Compensatory Regeneration as a Mechanism for Renal Tubule Carcinogenesis of Fumonisin B_1 in the F344/N/Nctr BR Rat

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Fumonisin B_1 (FB_1) is a fungal metabolite of *Fusarium verticillioides* (= *F. moniliforme*), a fungus that grows on many crops worldwide. Previous studies demonstrated that male BD IX rats consuming diets containing 50 ppm fumonisin B_1 developed hepatocellular carcinomas. In our recent studies, diets containing FB_1 at 50 ppm or higher concentrations induced renal tubule carcinomas in male F344/N/Nctr BR rats and hepatocellular carcinomas in female B6C3F_1/Nctr BR mice. The carcinogenicity of FB_1 in rats and mice is not due to DNA damage, as several laboratories have demonstrated that FB_1 is not a genotoxin. FB_1 induces apoptosis in cells *in vitro*. Including FB_1 in the diets of rats results in increased hepatocellular and renal tubule epithelial cell apoptosis. In studies with F344/N/Nctr BR rats consuming diets containing up to 484 ppm FB_1 for 28 days, female rats demonstrated more sensitivity than male rats in the induction of hepatocellular apoptosis and mitosis. Conversely, induction of renal tubule apoptosis and regeneration were more pronounced in male than in female rats. Induction of renal tubule apoptosis and hyperplasia correlated with the incidence of renal tubule carcinomas that developed in the 2-year feeding study with FB_1 in the F344/N/Nctr BR rats. The data are consistent with the hypothesis that the induction of renal tubule carcinomas in male rats could be partly due to the continuous compensatory regeneration of renal tubule epithelial cells in response to the induction of apoptosis by fumonisin B_1. *Key words:* apoptosis, fumonisin B_1, renal tubule, rodent bioassay. — *Environ Health Perspect* 109(suppl 2):309–314 (2001).
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Fusarium fungi are among the most abundant fungi that infect crops such as wheat, corn, and barley (*1–3*). The presence of *Fusarium* fungi in dietary corn has been associated with elevated incidences of esophageal cancer among inhabitants of the Transkei region of South Africa (*4*), the Henan province of the People's Republic of China (*5*), and the Pordenone province of Italy (*6*). These studies did not find an association of fungal contamination with other human cancers (e.g., liver or kidney). The mycotoxin fumonisin B_1 (FB_1) was purified from cultures of *Fusarium verticillioides* (= *F. moniliforme*) isolate MRC 826 as a compound responsible for the induction of liver cancer in BD IX rats (*7–9*).

The role of FB_1 as a tumor initiator or promoter was investigated by Gelderblom and colleagues (*10*). Including 1,000 ppm FB_1 in the diet of male BD IX rats for 4 weeks resulted in the formation of hepatocellular necrosis, mitotic figures, karyomegaly, bile ductile proliferation and fibrosis, and some necrosis in the proximal convoluted tubules in the kidney (*10*). When the diet of male Fischer 344 (F344) rats included 1,000 ppm FB_1 for 26 days, γ-glutamyl transpeptidase-positive (GGT+) foci formed in the livers (*11*). Subsequent treatment of the rats with partial hepatectomy and administration of 2-acetylaminofluorene and carbon tetrachloride (*12,13*) resulted in increased formation of GGT+ foci compared to rats on control diets (*11*). In another part of the study (*11*), male F344 rats were given partial hepatectomies followed 18 hr later by treatment with 30 mg/kg diethylnitrosamine, 100 mg/kg FB_1, or vehicle and treated 2 weeks later with the Solt/Farber protocol. Treatment with diethylnitrosamine resulted in the formation of GGT+ foci, whereas including FB_1 in the treatment did not result in an increase in foci formation compared to controls. The intraperitoneal administration of FB_1 either 4 hr before and 18 hr after the partial hepatectomy or 18 and 24 hr after the partial hepatectomy did not result in increased formation of GGT+ foci. These results suggest that FB_1 is not a tumor initiator. In an initiation/promotion study (*14*), male F344 rats were administered 200 mg/kg diethylnitrosamine, then fed diets containing 0, 10, 50, 100, 250, or 500 ppm FB_1 for 21 days. GGT+ foci were increased in the livers of the rats that received the diets containing 250 or 500 ppm FB_1. Foci of the placental form of glutathione *S*-transferase were increased in the rats that received 100, 250, and 500 ppm FB_1. These results suggest FB_1 may act as a tumor promoter in cancer development.

