Reaction with Fructose Detoxifies Fumonisin B1 while Stimulating Liver-Associated Natural Killer Cell Activity in Rats

Z. Lu
Iowa State University

W. R. Dantzer
Iowa State University

E. C. Hopmans
Iowa State University

V. Prisk
Iowa State University

Joan E. Cunnick
Iowa State University, jcunnick@iastate.edu

Follow this and additional works at: http://lib.dr.iastate.edu/fshn_ag_pubs

Part of the Animal Sciences Commons, Food Science Commons, Human and Clinical Nutrition Commons, Other Microbiology Commons, and the Other Veterinary Medicine Commons

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/fshn_ag_pubs/88. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.
Reaction with Fructose Detoxifies Fumonisin B1 while Stimulating Liver-Associated Natural Killer Cell Activity in Rats

Abstract
Fumonisin B1 (FB1) was reacted with fructose in an attempt to detoxify this mycotoxin. Fischer 344/N rats were initiated with diethylnitrosamine (15 mg/kg body weight) and then fed 69.3 μmol FB1/kg diet or 69.3 μmol FB1 reacted with fructose (FB1–fructose)/kg diet for 4 weeks. In comparison with the rats fed basal diet or FB1–fructose, the FB1-fed rats had significantly increased plasma cholesterol ($P < 0.01$), plasma alanine aminotransferase activity ($P < 0.05$), and endogenous hepatic prostaglandin production ($P < 0.05$). Placental glutathione S-transferase-positive and γ-glutamyl transferase-positive altered hepatic foci occurred only in the FB1-fed rats. Liver-associated natural killer (NK) cell activity was significantly decreased in the FB1-fed rats and increased in the group fed FB1-fructose, as compared with the basal group ($P < 0.03$). Therefore, modifying FB1 with fructose seems to prevent FB1-induced hepatotoxicity and promotion of hepatocarcinogenesis while stimulating liver-associated NK cell activity in rats.

Keywords
Department of Microbiology, Immunology and Preventive Medicine, Fumonisin B1, Frutose, Prostaglandins, NK cell, Placental glutathione S-transferase

Disciplines
Animal Sciences | Food Science | Human and Clinical Nutrition | Other Microbiology | Other Veterinary Medicine

Comments

Rights
One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions).

Authors
Z. Lu, W. R. Dantzer, E. C. Hopmans, V. Prisk, Joan E. Cunnick, Patricia A. Murphy, and Suzanne Hendrich
Fumonisin B1 (FB1) was reacted with fructose in an attempt to detoxify this mycotoxin. Fischer 344/N rats were initiated with diethylnitrosamine (15 mg/kg body weight) and then fed 69.3 \( \mu \)mol FB1/kg diet or 69.3 \( \mu \)mol FB1 reacted with fructose (FB1-fructose)/kg diet for 4 weeks. In comparison with the rats fed basal diet or FB1-fructose, the FB1-fed rats had significantly increased plasma cholesterol (\( P < 0.01 \)), plasma alanine aminotransferase activity (\( P < 0.05 \)), and endogenous hepatic prostaglandin production (\( P < 0.05 \)). Placental glutathione S-transferase-positive and \( \gamma \)-glutamyl transferase-positive altered hepatic foci occurred only in the FB1-fed rats. Liver-associated natural killer (NK) cell activity was significantly decreased in the FB1-fed rats and increased in the group fed FB1-fructose, as compared with the basal group (\( P < 0.03 \)). Therefore, modifying FB1 with fructose seems to prevent FB1-induced hepatotoxicity and promotion of hepatocarcinogenesis while stimulating liver-associated NK cell activity in rats.

