Fumonisin B_1 induced renal toxicity in Sprague-Dawley rats

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Abstract. We investigated the effect of a single intravenous dose of Fumonisin B_1(FB_1) of rat kidney on the time sequence. Male Sprague-Dawley rats were intravenouslyin jected with FB_1 at 1.25 ㎎/㎏ and were euthanized at 12 hrs, 1, 2, 4, and 6 days after the injection. In FB_1 treated rats, serum BUN and creatinine were elevated from 12 hrs. Microscopically, the initial target site was tubules of inner stripe, with mild degenerative and necrotic changes at 12 hrs, but the tubules recovered on day 4. In outer stripe, there were only a few scattered necrotic cells on day 1. These changes became more obvious over the time passed and most severe on day 4. On day 6, regeneration occurred, manifest as hypertrophic, basophilic tubular cells. The dying cells were proved to necrotic cells instead of apoptotic cells by TUNEL. Ultrastructural changes were cytoplasmic vacuole, dilated endoplasmic reticulum, swollen mitochondria, ballooned microvilli of the tubular cell in the outer stripe. These results showed that the renal tubules of outer medulla were the target to FB_1-induced nephrotoxicity in the rat. However, initial target was inner stripe of medulla.

Key words : Fumonisin B_1, inner stripe, outer stripe, nephrotoxicity

Introduction

Fumonisins are toxic fungal metabolites of *Fusarium moniliforme*, and other *Fusarium* species commonly found in corn.[1,2,3] Fumonisins have been reported in animal feeds and corn-based human food-stuffs.[4,5] Although hepatic injury has been induced experimentally in all vertebrate species studied to date, other target organs appear to be more species-specific; equine leukoencephalomalacia,[6] porcine pulmonary edema,[7] and epidemiological data of human esophageal cancer.[8] Additional concerns related to human health are the ability of fumonisin to induce hepatocellular carcinomas.[9]

The real mechanism of fumonisin toxicity is poorly understood, but it appears to be related to interference with sphingolipid biosynthesis in multiple organs. The fumonisin backbone strongly resembles that of the sphingoid bases, sphinganine and sphingosine, and FB_1 inhibits sphinganine N-acyltransferase, a critical enzyme in the biosynthesis of sphingolipid.[10] Nephrotoxicity of FB_1 has been reported in several species. In horse, renal lesions has been described variously as hydropic degeneration, nephrosis, or individual cell necrosis.[11,12] In pig, mild to moderate renal tubular necrosis was reported.[13] In rat and rabbit, the kidney appears to be the most sensitive target organ.[14,15]

The objective of this study was to elucidate the histopathologic lesions of the kidney as fumonisin toxicity in the rat according to the base of time sequence.

Materials and Methods

Animals

Three-week-old male Sprague-Dawley rats were obtained from Korea Research Institute of Chemical Technology and reared until the body weights were from 170 to 200 g in our laboratory. The rats were allowed to acclimate for 1 week prior to this study

Treatment protocol

FB_1(purified to > 98%; Sigma) was dissolved in sterile phosphate buffered saline (PBS) before use. Rats were given a single intravenous injection(maximum volume 0.5 ㎖) of FB_1 at 1.25 ㎎/㎏ or an equivalent amount of PBS(vehicle) into the tail vein on day 0. This dose was based on previous studies[12]. Animals were observed daily for the check of clinical signs such as behavior or vitality. Three control and three FB_1-treated rats were euthanized at 12 hrs, 1, 2, 4, and 6 days, respectively. Blood samples were collected from the abdominal vena cava.

Serum analysis

Serum samples were analyzed using an autoanalyzer(Spotchem SP-4410, Daiichi kagaku Co., Kyoto, Japan) for blood urea nitrogen(BUN) and creatinine.

Pathologic observation

After euthanasia, animals were immediately necropsied and left kidneys were fixed in 10 % neutral buffered formalin, routinely processed, sectioned at 3-4 ㎛, stained with hematoxylin and eosin(HSE), and examined by light microscope.

The method of TdT-mediated dUTP-biotin nick end labeling(TUNEL) was performed on kidney sections using TACSTM in situ apoptosis detection kit(Trevigen, Gaithersberg, USA). Small pieces(1 mm^3) of right kidney of each rat were taken for electron microscopic examination. Sections were pre-fixed in 3 % glutaraldehyde in 0.1 M phosphate buffer(pH 7.4), post-fixed in 1 % osmium tetroxide, then processed routinely, and observed under a JEOL JEM 1010 transmission electron microscope(JEOL, Tokyo, Japan).

Statistical analysis

Quantitative data were analyzed using the paired Student's t-test and were considered significant at $P<0.05$

Results

General condition and serum analysis

All animals survived to the end of the study. On day 4 after dosing, FB_1 treated rats were inactive and showed bristling of hairs. Serum BUN and creatinine were elevated in FB_1-treated rats beginning on 12 hrs. BUN were progressively increased and peaked on day 4 in FB_1-treated rats; only decreased on day 1(Table. I).