Research to date indicates that FB_1 is not genotoxic. Including 693 nmole FB_1 in bacterial mutation assays using *Salmonella typhimurium* TA98 and TA100 did not result in any increased mutagenesis (*15*). FB_1 also did not induce an SOS response in *Escherichia coli* and did not alter the survival of *E. coli* strains that differed in DNA repair when 693 nmole of FB_1 was added per plate (*15*). Including 250 μM FB_1 in primary cultures of male Sprague-Dawley rat hepatocytes did not induce any DNA repair based on levels of [^{3}H]thymidine incorporated into the DNA (*16*). When 140 μM FB_1 was added to primary cultures of female F344 rat hepatocytes, there were no changes in the formation of micronuclei or in the mitotic index (*15*); however, increases in chromosomal aberrations were detected at 1.4, 14, and 140 μM FB_1 (*15*). Therefore, the *in vivo* initiation/promotion studies in rats and the *in vitro* mutagenicity studies are consistent and suggest that FB_1 tumorigenesis involves a nongenotoxic mechanism.

The carcinogenicity of FB_1 was first demonstrated in a single-dose study in which FB_1 was included at 50 ppm in the diet of male BD IX rats (*9*). Five rats on control diets and five rats fed 50 ppm FB_1 were sacrificed at 6, 12, 20, and 26 months and histochemically examined. Liver regenerative nodules and cholangiofibrosis were present in all rats fed FB_1 for 12 months. Liver cirrhosis and hepatocellular carcinomas were present in 100 and 70% of the rats fed FB_1 for 20 and 26 months, respectively. In a subsequent study, including 25 ppm FB_1 in the diet did

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not result in induction of any tumors in male BD IX rats (*10*). These results indicate that FB_1 is a complete carcinogen when included in the diet of male BD IX rats. Additionally, the dietary level of FB_1 required to induce hepatic tumors in male BD IX rats is between 25 and 50 ppm.

Including FB_1 in the diets of male and female F344/N/Nctr BR rats for 2 years resulted in formation of renal tubule adenomas and carcinomas in male rats (*11,12*). The doses of FB_1 ranged from 5 to 150 ppm in male F344 rats and from 5 to 100 ppm in female F344 rats. The renal tubule adenomas and carcinomas were present in male rats consuming 50 and 150 ppm FB_1; there were no dose-related tumors in the female rats. As part of this same study, male and female mice were given diets containing FB_1 for 2 years (*11,12*). The incidence of hepatocellular adenomas and carcinomas was increased in female mice fed 50 and 80 ppm FB_1, whereas there were no increases in any tumors in male mice fed diets containing as high as 150 ppm FB_1. Therefore, results of studies with F344/N/Nctr BR rats, BD IX rats, and $B6C3F_1$/Nctr BR mice suggest that FB_1 is a carcinogenic compound when included in the diet at 50 ppm or higher.

The effects of FB_1 in cultures of human cells *in vitro* were studied in an attempt to understand the mechanism of action of FB_1 in cell homeostatsis (*17*). FB_1 induced apoptosis in primary human keratinocytes in a dose-dependent manner with a dose as small as 1 μM (*17,18*). The apoptosis was characterized by morphologic characteristics, the presence of fragmented DNA, histochemical staining techniques to demonstrate disorganization of the nucleus, and electron microscopic examination of cellular structure (*17,18*). FB_1 also induced apoptosis in normal human fibroblasts, HepG2 human hepatoblastoma cells, and immortalized human esophageal epithelial cells (*17*). Induction of apoptosis or inhibition of the growth of various cell lines *in vitro* has also been described for other cultured mammalian cells (*19–23*).

Administration of FB_1 to rats or mice results in induction of hepatocellular and renal tubule epithelial cell apoptosis. This was first described as single-cell necrosis in livers of rats and mice treated with FB_1 (*24,25*) and later confirmed as apoptosis (*26*). FB_1-induced hepatocellular and renal tubule apoptosis is reviewed elsewhere in this issue (*27*).

Because FB_1 is a nongenotoxic carcinogen and apoptosis has been suggested as the principal cellular consequence of exposure to FB_1, we sought to determine whether the incidence of apoptosis and cell proliferation in F344/N/Nctr BR rats treated for 28 days with diets containing FB_1 correlated with the induction of tumors in the 2-year FB_1 feeding study.