**Keywords:** Fumonisin B1; fructose; prostaglandins; NK cell; placental glutathione S-transferase

**INTRODUCTION**

Fumonisin B1 (FB1), a mycotoxin produced by the commonly occurring corn fungi, Fusarium moniliforme and Fusarium proliferatum, was hepatocarcinogenic in rats when fed in amounts of 69.3 \( \mu \)mol/kg diet for approximately 2 years (Gelderblom et al., 1991). Human esophageal carcinoma rate was high in areas where FB1 concentration in corn reached approximately 11.1 \( \mu \)mol/kg (Sydenham et al., 1990a). Corn products for human and animal consumption were determined to contain 0.3–4.2 \( \mu \)mol FB1/kg (Hopmans and Murphy, 1993; Murphy et al., 1993; Sydenham et al., 1990b).

The hepatocarcinogenic effect of FB1 can be evaluated in vivo by measuring changes in placental glutathione S-transferase (PGST) (Lebepe-Mazur et al., 1995) and \( \gamma \)-glutamyl transferase (GGT) activity (Gelderblom et al., 1988), which are markers of altered hepatic foci (AHF) (Sato et al., 1984; Goldsworthy et al., 1985). Also, plasma alanine aminotransferase (ALT) activity was related to fumonisin hepatotoxicity (Voss et al., 1992) and hepatocarcinogenesis in rats (Hendrich et al., 1993). Increased plasma total cholesterol was also an indicator of FB1 toxicity in vervet monkeys (Fincham et al., 1992) and in rats (Hendrich et al., 1993). Production of prostaglandin E2 (PGE2) and other eicosanoids from the cyclooxygenase pathway was stimulated in Yoshida hepatoma cells in rats and in human hepatocellular carcinomas (Trevisani et al., 1980; Hanai et al., 1993). Effects of FB1 on PG production in rat liver have not been investigated yet.

Fumonisin B1 can alter immunological functions mediating antitumor mechanisms. Macrophage structure and phagocytic function were down-regulated in vitro by FB1 (Chatterjee and Mukherjee, 1994; Chatterjee et al., 1995) as was lymphocyte proliferation in response to lipopolysaccharides (LPS) (Dombrink-Kurtzman et al., 1994). The effects of in vivo administration of FB1 produced both increases and decreases in plaque-forming cell response in BALB/C mice depending upon the timing of the fumonisin injections and the number of injections (Martinova and Merrill, 1995).

Efforts have been devoted to detoxifying FB1 in several ways. Thermostability of FB1 proved to be great, because nearly 85% of FB1 was recovered after different heat treatments: 75 °C for 135 min, 100 °C for 45 min, and 125 °C for 5 min (Dupuy et al., 1993). Treatment of fumonisin-contaminated corn with 2% ammonia for 4 days, a process that detoxified aflatoxin B1, led to slight reduction in the concentration of FB1 without decreasing the toxicity in rats (Norred et al., 1991). Hydrolyzed FB1, which was produced by boiling F. proliferatum-contaminated corn in 1.2% calcium hydroxide solution for 1 h, was similar in toxicity to FB1 when the nutritional status of rats was adequate (Hendrich et al., 1993). In vitro toxicity studies on several analogs of FB1 showed that the analogs containing FB1's amine group and the tricarballylic side chains were more toxic than FB1, whereas the analog containing only the tricarballylic side chains was not toxic (Kraus et al., 1992). The N-acetyl derivative of FB1 at 1 mM was less toxic in primary rat hepatocytes than FB1 at the same concentration. In addition, rats fed N-acetyl-FB1 at about 1.3 mmol/kg diet did not exhibit hepatic neoplastic nodules or increased hepatic GGT activity (Gelderblom et al., 1993). Therefore, FB1's amine group is likely to be critical for its toxicity. The N-acetyl-FB1 may be produced during the isolation and purification of FB1 from corn cultures of F. moniliforme. This paper describes a dedicated and more practical method to block FB1's amine group by reacting the amine group with reducing sugars such as fructose in a Maillard reaction. It was hypothesized that modifying FB1 with fructose would reduce or prevent promotion of hepatocarcinogenesis and hepatotoxicity in rats.