Pathologic findings

On gross, kidneys were pale in FB_1 treated group at necropsy. Histopathologically, the renal lesions were predominantly located in the outer medulla of the kidney. Renal tubular cells of inner stripe showed normal architecture in control group(Fig. 1a). Kidney lesions were first detected at 12 hour, in which the tubular cells of inner stripe were degenerate and necrotixed (Fig. 1b). Thereafter, the affected portion of the tubules returned to almost normal structure showing mitotic figures on day 4 and 6 (Fig. 1c, 1d).

Tubular cells of outer stripe showed normal architecture in control group (Fig. 2a). Cell death was initially observed on day 1 and increased in severity until day 4 (Fig. 2b). Some dying cells, characterized by marginated

Table 1. Effects of a Single Intravenous Dose of Fumonisin B1 at 1.25 ㎎/㎏ (BW) on Serum Urea nitrogen and Creatinine Values in Rats†

Groupa	Urea nitrogen(㎎/dℓ)	Creatinine(㎎/dℓ)
Hour 0		
Control	22.33 ± 2.96	0.77 ± 0.09
Treated	—	—
Hour 12		
Control	23.33 ± 0.88	0.97 ± 0.12
Treated	24.00 ± 0.58	1.00 ± 0.06
Day 1		
Control	24.00 ± 1.16	0.77 ± 0.07
Treated	20.33 ± 2.73	0.93 ± 0.03*
Day 2		
Control	24.33 ± 1.45	0.83 ± 0.17
Treated	28.00 ± 1.00	0.83 ± 0.19
Day 4		
Control	21.33 ± 2.03	0.93 ± 0.12
Treated	31.67 ± 0.67*	1.10 ± 0.10
Day 6		
Control	—	—
Treated	31.33 ± 3.28	1.00 ± 0.06

† Data expressed as mean ± SE (n = 3)
* Significantly different from control; *P < 0.05
a Time post-treatment with fumonisin B1

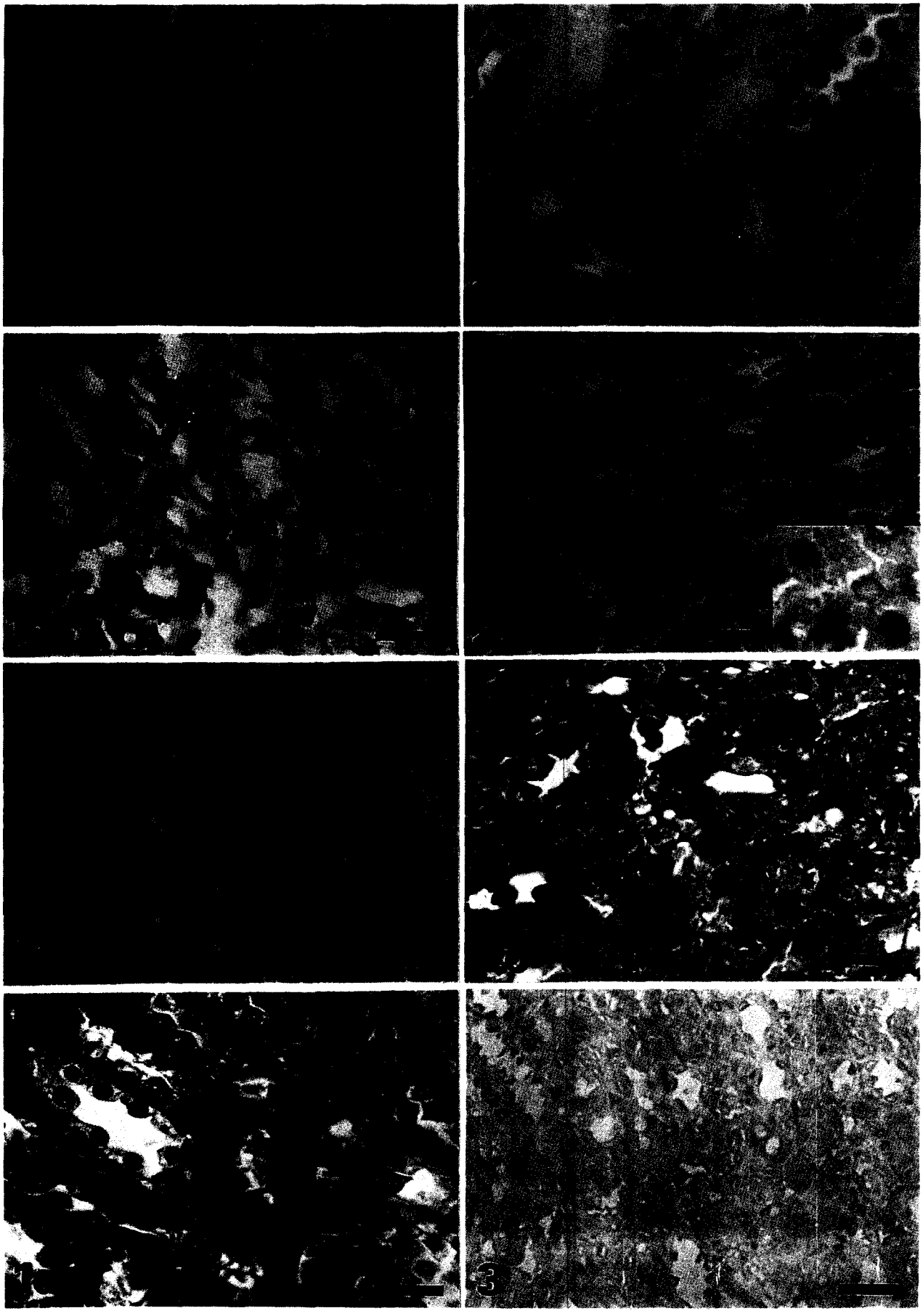
chromatin (Fig. 2b inlet), stained negatively by TdT-mediated dUTP-biotin nick end labeling(TUNEL) (Fig. 3). On day 6, some dying cells were sloughed into the tubular lumens. However, most cells were regenerated, manifest as hypertrophic, basophilic tubular cells (Fig. 2c).