Materials and Methods

Study Material and Feed

Two different preparations of FB_1 were used in the studies summarized in this article. FB_1 was produced by aqueous cultures of *F. proliferatum* on corn (P.E. Nelson, Pennsylvania State University, State College, PA, USA). FB_1 was extracted from the lyophilized culture material using methanol. The FB_1 used in the 28-day feeding study was purified as free acid to a purity of 92.5% using high-performance liquid chromatography (HPLC) (*28*). The FB_1 used in the 2-year feeding study was purified as ammonium salt to a purity of > 96% using HPLC. The purity of the FB_1 was established using spectroscopic and HPLC techniques (*28*).

Autoclaved powdered NIH-31 rodent feed (Purina Corp., St. Louis, MO) was the test diet in the study, and FB_1 was added as a water-based component using a Patterson-Kelley V-blender (Patterson-Kelley Co., East Stroudsburg, PA, USA). The FB_1 content of the control diet was below 0.06 ppm feed.

Animals and Housing

Female and male F344/N/Nctr BR rats were obtained from the breeding colony at the National Center for Toxicological Research at 4 weeks postpartum. Rats were allocated to study dose groups in a random manner to control for weight bias and to reduce sibling allocation to any dose group. Powdered feed was available *ad libitum* in feeders custom designed for powdered feed, and water was available *ad libitum*. Cages and water were changed twice weekly.

Study Design

Rats (10/dose/sex) were fed diets containing FB_1 for 28 consecutive days. The doses used in this study were 0, 99, 163, 234, and 484 ppm FB_1 (*26,28*). Rats were fasted overnight and euthanized by asphyxiation with carbon dioxide and bled via the orbital sinus before necropsy. Organ weights were determined; tissues were fixed in 10% neutral buffered formalin and processed as described (*28*). All tissues were examined in rats that received the control diets and the highest FB_1 dose (484 ppm); livers and kidneys were examined for all rats in the study.

The design of the 2-year study has been described previously (*28*). Rats and mice were randomly allocated to dose groups, the study was conducted, and the tissues were analyzed for tumors in accordance with the guidelines of the U.S. National Toxicology Program (*29*) and U.S. Food and Drug Administration (*30*).

Determination of Cell Cycle Using Anti-PCNA Immunohistochemical Methods

The proportion of cells in S phase was determined immunohistochemically with minor modification of established methods (*31,32*). Paraffin-embedded kidney sections that had been fixed for 48 hr in 10% neutral buffered formalin and subjected to heated-citrate antigen retrieval were used. Mouse monoclonal

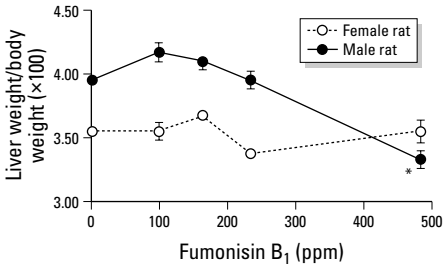

Figure 1. Graphic representation of the relative liver weights of the rats fed diets containing up to 484 ppm FB_1 for 28 days. Data presented are liver weights as percent of total body weight (mean ± standard error, 10 rats per sex) for female and male rats. Asterisk (*) indicates the value is significantly different ($p < 0.05$) from the value for rats consuming control diets.

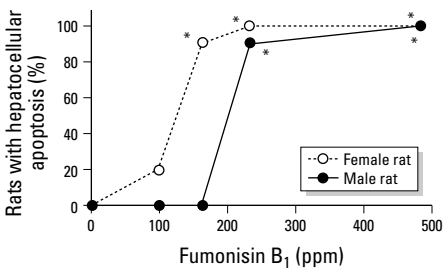

Figure 2. Incidence of hepatocellular apoptosis was determined for female and male rats fed FB_1 for 28 days. The incidence of apoptosis was determined morphologically in hematoxylin-eosin–stained slides of hepatic tissue. There were 10 rats per dose per sex. Asterisk (*) indicates the value is significantly different ($p < 0.05$) from values in control groups.