**Keywords:** Fumonisin B1; fructose; prostaglandins; NK cell; placental glutathione S-transferase
MATERIALS AND METHODS

Preparation of Crude Fumonisin B1. Fumonisin B1 is a class 2B carcinogen (International Agency for Research on Cancer, 1993), and standard safety precautions were taken during its handling. F. proliferatum strain M5991 (from Dr. Paul Nelson, Pennsylvania State University, College Station, PA) predominantly produces FB1. Sterile, aflatoxin-free corn was inoculated with lyophilized cultures of F. proliferatum strain M5991 which had been reconstituted in pH 7.4 phosphate-buffered saline. This corn culture was incubated at 22 °C in the dark for 3 weeks. After incubation, freeze-dried corn culture was ground and extracted with 1:1 acetonitrile:water. The extract was partitioned with ethyl acetate, after which the water phase was loaded onto a 1 kg XAD-16 column (7.5 × 100 cm, Sigma Chemical Co., St. Louis, MO). The column was washed with water, and FB1 was eluted with 4 L of methanol. The eluate was dried under vacuum, reconstituted in water, and loaded onto a Lobar LiChroprep RP-8 column (25 × 100 mm; EM Separations, Gibbstown, NJ). The column was washed with 20% acetonitrile:80% water containing 0.1% trifluoroacetic acid (TFA), and FB1 was eluted with 50% acetonitrile:50% water containing 0.1% TFA. The purity of the isolated FB1 was determined by analytical HPLC of the o-phthalaldehyde (OPA) derivative (Hopmans and Murphy, 1993). The FB1 standard curve was prepared with FB1 generously donated by Dr. P. G. Thiel (Research Institute for Nutritional Diseases, South African Medical Research Council, South Africa). The isolated FB1 was approximately 40% pure, containing 1.5 g of FB1. The preparation also contained trace amounts of fumonisins B2 and B3.

Preparation of Highly Purified Fumonisin B1. Liquid cultures were prepared as in Dantzer et al. (1996a) by inoculating capped baffled Erlenmeyer flasks containing 500 mL of modified Myro medium with a 4 day shake flask culture of F. proliferatum strain M5991. Fumonisin B1 was isolated and purified by the procedure as described in Dantzer et al. (1996b). Briefly, harvested liquid cultures were filtered and run over a series of chromatographic steps, including Amberlite XAD-16, reverse phase Lobar C8, and purified by the procedure as described in Dantzer et al. (1996b).

Preparation of Fumonisin B1- Fructose Adduct. Both crude and highly purified FB1 were conjugated with fructose as described by Murphy et al. (1995). Briefly, 725 µM FB1 in 50 mM potassium phosphate buffer, pH 7.0, containing 1 M fructose, was heated for 48 h at 80 °C. Less than 5% of the FB1 was left unreacted with fructose. Fumonisin B1- fructose was hydrolyzed by refluxing in 2 N KOH for 2 h at 100 °C, after which the pH was adjusted to 2.8 with 2 N HCl. All of the FB1 could be recovered as hydrolyzed FB1. In addition, FB1 was heated under the same conditions without fructose and retained 100% reactivity with OPA.

Diets. Three experimental diets were fed to rats in each of the two studies. Basal diet supplying 40% of energy as fat was modified from AIN-76 (American Institute of Nutrition, 1993) in the study feeding crude FB1, or from AIN-93G (American Institute of Nutrition, 1993) in the study feeding highly purified FB1 (Table 1). Crude or highly purified FB1 containing diets were prepared by incorporating 69.3 µmol FB1/kg diet into the basal diets. Crude or highly purified FB1 reacted with fructose were incorporated into the basal diets at a level equivalent to 69.3 µmol FB1/kg diet. On the basis of the proposed reaction between FB1 and a reducing sugar such as fructose (Murphy et al., 1996), it was estimated that approximately 17 g of unreacted fructose was added per kilogram of FB1-fructose diet. All the diets were stored at 4 °C.