Ultrastructurally, some myelin bodies, swollen mitochondria and variable sized cytoplasmic vacuoles were observed in affected tubular cells of outer stripe on day 4 (Fig. 4a). Those epithelial cells had less electron-density,

Fig.1. Kidney; rat-treated FB1. Inner stripe of outer medulla. 1a. Tubules of control show normal structure. 1b. Degenerative changes consist of cells showing eosinophilic cytoplasm. Pyknotic nuclei with hypereosinophilic cytoplasm are scattered throughout the affected portion of the tubules at 12 hrs. 1c. On day 4, The mitotic figure is shown. 1d. Lesions are recovered to almost normal tubular architectures on day 6. H&E, Bar=23㎛

Fig.2. Kidney; rat. Outer stripe of outer medulla. 2a. Tubules of control show normal structure. 2b. Disorganization of renal tubule with necrotic tubular epithelia is shown on day 4. Some apoptotic-like cells with margination of chromatin (inlet, 650X). 2c. On day 6, Most cells were regenerated, manifest as hypertrophic, basophilic tubular cells with sporadic mitotic figures. Some dying cells are sloughed into the tubular lumens. H&E, Bar=23㎛

Fig.3. Kidney; rat. With TdT-mediated dUTP-biotin nick end labeling (TUNEL), Affected portions of the tubules show negative stain on day 6. TdT-mediated dUTP-biotin nick end labeling (TUNEL), Methyl green counter-stain. Bar=53㎛



ballooned microvilli (Fig. 4b). The cell characterized margination of chromatin showed swollen mitochondria (Fig. 4c).

Discussion

Previous studies have shown that fumonisin is hepatotoxic to most species studied, and also that it induces species-specific toxicity in various target organs.[16] In both the rat and rabbit, the kidney and liver are target organs of FB_1 toxicity with the kidney being most susceptible.[14,17] Our studies demonstrate that the outer medulla is the initial target exposed to FB_1 where was degenerative and necrotic changes.

The values of BUN and creatinine were elevated above control values in FB_1-treated rats beginning on 12 hours and mild but progressively increased as reported previously[15]. Histologically, the early pathologic lesion was detected in the inner stripe, but later damage was predominantly found on proximal tubules in outer stripe at the late time point. The injury of epithelial cells in the outer stripe which was peaked on day 4 recovered on day 6.

Liver and kidney of rat had the highest concentrations of [^{14}C]fumonisin B_1 following both intragastric and intravenous administration[18]. The FB_1 label peaked within several hours in these tissues and persisted for the experiment period (96 hr). The localization of renal injury to the outer medulla in this study was similar to that described previously in the rat[17].

The dominant early morphological change in the kidney and liver in fumonisin toxicity was apoptotic cell death.[15,19] In vitro system, apoptosis was induced.[20,21] However, this type of cell death was not observed in this study. Hisologically, several dying cells were charcterized by margination of nuclear chromatin. These cells stained negatively with the apoptotic detection system of TUNEL and their ultrastrucural organells showed dilated rough endoplasmic reticulum, dilated mitochondria, and ballooned microvilli, which means necrotic cell death.