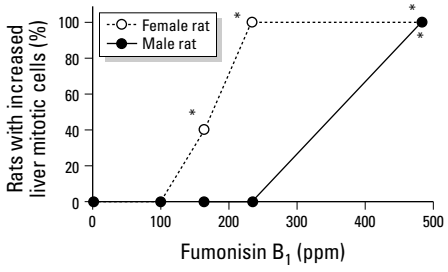

Figure 3. The incidence of hepatocellular mitosis was determined for female (○) and male (●) rats fed fumonisin B_1 for 28 days. The incidence of hepatocellular mitosis was determined morphologically in hematoxylin-eosin–stained slides of hepatic tissue. There were 10 rats per dose per sex. Asterisk (*) indicates the value is significantly different ($p < 0.05$) from values in control groups.

antiproliferating cellular nuclear antigen (anti-PCNA) (clone PC 10, Dako Corp., Carpenteria, CA) was localized with a biotinylated antimouse second antibody followed by streptavadin-peroxidase, with diaminobenzidine as chromogen. Nuclei were counterstained with hematoxylin. Approximately 2,000 nuclei per kidney cortex were evaluated using digitized microscopy and proprietary image-analysis software (Optimas, Media Cybernetics, Silver Springs, MD) that semiautomatically tallied the nonblue (brown) nuclei representing cells in S phase. The results are reported as the percent of nuclei in S phase.

Statistical Analysis

Comparison of dosed rats with altered parameters to rats on the control diets was accomplished using either analysis of variance or Fisher's exact test (SigmaStat, Jandel Scientific, San Rafael, CA, USA). Levels of significant differences were tested at the 95% confidence level ($p < 0.05$).

Results

Male and female F344 rats were fed diets containing FB_1 for 28 days and their body weights monitored weekly. Female rats fed a diet containing 484 ppm FB_1 had body weights 9.8 and 10.5% less than rats on control diets after 3 and 4 weeks, respectively; female rats fed the diet containing 234 ppm FB_1 had body weights 6.3% less than control rats at 4 weeks. Male rats fed diets containing 484 ppm FB_1 had body weights 13 and 14.7% less than male rats on control diets after 3 and 4 weeks in the study, respectively, whereas rats fed other doses did not have any differences in body weights from those of control rats.

Several studies have shown that the liver is a target organ for FB_1 toxicity. Liver weights of female rats were unaffected by inclusion of FB_1 in the diets; however, liver weights of male rats were decreased in the rats fed diets containing 484 ppm FB_1 (Figure 1).

Histopathologic examination was conducted on livers of the rats fed FB_1 for 28 days. The most predominant lesions noted were increased incidence of hepatocellular apoptosis and mitosis. Apoptosis was morphologically distinguishable as hepatocytes with decreased cell volume and withdrawal from neighboring hepatocytes. The apoptotic cells were eosinophilic with condensed and marginated nuclei. There was no apparent necrosis in the livers; however, there was disorganization of the sinusoidal structure as a result of the apoptosis. An *in situ* hybridization method for detection of DNA fragments was used to confirm the identification of some of the apoptotic cells [TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay (*28*); data not presented]. The incidence of morphologically distinguishable hepatocellular apoptosis is summarized in Figure 2. The number of female rats with hepatocellular apoptosis increased from 0 at 0 ppm, to 2 of 10 rats at 99 ppm, 9 of 10 rats (90%) at 163 ppm, and all 10 of female rats at 234 and 484 ppm FB_1. Male rats evidently were less sensitive to the effects of FB_1 because hepatocellular apoptosis was not present in the males fed 0, 99, and 163 ppm FB_1 (Figure 2). Hepatocellular apoptosis was present in all male rats fed diets 234 and 484 ppm FB_1 (Figure 2). The severity of the hepatocellular apoptosis was graded minimal (1), mild (2), moderate (3), or marked (4). In the female rat livers, the median severity of the apoptosis was 1.2, 1.9, and 2.2 for rats receiving 163, 234, and 484 ppm FB_1, respectively. The median severity of apoptosis in the male rat livers was 1.1 and 2.3 for the rats in the 234- and 484-ppm dose groups, respectively.