Animals. The use of animals and the experimental procedures were approved by the Iowa State University Animal Care Committee. In the experiment with crude FB1, 20 10-day-old male F 344/N rats obtained from Harlan Sprague-Dawley (Madison, WI) were injected intraperitoneally with diethylnitrosamine (DEN, 15 mg/kg body weight) in 0.1 mL of corn oil. At 3 weeks of age, the weaned rats were randomly assigned into one of the three treatment groups with six or seven rats each. In the experiment with highly purified FB1, 39 10-day-old female F 344/N rats obtained from Harlan Sprague-Dawley were initiated as described above, randomly assigned into the treatments with 12–15 rats each. In both experiments, rats were given free access to the experimental diets and water for 4 weeks in an animal facility with a 12-h light/dark cycle maintained at 22–25 °C and 50% humidity. Body weight and feed intake of the female rats were recorded weekly.

Plasma and Liver Sample Preparations. Part of the plasma obtained from heparinized blood was analyzed within 24 h for ALT activity. The remaining plasma was stored at −80 °C and later analyzed for plasma total cholesterol. The liver was perfused with 40 mL of Hank’s balanced salt solution containing 1% EDTA and 25 mM HEPES. The perfusate (10–12 mL) containing red blood cells, Pitt cells, and other leukocytes was used as the source of effectors in the NK cell activity assay.

Each of the three largest lobes of the liver was sliced into 1 cm slices. Three slices, one from each lobe, were immediately frozen as a block on dry ice and stored at −80 °C. From each of the frozen liver blocks, two 10-µm serial sections were cut with a Histostat Microtome (Model 855, Leica Inc, Deerfield, IL) for later stainings for GGT activity and PGST.

Table 1. Basal Diet Composition*

<table>
<thead>
<tr>
<th>ingredient</th>
<th>crude FB1 (g/kg)</th>
<th>purified FB1 (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>beef tallow</td>
<td>139.6</td>
<td>139.6</td>
</tr>
<tr>
<td>corn oil</td>
<td>66.5</td>
<td>66.5</td>
</tr>
<tr>
<td>soybean oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>casein</td>
<td>224.1</td>
<td>224.1</td>
</tr>
<tr>
<td>corn starch</td>
<td>228.5</td>
<td>228.5</td>
</tr>
<tr>
<td>dextrose</td>
<td>224.1</td>
<td>224.1</td>
</tr>
<tr>
<td>cellulose</td>
<td>56.0</td>
<td>56.0</td>
</tr>
<tr>
<td>vitamin mix(AIN-76)</td>
<td>11.2</td>
<td>10.0</td>
</tr>
<tr>
<td>vitamin mix(AIN-93G-VX)</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>mineral mix(AIN-76)</td>
<td>39.2</td>
<td>39.2</td>
</tr>
<tr>
<td>mineral mix(AIN-93G-MX)</td>
<td>39.2</td>
<td>39.2</td>
</tr>
<tr>
<td>CaCO3</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>choline chloride</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>L-methionine</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>ascorbate</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>L-cystine</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>choline bitartrate</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>TBHQ</td>
<td>0.014</td>
<td></td>
</tr>
</tbody>
</table>

* Basal diet modified from AIN-76 was used in crude FB1 experiment; basal diet modified from AIN-93G was used in highly purified FB1 experiment.

Table 2. Differences in Body Weight but Not Feed Intake among Female Rats Fed Fumonisin B1 (FB1), FB1 Reacted with Fructose (FB1-Fructose), or a Basal Diet

<table>
<thead>
<tr>
<th>group</th>
<th>basal diet</th>
<th>FB1-Fructose</th>
<th>FB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>total feed intake (g)</td>
<td>366 ± 37</td>
<td>354 ± 43</td>
<td>320 ± 25</td>
</tr>
<tr>
<td>average daily intake (g/d)</td>
<td>13 ± 1</td>
<td>13 ± 2</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>final body weight (g)</td>
<td>191 ± 19</td>
<td>191 ± 10</td>
<td>171 ± 14*</td>
</tr>
<tr>
<td>body weight increase (g)</td>
<td>128 ± 15</td>
<td>128 ± 8</td>
<td>108 ± 15*</td>
</tr>
</tbody>
</table>

* Significantly different from the basal group, P < 0.05.