The metabolism of the fumonisins is poorly understood. The fumonisins are structurally similar to sphingosine, the major long-chain base backbone of cellular sphingolipids, and block *de novo* sphingolipid biosynthesis, as indicated by significant elevation of the sphinganine to sphingosine ratio.[10] This hypothesis may be one of the mechanism of fumonisin toxicity. In the rat kidney, there were significantly elevated concentration of sphingoid bases.[22] However, In other animal studies, the correlation between fumonisin-induced accumulation of sphingoid bases and morphologic injury is not consistent. So, there is still something else besides that is involved. Thus, initial injury site of fumonisin, inner stripe of kidney as shown in this study, can be linked other factors.

In this study we have confirmed that the kidney is the most sensitive organ to FB_1-induced toxicity in the rat and renal tubules of inner stripe is the initial target site in FB_1-induced nephrotoxicity.

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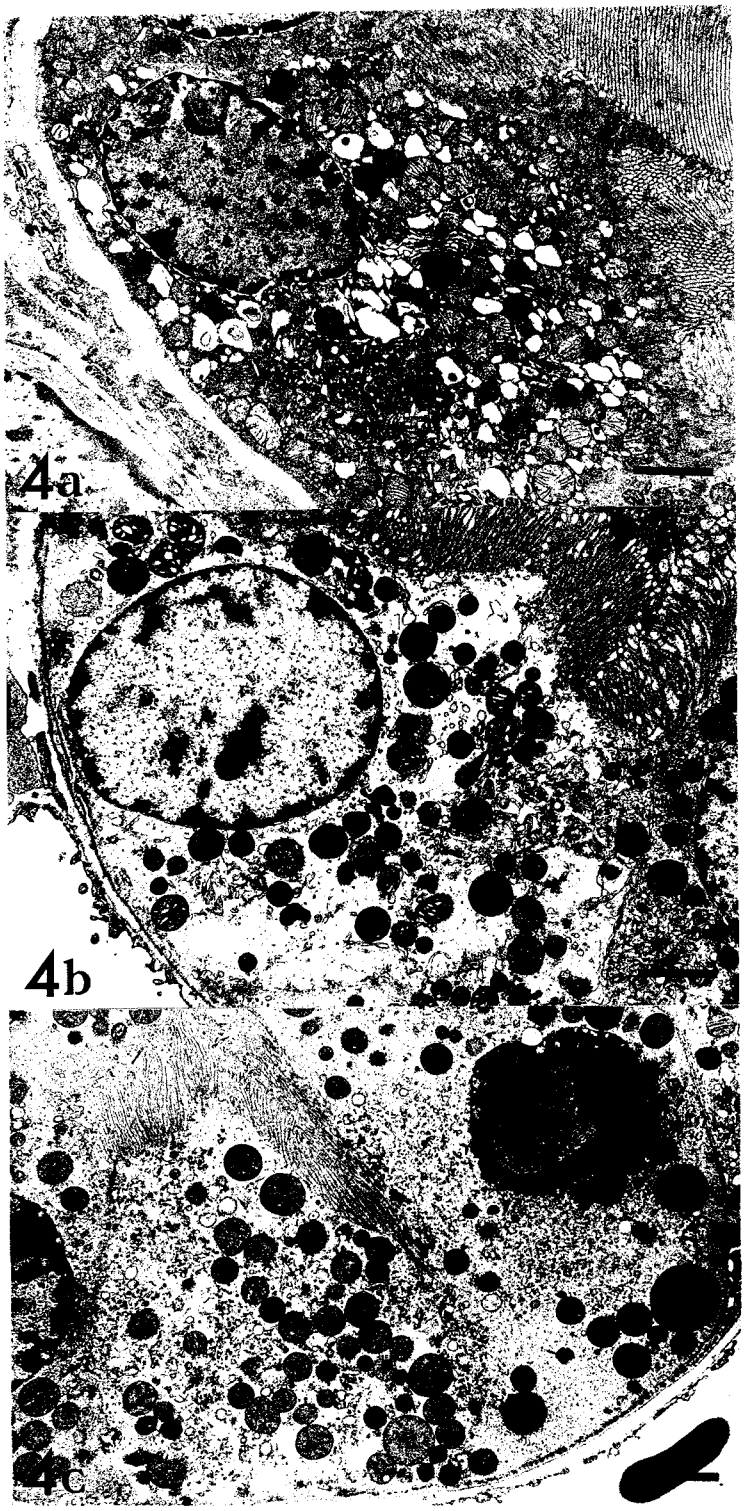

Fig. 4. Kidney; rat treated FB_1 on day 4. 4a. Some myelin bodies, numerous swollen mitochondria and variable sized cytoplasmic vacuoles are observed. 4b. The cytoplasm has less electron density, dilated rough endoplasmic reticulum, and ballooned microvilli. 4c. The cell is characterized margination of chromatin and swollen mitochondria. Uranyl acetate & Lead citrate, Bar=2㎛

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