Induction of hepatocellular apoptosis in the rats was accompanied by increases in morphologically distinguishable hepatocellular mitosis; results are shown in Figure 3 as the percent of rats with increased mitoses. Hepatocellular mitoses were increased in the female rats at 163, 234, and 484 ppm FB_1 and were increased in the male rats only at 484 ppm FB_1. Induction of both hepatocellular proliferation (Figure 3) and hepatocellular apoptosis (Figure 2) occurred at lower doses in the female rats than in the male rats. Hepatocyte proliferation was also determined using an immunohistochemical method for the detection of PCNA. The percent of hepatocytes in S phase in male rats on the control diet was 0.007%, and increased to 0.18, 0.29, and 1.32% at 163, 234, and 484 ppm FB_1, respectively (Figure 4). The percent of hepatocytes in S phase of the cell cycle in female rats was 0.2% in the control group and did not increase until 234 and 484 ppm FB_1, whereas the percentage of cells in S phase increased to 0.86 and 0.74%, respectively (Figure 4).

The effect of dietary FB_1 on the weights of the kidneys is presented in Figure 5. The data are expressed as weight of the kidneys relative to total body weight. Relative kidney weights were reduced in all the rats that consumed diets containing FB_1. Relative weights of kidneys in male rats fed 484 ppm FB_1 were reduced by 19% from the values of male rats on control diets, whereas relative kidney weights in female rats fed 484 ppm FB_1 were reduced by 11% compared to those of controls (Figure 5).

The most prevalent morphologic changes in the kidneys of the rats were increased rates of renal tubular epithelial cell apoptosis in the cortico-medullary area. Apoptotic renal tubule epithelial cells were noted by cellular shrinkage from adjacent cells, eosinophilic cytoplasm, and chromatin condensation and margination in the nucleus. Although apoptotic bodies were not detected, clear morphologic markers of apoptosis were present. The morphologic diagnosis of apoptosis was confirmed using the TUNEL assay for detecting fragmented DNA (not shown). Apoptotic renal tubule epithelial cells were present in the kidneys of all male rats treated with 99, 163, 234, and 484 ppm FB_1 (Figure 6). The median severity of apoptosis was mild (2.0) in all kidneys of the male rats. Apoptotic renal tubule cells were not detected in kidneys of

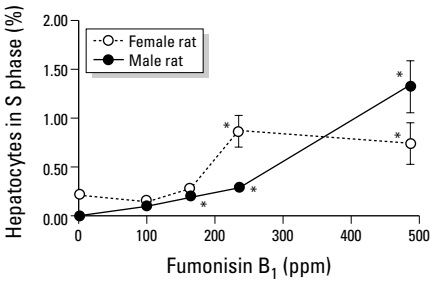

Figure 4. The percent of proliferating hepatocytes was determined using immunohistochemical methods for the detection of PCNA. Approximately 2,000 cells were evaluated on each slide for the presence of PCNA-positive cells in S phase. The data are presented as the mean ± standard error for 10 rats per dose for males and females. Asterisk (*) indicates the value is significantly different ($p < 0.05$) from the control value for the same sex.

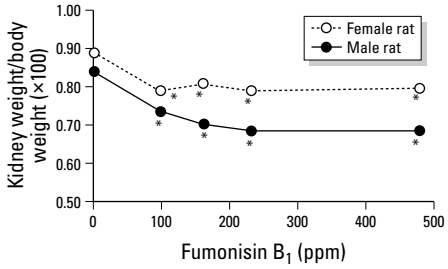

Figure 5. Graphic representation of the relative kidney weights of rats fed diets containing up to 484 ppm fumonisin B_1 for 28 days. Data presented are kidney weights as percent of total body weight (mean ± standard error, 10 rats per sex) for female rats and male rats. Asterisk (*) indicates the value is significantly different ($p < 0.05$) from the value for rats consuming control diets.

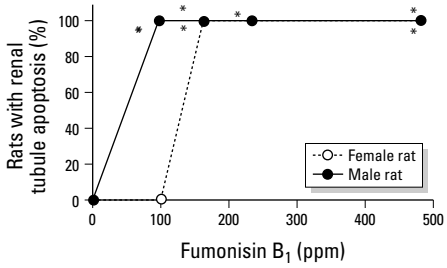

Figure 6. The incidence of renal tubule epithelial cells apoptosis was determined in female and male rats fed FB_1 for 28 days. The incidence of apoptosis was determined morphologically in hematoxylin-eosin–stained slides. There were 10 rats per dose per sex. Asterisk (*) indicates the value is significantly different ($p < 0.05$) from values in control groups.

female rats fed 99 ppm FB_1 but were present in all kidneys of female rats that received 163, 234, and 484 ppm FB_1 (Figure 6). The median severity of the apoptosis in female rats increased from 1.0 at 163 and 234 ppm to 2.4 at 484 ppm. These data demonstrate that renal tubule apoptosis was induced in both sexes; however, male rats were more responsive than female rats to the induction of renal tubule apoptosis by dietary FB_1.