Table 1. Basal Diet Composition*
Potter-Elvehjem homogenizer in 5 mL, pH 7.4, 50 mM potassium phosphate buffer containing 4.2 mM acetylsalicylic acid. The liver homogenates were frozen on dry ice and stored at −80 °C for later analyses of endogenous hepatic PGF2α and PGE2 levels.

**Plasma Total Cholesterol Concentration and Alanine Aminotransferase Activity.** Plasma total cholesterol concentration was determined by using Sigma diagnostic kit, procedure 352-3 (Sigma). Plasma ALT activity was measured by using Sigma diagnostic kit for glutamate/pyruvate transaminase. Endogenous liver PGF2α and PGE2 were measured by radioimmunoassay.

**Histochemical Staining and Computerized Stereology of AHF.** One of the frozen serial sections was stained for the presence of PGST-positive altered hepatic foci (AHF). Placental glutathione S-transferase was detected by the peroxidase-anti-peroxidase (PAP) method using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) connected to an LA120 printer (Digital Equipment Corporation, Maynard, MA) to quantify AHF.

**Radioimmunoassay for Endogenous Liver Prostaglandin F2α and E2 Levels.** Endogenous liver PGF2α and PGE2 levels were determined by radioimmunoassay according to the method of McCosh et al. (1976). Anti-rat PGF2α rabbit antiserum was purchased from Sigma. Anti-rat PGE2 rabbit antiserum was a gift from Dr. J. Aqueline Dupont, USDA-ARS, Beltsville, MD. Goat anti-rabbit gamma globulin (ARGG) was obtained from Western Chemical Research Corp., Fort Collins, CO. Concentrations of [3H]PGF2α and [3H]PGE2 with specific activities of 168.0 and 154.0 Ci/mmol, respectively, were purchased from Dupont New England Nuclear, Boston, MA. Standards of PGF2α and PGE2 were obtained from Sigma. Scintiverse BD (Fisher Chemical, Fair Lawn, NJ) was added to each tube before counting. Sample-containing tubes were run in duplicate. Total count tubes and background tubes were counted in triplicate. Six replicates were made to determine total binding. Along with the samples, a standard curve was run in duplicate, one set at the beginning of the sample run and the other set at the end. Radioactivity was determined as cpm using a Packard liquid scintillation analyzer model 1900 TR (Packard Instrument Co., Downers Grove, IL).

**Natural Tumor Cytotoxicity Assay.** In the experiment with purified FB1, tumor cytotoxicity of liver NK cells was assessed on the first seven animals sacrificed from each group due to time constraints in performing the assay. The liver perfusate containing Pit cells was layered over 3 mL of Accupaque (Accurate Chemical Co., Westbury, NY). The cells were centrifuged for 15 min at 15,000 rpm in a swinging bucket centrifuge (Model CR312, J. ouen, Winchester, VA). The cells at the interface of the density gradient media were collected, washed twice with complete media (RPMI-1640 supplemented with 50 µg/mL gentamicin, 25 mM HEPES, 2 mM L-glutamine (all from Life Technologies, Gaithersburg, MD) and 10% fetal bovine serum (FBS) (JRH Scientific, Lenexa, KS)), and enumerated on a Celltrack II (NOVA Biomedical, Waltham, MA). The cells from each sample were diluted to 5 × 10^6 and plated in 96-well plates (Model 3595, Costar, Cambridge, MA) to obtain 20, 10, 5, and 2.5 × 10^4 cells (effectors)/well in triplicate. The targets for the assay were YAC-1 cells, which had been labeled with 200 µCi of ^51^Cr (401 mCi/mg, Du Pont New England Nuclear) for 70 min, and maintained in complete media. The targets were washed three times prior to dilution, and 10^6 targets were plated in each well of the NK assay including control wells to determine spontaneous and maximum release. The plates were incubated for 4.5 h in a humidified CO2 incubator (5% CO2, 95% air) (Fisher Scientific). At the end of the incubation the plate was centrifuged at 500 rpm for 5 min, and 100 µL of supernatant was collected to determine the amount of ^51^Cr released by dying cells using a Gamma Trac 1191 (TM Analytic, Inc., Elk Grove Village, IL). The cpm detected were used to calculate the lytic units (LU) of activity at 20% lysis in 10^5 effectors using a computer program.

