

Non-genotoxic carcinogens can act through alteration of cell proliferation, either directly by stimulating mitogenic activity of indirectly by regenerative cell proliferation following cytotoxicity, or by induction or inhibition of apoptosis (Cohen *et al.*, 1990; Bissonnette *et al.*, 1994). There are still controversy results *in vitro* that FB_1 can be mitogenic or inhibitory to cell proliferation depending on the cell line and conditions of culture (Norred *et al.*, 1992b; Schroeder *et al.*, 1994). Since 1994 the National Center for Toxicology Research, USA is conducting a 2-year chronic tumor studies of FB_1 in mice and rats. It will give more information to make an appropriate risk assessment, and understand the mechanism of carcinogenesis associated with fumonisins.

In the preliminary limited survey of FB_1 contents, the natural occurrence was detected in Korean-native corn samples from the 1994 harvests intended for human consumptions, up to 1.3 ppm (Lim *et al.*, 1995). Additional survey is required to determine the significance of FB_1 content in native and imported agricultural commodities.

This preliminary experiment suggest that under the exposure conditions used, effect of FB_1 occurs in liver earlier than in kidney. It also stimulates mitosis in liver.

Acknowledgements

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Fumonisin B_1의 SD흰쥐에 대한 세포분열과 세포독성작용

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적 요

Fumonisin B_1(FB_1)은 모든 동물종에서 간독성을 나타내며 흰쥐에서는 간암과 신장독성을 일으키는 발암물질이다. 본 연구에서는 **FB_1**의 반복투여 효과를 관찰하고자 수컷 **Sprague-Dawley** 흰쥐에 **1 mg FB_1/kg**을 **1일(T_1)** 혹은 **2일(T_2), 3일(T_3)**간 반복주사하였다. **T_1**군에서는 간장과 신장 다같이 독성작용이 발견되지 않았으나 간장에서는 대조군에 비해 **5.5**배의 증가된 세포분열상이 관찰되었다. 한편 **T_3**군에서는 병리조직학적 관찰과 혈청검사에서 간소엽 중심부에 병변이 있는 간독성이 관찰되었으며, 신장은 수질부 **outer stripe**에서 세포변성이 인정되었다. **T_3**군에서는 간효소를 포함하는 혈청검사치가 증가되었는데 특히 **Cholesterol**이 증가되었다. 따라서 **FB_1**의 반복노출시에 신장보다는 간장에서 먼저 세포독성이 관찰되었으며 세포분열 역시 증가되었다.