Renal tubule cell proliferation at the cortico-medullary junction was determined using immunohistochemical detection of nuclear PCNA (Figure 7). The number of renal tubule epithelial cells in S phase was elevated at all doses of FB_1 in male rats and increased in a dose-dependent manner (Figure 7). In female rat kidneys, the percentage of cells in S phase also increased in a dose-dependent manner at all doses of FB_1; however, induction of proliferation at 99 and 163 ppm FB_1 was less in female than in male rat kidneys (Figure 7).

The incidence of tumors in male and female F344 rats treated for up to 2 years with FB_1 has been reported (*28,33*). No liver tumors were detected in the male and female F344 rats in the 2-year study; however, renal tubule adenomas and carcinomas were induced in male rats fed diets containing 50 and 150 ppm FB_1. Relative weights of the rat livers and kidneys in that study are shown in Figures 8 and 9. Relative liver weights of the male rats fed FB_1 for 2 years were decreased at all doses of FB_1 (5, 15, 50, and 150 ppm) compared to relative liver weights in rats receiving control diets (Figure 8). In contrast, relative liver weights in female rats were not affected by inclusion of FB_1 in the diets (Figure 8). Relative kidney weights in the male rats were decreased in the groups that received 15, 50, and 150 ppm FB_1 for 2 years (Figure 9). This decrease in relative kidney weight plateaued at approximately 21% in male and female rats consuming diets containing 50–150 ppm FB_1 (Figure 9).

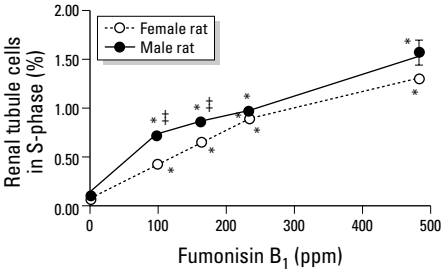

Figure 7. The percent of proliferating renal tubule epithelial cells was determined using immunohistochemical methods for the detection of PCNA. Approximately 2,000 cells at the cortico-medullary junction were evaluated on each slide for presence of PCNA-positive cells in S phase. The data are presented as the mean ± standard error for 10 rats/dose for males and females. Asterisk (*) indicates the value is significantly different ($p < 0.05$) from the control value for the same sex. A double-cross (‡) indicates the values for the males were higher than the values for the females at that dose.

Discussion

Inclusion of FB_1 in the diets of F344/N/Nctr BR rats for 2 years resulted in the formation of renal tubule adenomas and carcinomas in male but not in female rats (*28,33*). We examined kidneys and livers of rats fed diets containing up to 484 ppm FB_1 for 28 days to determine whether the presence of apoptosis and proliferation correlated with the incidence of renal tubule epithelial cells tumors by FB_1 in the 2-year feeding study.

Consumption of 234 and 484 ppm FB_1 for 28 days decreased the total body weight in female rats, and to a lesser extent, in male rats but only at 484 ppm. This toxicity was not reflected in the relative liver weights in male and female rats; a decrease in relative liver weight was detected only in male rats fed 484 ppm FB_1 (Figure 1). The presence of hepatocellular apoptosis in female rats was increased by 163, 234, and 484 ppm FB_1, whereas higher doses of FB_1 were required for induction of hepatocellular apoptosis in male rats (Figure 2). This trend of hepatocytes in the female rat being more sensitive than those in the male rat to the toxicity of FB_1 additionally was reflected in the percentage of rats that had morphologically distinguishable hepatocellular mitoses (Figure 3) and hepatocytes in the S phase of the cell cycle (Figure 4).