**Statistical Analysis.** The liver PGF2α and PGE2 concentrations were determined by a computer program based on a logit transformation of the standard curve (Duddleson et al., 1972). One-way ANOVA was performed to analyze feed consumption, body weight, plasma total cholesterol level and ALT activity, endogenous hepatic PGF2α and PGE2 concentrations, and natural tumor cytotoxicity, using the Statistical Analysis System (Cary, NC). Student's t-test was performed to compare group differences after ANOVA. A P value of <0.05 was considered to be statistically significant. The analysis of computerized stereology of AHF, only the means and standard deviations were given because the means equalled zero in basal and FB1-fructose groups.

**RESULTS**

**Experiment 1. Male Rats Fed Crude Fumonisin B1.** In comparison with the rats fed basal diet and the rats fed FB1-fructose, the FB1-fed rats had significantly increased plasma total cholesterol concentration and ALT activity by 85% (P < 0.01) and 100% (P < 0.05), respectively (Table 3).

The PGF2α concentration was significantly increased by approximately 51% in the rats fed FB1 as compared with those in the other groups (P < 0.05) (Table 3). There were no significant differences in PGE2 levels among the groups (Table 3).

All FB1-fed rats had both PGST- and GGT-positive AHF. The average PGST-positive AHF area percentage
was 5.0 ± 6.3, and the average GGT-positive AHF area percentage was 1 ± 1 in the rats fed FB1 at 69.3 µmol/kg diet (Table 4). There were no detectable PGST- or GGT-positive AHF in the group fed 69.3 µmol/kg FB1 reacted with fructose or in the control group (Table 4).

**Experiment 2. Female Rats Fed Highly Purified Fumonisins B₁.** The average daily feed intake in FB₁ group, FB₁-fructose group and basal group was 11 ± 1, 13 ± 2, and 13 ± 1 g, respectively. There were no significant differences in the total feed intake or the average daily feed intake among the three groups (Table 2).

The rats in the FB₁-treated group had significant reductions in both body weight (by 12%, P < 0.05) and body weight gain (by 16%, P < 0.05) as compared with the rats in the groups fed FB₁-fructose and basal diet (Table 2).

In comparison with the basal group and the group fed FB₁-fructose, rats fed FB₁ at 69.3 µmol/kg diet had significantly increased plasma total cholesterol concentration and ALT activity by about 2.3-fold (P < 0.01) and 1.7-fold (P < 0.05), respectively (Table 3).

The PGF₂α and PGE₂ concentrations were both significantly increased by 2-fold in rats fed 69.3 µmol/kg FB₁-containing diet as compared with those in the other groups (P < 0.05) (Table 3).

All of the rats fed FB₁ developed PGST- and GGT-positive AHF. The average number of PGST-positive AHF and the average PGST-positive AHF area percentage were 50 ± 17 and 68 ± 18, respectively, and the average number of GGT-positive AHF and the average GGT-positive AHF area percentage were 45 ± 13 and 61 ± 13, respectively, in the rats fed FB₁ (Table 4). There were no detectable PGST- or GGT-positive AHF in either the group fed FB₁-fructose or basal diet (Table 4).

Compared with the control rats, tumor cytotoxicity was significantly suppressed in animals fed FB₁ (P < 0.03) and significantly elevated in animals fed FB₁-fructose diet (P < 0.01) (Figure 1). Thus, feeding highly purified FB₁ or FB₁-fructose induced significant and opposing changes in liver tumor cytotoxicity.

**DISCUSSION**

These studies demonstrated that subjecting FB₁ to nonenzymatic browning conditions with fructose eliminated FB₁ toxicity as reflected in body weight, plasma total cholesterol concentration, and ALT activity, development of GGT- and PGST-positive AHF, and concentrations of endogenous hepatic PGF₂α and PGE₂. Loss of amine group reactivity toward OPA was taken as an indication that the amine had condensed with fructose (Murphy et al., 1995). Results with either crude or highly purified FB₁ were generally similar, showing detoxification of FB₁, because in both cases FB₁ reacted with fructose did not promote hepatocarcinogenesis in rats and did not increase plasma cholesterol concentration, plasma ALT activity, or hepatic PGF₂α.