In the 2-year tumor study where FB_1 was fed to male and female F344/N/Nctr BR rats (*28,33*), relative liver weights in the male rats were reduced in a dose-dependent manner at all FB_1 doses, whereas relative liver weights in the female rats were not affected by dietary FB_1 (Figure 8). These doses did not induce formation of hepatic tumors in F344/N/Nctr BR rats (*28,33*). Consequently, a clear role for hepatocellular apoptosis and proliferation in rat liver tumor development cannot be determined from these studies. In contrast, hepatocellular carcinomas have been reported in BD IX rats fed 50 ppm FB_1 for up to 26 months (*9*), and similar doses of FB_1 have been shown to induce foci of altered expression of γ-glutamyl transpeptidase and placental glutathione *S*-transferase activity (*11,14,34*). Reasons for differences in responsiveness of F344/N/Nctr BR and other rats to the hepatotoxic and hepatocarcinogenic effects of FB_1 remain to be determined.

In contrast to liver, toxicity of dietary FB_1 in kidneys is more pronounced in male than in female F344/N/Nctr BR rats. Reduction in relative kidney weights was approximately 19% in male rats consuming 484 ppm FB_1 compared to relative kidney weights in control rats (Figure 5). In female rats, this reduction in relative kidney weight was only 11% (Figure 5). Reduction in relative kidney weights paralleled the induction of renal tubule epithelial cell apoptosis in rats (Figure 6); the incidence of apoptosis was greater in male rats than in female rats at 99 ppm FB_1. Similiarly, induction of renal tubule proliferation, most probably a consequence of apoptosis, was increased in male rats at 99 and 163 ppm FB_1 compared to that in female rats at the same doses (Figure 7). Induction of renal tubule epithelial cell proliferation was equivalent in male and female rat kidneys at 234 and 484 ppm FB_1 (Figure 7).

Inclusion of FB_1 in the diet of rats for 2 years resulted in formation of renal tubule epithelial cell adenomas and carcinomas in 19 and 31% of male rats fed 50 and 150 ppm, respectively (*28,33*). The FB_1-dependent increase in renal tumors was accompanied by an increased incidence of renal tubule hyperplasia in 29 and 17% of male rats fed 50 and 150 ppm FB_1, respectively (*28*). Female rats did not develop renal tubule hyperplasia, adenomas, or carcinomas in response to inclusion of up to 100 ppm FB_1 in the diet (*28,33*). Therefore, it appears that a consistent sex difference exists in the response of rats in the 28-day and 2-year feeding studies, in which male rats are more sensitive than female rats to the toxicity (apoptosis) of

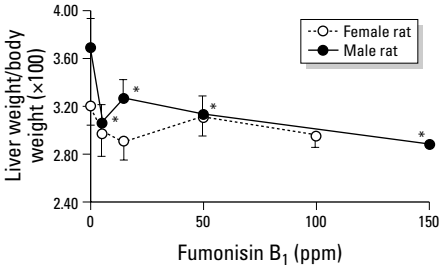

Figure 8. Graphic representation of the relative liver weights of rats fed diets containing 0–150 ppm FB_1 for 2 years. The data presented are liver weights as percent of total body weight (mean ± standard error) for female rats and male rats. An asterisk (*) indicates the value is significantly different ($p < 0.05$) from the value for rats consuming control diets.

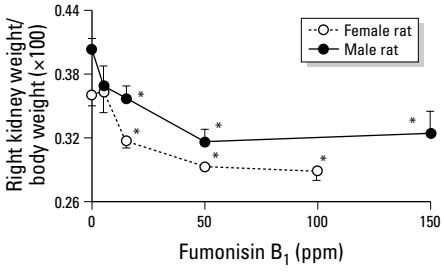

Figure 9. Graphic representation of the relative right kidney weights of rats fed diets containing 0–150 ppm FB_1 for 2 years. The data presented are right kidney weights as percent of total body weights (mean ± standard error) for the female rats and male rats. An asterisk (*) indicates the value is significantly different ($p < 0.05$) from the value for rats consuming control diets.

dietary FB_1; however, relative kidney weights were decreased in the 28-day (Figure 5) and 2-year (Figure 9) feeding studies for both male and female rats, suggesting that the sex difference in response to FB_1 is not absolute.

The mechanism of development of renal tubule adenomas and carcinomas in response to dietary FB_1 is not understood at this time. Induction of apoptosis in rat kidneys has been documented by several groups using different strains of rats. FB_1 is an inhibitor of ceramide synthase (*35–38*), an enzyme involved in the *de novo* synthesis of sphingolipids. Inhibition of this enzyme results in increased intracellular sphinganine and decreased intracellular ceramide and complex sphingolipid levels (*17,18,22,39*). Alterations in these bioactive compounds have been implicated as the mechanism for the induction of apoptosis in cells *in vitro* (*23,37,40*).

It is known that FB_1 induces apoptosis in rat kidney renal tubule epithelial cells; therefore, we propose that FB_1 induces tumors in male rat kidneys through induction of compensatory renal tubule epithelial cell hyperplasia in response to FB_1-induced apoptosis.

Increased cell proliferation in the induction of kidney cancer may play a role in the development of spontaneous and chemical-induced renal tumors in the Eker rat. Formation of renal cell carcinomas in the Eker rat was first described as an autosomal dominant trait (*41*) and involves a mutation in the *Tsc2* gene (*42,43*). The presence of homozygous mutant *Tsc2* genes is embryolethal (*44*); however, animals bearing heterozygous *Tsc2* genes develop spontaneous renal cell carcinomas by 1 year of age (*44*). The *Tsc2* gene codes for tuberin (*45*), which regulates the GTP-ase activity for Rap-1 and rab-5, two proteins involved in *ras* signal transduction (*46,47*). Inhibition of tuberin expression in cultured cells resulted in a decreased G_1 transit time, and an induced proliferation of G_0-arrested cells (*48*). Transgenic insertion of wild-type *Tsc2* into Eker rats resulted in the elimination of the embryolethality in homozygous animals and reduced the ability of *N*-ethyl-*N*-nitrosourea to induce renal tumors in heterozygotics (*49*). Therefore, loss of control at critical cell cycle checkpoints through loss of tuberin expression, and the sustained hyperplasia results in eventual renal tubule tumor formation in the Eker rat.

Sustained cellular hyperplasia has been proposed as a model for the induction of renal cell tumors in rats by chloroform and by compounds that induce $\alpha_{2\mu}$-globulin accumulation in renal tubule cell lysosomes of male rats. In the latter case, chemicals that bind to $\alpha_{2\mu}$-globulin (e.g., D-limonene) apparently interfere with $\alpha_{2\mu}$-globulin protein degradation in the lysosomes (*50–52*). The modified $\alpha_{2\mu}$-globulin accumulates in the lysosomes, resulting in cell death (hyaline droplet nephropathy). This loss of tubule cells is followed by compensatory cell proliferation in the tubules (*53*) and eventual formation of renal tumors. In support of the role of this mechanism of tumor formation, NCI Black Reiter rats do not produce $\alpha_{2\mu}$-globulin (*54*). Compounds that induce renal tumors in conventional rats through $\alpha_{2\mu}$-globulin accumulation and hyaline droplet nephropathy do not induce nephropathy or tumors in the NCI Black Reiter rats (*54*). The role of $\alpha_{2\mu}$-globulin nephropathy and sustained proliferation in the induction of renal cell tumors was substantiated in the mouse where transgenic expression of $\alpha_{2\mu}$-globulin in mice resulted in development of hyaline droplet nephropathy (*55*).

Continuous cell proliferation has also been associated with increased risk for tumor development in other tissues. Several compounds have been shown to induce urinary bladder regenerative hyperplasia and eventually cancer in rodents (*56*). The apparent mechanism of action is the formation of insoluble calculi, and the physical trauma of the interaction of the calculi with the urinary bladder epithelium results in cell death, sustained hyperplasia, and eventual tumor formation (*56,57*). This is the mechanism in the induction of urinary bladder tumors in male rats by high doses of sodium saccharin due to the high concentrations of protein and alkalinity of the urine (*56,58,59*).

In conclusion, we believe there is sufficient evidence to support the hypothesis that sustained renal tubule regeneration in response to FB_1-induced renal tubule epithelial cell apoptosis participates in the development of renal tubule tumors in male F344/N/Nctr BR rats fed FB_1. However, the increased hyperplasia may not be solely responsible for the induction of the tumors. The decrease in relative kidney weights (Figure 5) and the induction of renal tubule proliferation (Figure 7) in male and female F344/N/Nctr BR rats and the induction of renal tubule apoptosis and hyperplasia in male but not female rats fed FB_1 for 2 years support the hypothesis. It is anticipated that ongoing studies concerning the role played by signal transduction pathways in the induction of apoptosis will shed additional light on the molecular mechanism of FB_1-induced renal tubule apoptosis and tumor formation in male rat kidneys.

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