Figure 1. Tumor cytotoxicity of liver-associated NK cells against YAC-1 target cells, expressed as mean lytic units (LU) ± SE (n = 7/group), in female rats fed fumonisin B₁ (FB₁), FB₁ reacted with fructose (FB₁-fructose), or a basal diet. Treatments marked by different letters were significantly different from basal group: b, P < 0.03; c, P < 0.01.

The chemical modification of FB₁ by fructose by a Maillard reaction probably caused the formation of a Schiff's base, FB₁-fructose adduct, in which the FB₁ amine group was combined with the fructose keto. The addition of the bulky fructose did not cause FB₁ to be less absorbed in the intestine even though this may increase both water solubility and molecular size (Hopmans et al., submitted). At the molecular level, FB₁ toxicity is most likely due to its inhibition of ceramide synthase, a key enzyme in de novo sphingolipid biosynthesis (Wang et al., 1991). Perhaps the presence of fructose blocked the inhibitory binding of FB₁ to ceramide synthase.

The putative effectiveness of the detoxification of FB₁ by reaction with fructose suggested that the amine group of FB₁ was critical to FB₁ toxicity. This was in accordance with another study in which the N-acetyl
derivative of FB1 showed no hepatotoxicity and no hepatocarcinogenicity (Gelderblom et al., 1993).

The significant reductions in body weight and body weight gain of female rats fed FB1 compared with the other groups were accompanied by normal feed consumption (Table 2). This did not agree with another study in which both body weight and feed intake in male Sprague-Dawley rats were significantly decreased after intraperitoneal injection of FB1 (Bondy et al., 1995). Neither body weight nor feed intake was significantly decreased after FB1 consumption by male and female Fischer 344 rats (Voss et al., 1995). The difference between our results and the others cannot be readily explained.

Fumonisin B1-induced hypercholesterolemia as observed in the present studies (Table 3) was reported in vervet monkeys (Fincham et al., 1992), as well as in rats fed FB1 (Hendrich et al., 1993). Hypercholesterolemia is an observed effect of other tumor-promoting agents and toxicants, such as phenobarbital (Katayama et al., 1991). The hypercholesterolemic mechanism of FB1 is unknown.

In agreement with the findings of Hendrich et al. (1993), FB1 fed to rats at 69.3 μmol/kg diet for 4 weeks increased not only plasma total cholesterol but plasma ALT activity as well (Table 3). Elevated plasma ALT activity indicated hepatocyte membrane damage which led to the leakage of ALT into the blood. Such damage was associated with the development of AHF caused by FB1 (Hendrich et al., 1993).

Placental glutathione S-transferase-positive AHF and GGT-positive AHF were found only in FB1-fed rats in both studies (Table 4). Placental glutathione S-transferase and GGT are useful markers of FB1 hepatocarcinogenesis. The induction of PGST could be demonstrated in single putatively initiated hepatocytes within 48 h after DEN treatment (Moore et al., 1987). Both PGST and GGT persisted during hepatocarcinogenesis (Hendrich and Pitot, 1987). In the present study, placental glutathione S-transferase- and GGT-positive AHF were detected after 4 weeks of FB1 treatment. This indicated that both PGST and GGT were induced at an early stage of hepatocarcinogenesis and could serve as sensitive markers of FB1-induced hepatocarcinogenesis. Because PGST- and GGT-positive AHF were virtually undetectable in DEN-initiated rats fed FB1 reacted with fructose, FB1 promotion of carcinogenesis may be blocked by modifying FB1 with fructose.

Only FB1-treated rats showed significantly greater amounts of endogenous PGF2α and PGE2 compared with the control group, and PGST- and GGT-positive AHF were only present in FB1-fed rats. The elevation of hepatic PGF2α and PGE2 concentrations paralleled the induction of AHF in the liver. Therefore, increased PG production is related to promotion of rat hepatocarcinogenesis caused by FB1. Both PGF2α and PGE2 may contribute to cell proliferation in the liver during FB1 treatment. Prostaglandins of the F series stimulated the proliferation of neonatal rat hepatocytes (Armato and Andreis, 1983). Prostaglandins including PGF2α and PGE2 also played a role in proliferation of hepatoma cells (Trevisani et al., 1980). Direct evidence of stimulatory effect of PGs on the proliferation of hepatoma cells was shown by the reversal of decreased hepatoma cell numbers by PGE2 in indomethacin-treated rats (Trevisani et al., 1980).

Prostaglandins may also exert indirect effects on proliferation of tumor cells by suppressing the local immune response. Prostaglandins were able to make macrophages and/or lymphocytes less sensitive to various stimuli (Pelus and Strauss, 1977; Schultz et al., 1978). Moreover, prostaglandin F2α suppressed NK cells and lymphokine-activated killer (LAK) cells (Ohnishi et al., 1991; Roth and Golub, 1993; Baxevanis et al., 1993). The present studies showed that hepatic PGE2 concentrations were significantly increased in FB1-induced promotion of rat hepatocarcinogenesis (Tables 3 and 4), and in the female rats fed purified FB1, liver-associated antitumor response was decreased (Figure 1). However, the mechanism for enhancement of tumor cytotoxicity in the FB1–fructose-fed group is not clear as this group did not differ from the basal diet group in any of the other measures. The tumor cytotoxic cells of the liver are Pitt cells, which are similar to blood NK cells, but with higher lytic activity. The possible immune-enhancing effect of FB1–fructose deserves further study.

The mechanism of FB1-induced carcinogenesis is still not fully understood. Fumonisin B1 is an inhibitor of sphingolipid synthesis (Wang et al., 1991). Sphingolipids may be anticarcinogenic because they are down-regulators of protein kinase C (Merrill, 1991), which could affect many enzyme activities after it is activated by carcinogens (Weinstein, 1987). It is not known whether FB1 can stimulate phospholipase A2 which hydrolyzes phospholipids to release the PG precursor, arachidonate, or activate cyclooxygenase to produce more PGs in the liver. Prostaglandin F2α was shown to stimulate the formation of intracellular diacylglycerol in Swiss 3T3 cells (Macphee et al., 1984), which can in turn activate protein kinase C. Perhaps FB1-induced alteration of sphingolipid metabolism stimulates PG production, by an unknown mechanism.

Possibly decreased inhibitory binding of FB1 to ceramide synthase as a result of FB1 reaction with fructose may be the mechanism of FB1 detoxification in this experiment. Further study is needed to identify the structure of the putative FB1–fructose conjugate and investigate its stability, possible processing occurrence, bioavailability and toxicity.

ABBREVIATIONS

Used FB1, fumonisin B1; DEN, diethylnitrosamine; ALT, alanine aminotransferase; PG, prostaglandin; PGST, placental glutathione S-transferase; GGT, γ-glutamyl transferase; AHF, altered hepatic foci; NK cell, natural killer cell; and OPA, o-phthaldialdehyde.

ACKNOWLEDGMENT

We thank Dr. Paul Nelson of Pennsylvania State University for supplying Fusarium proliferatum strain M5991, Dr. J aqueline Dupont of USDA–ARS for donating anti-rat PGE2 rabbit antiserum, Dr. Pieter Thiel of South African Medical Research Council for donating pure FB1, and Dr. Ronald Platter for confirming the purity of our purified FB1.

LITERATURE CITED


Reaction with Fructose Detoxifies Fumonisin B₁


Received for review October 19, 1996. Accepted December 10, 1996. This research was supported by Center for Advanced Technology Development, Iowa State University, and Pioneer Hybrid International, Inc. This paper is published as Journal Paper J-16170, Iowa Agriculture and Home Economics Experiment Station, Project 2995, a contributing project to regional NC-129.

J F9607775