Glucose Reaction with Fumonisin B₁ Partially Reduces Its Toxicity in Swine

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Acute and subacute intraperitoneal doses of fumonisin B₁ (FB₁) were administered to test the efficacy of the FB₁-glucose reaction products in detoxifying FB₁ in swine. In the acute study at 11 µmol of FB₁/kg of body weight, five of six pigs administered FB₁ and four of six pigs administered FB₁-glucose died from acute pulmonary edema. Analysis of weight gain, serum aspartate aminotransferase and γ-glutamyltransferase, total cholesterol, and pathological evaluation did not provide evidence of protection against FB₁ toxicity by the FB₁-glucose reaction products. In the subacute study at 5.5 µmol of FB₁/kg of body weight, one pig administered FB₁ died from liver damage. Analysis of serum aspartate aminotransferase, γ-glutamyltransferase, and total bilirubin showed protection against FB₁ toxicity by the FB₁-glucose reaction products. The levels of sphinganine and sphinganine/sphingosine ratios in serum and liver as well as pathologic findings provided definitive evidence of protection against the FB₁ toxic effects by this detoxification procedure (p < 0.05).

KEYWORDS: Fumonisin B₁; fumonisin B₁ toxicity; detoxification; swine

INTRODUCTION

The fumonisins are a group of mycotoxins mainly produced by the fungi Fusarium verticillioides (= F. moniliforme) and Fusarium proliferatum in corn (1). Among other structurally related homologues, fumonisin B₁ (FB₁) is the most abundant natural contaminant of corn-based products intended for human or animal consumption throughout the world (2). This mycotoxin has been associated with a high incidence of human esophageal cancer in some regions of China and the Transkei in South Africa, where corn-based foods are the staple diet (3). Hence, FB₁ has been declared a class 2B carcinogen, a probable human carcinogen (4). Fumonisin B₁ has species-specific toxic effects. In rodents, FB₁ is hepatocarcinogenic, hepatotoxic, and nephrotoxic (5). Horses are the most sensitive species, known to develop leukoencephalomalacia (ELEM) after consuming corn contaminated with FB₁ at levels >0.001 µmol/g (6, 7). In pigs, subacute dietary levels, in the range of 0.1 µmol of FB₁/g, can cause liver disease in ~15 days, whereas acute dietary levels, >0.14 µmol of FB₁/g, may produce fatal porcine pulmonary edema (PPE) in <1 week (8, 9). In the United States, widespread large-scale outbreaks of ELEM and PPE occurred during the fall of 1989 and the winter of 1990. Significant numbers of horses and pigs died from consuming commercial mixed feeds containing FB₁-contaminated corn (10).

Fumonisin B₁ disrupts sphingolipid metabolism by potently inhibiting the enzyme ceramide synthase (sphinganine N-acetylatedtransferase) in the de novo biosynthesis pathway of major sphingolipids, thus causing a rapid increase in free sphinganine (Sa), one of the base precursors (11, 12). The levels of free Sa and sphingosine (So) in tissues and biological fluids may be compared by constructing a ratio of Sa to So (Sa/So ratio) that is used as an early biomarker of exposure to the mycotoxin (13). The inhibition of ceramide synthase is also likely to disrupt overall sphingolipid metabolism and, theoretically, sphingolipid-mediated regulation of important cell events such as apoptosis and mitosis, which are probably in part responsible for the cytotoxic and carcinogenic properties of the mycotoxin (11, 14).

In swine, the animal model used in the present study, FB₁ alters sphingolipid biosynthesis with the greatest alterations in Sa and So concentrations occurring in kidneys, liver, lungs, and heart (13). In a recent swine study, diets that were amended with a fungal culture of F. verticillioides to achieve FB₁ concentrations of 0.006 and 0.01 µmol/g caused a significant increase in the Sa/So ratio at days 15 and 8 of the study, respectively (15). Serum markers of liver injury, such as aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), total bilirubin (BIL), and total cholesterol (CHOL), increase after exposure to either acute or subacute levels of FB₁. Hepatic lesions consist of apoptosis, necrosis, and hepatocyte proliferation (8, 9). The toxicological effects of feeding 0.04 µmol of...
FB1/g of feed to weaned piglets for 28 days were recently evaluated. Animals presented typical signs of pulmonary edema with reduced feed consumption and weight gain as well as typical gross and microscopic lesions. Increases in erythrocyte count, hematocrit, BIL, total protein, CHOL, and activities of serum alkaline phosphatase (SAP), AST, and alanine aminotransferase (ALT) were detected (16).

The primary amine group of FB1 has been suggested as the site of toxicity for the molecule (> 17, 18). Detoxification of FB1 by blocking the amine group with a reducing sugar such as fructose or glucose via the nonenzymatic browning (NEB) or Maillard reaction has been suggested (19). This detoxification strategy was used to demonstrate a significant reduction in cancer promotion of diethylnitrosamine (DEN)-initiated Fischer rats fed diets containing no FB1, highly purified FB1 at 0.1 mol of FB1/g of feed, and FB1 reacted with glucose containing a residual 0.01 mol of FB1/g of feed, respectively. The Sa/So ratio in the FB1-glucose group was the same as in the controls, whereas the FB1 group presented a ratio that indicated an alteration in the synthesis of complex sphingolipids (25).

This study describes the effects of FB1-glucose reaction products in swine using acute and subacute intraperitoneal (IP) doses of FB1 in completely randomized designs. Toxicological endpoints were defined as PPE and liver damage, respectively. Our hypothesis was that the chemical reaction of FB1 with glucose would decrease FB1 toxicity in swine. The goal was to evaluate the efficacy of the FB1-glucose reaction products in the detoxification of acute and subacute FB1 poisoning of swine.

MATERIALS AND METHODS

Preparation of Purified Fumonisin B1. Liquid cultures of F. proliferatum strain M5991 were prepared following the method of Dantzer et al. (26) by inoculating capped baffled Erlenmeyer flasks containing 500 mL of modified Myro medium with a 4 day shake flask culture of the fungus for 100 days. FB1 was isolated and purified to > 95% according to the procedure of Dantzer et al. (27).

Preparation of Fumonisin B1-Glucose Adducts. Two batches of FB1-glucose adduct were prepared according to the method of Lu et al. (24). Briefly, 1.39 mM FB1 (total = 6.144 mmol or 4.435 g in the glucose adduct were prepared according to the method of Lu et al. (24). A recent study compared three groups of DEN-initiated Fischer rats fed diets containing no FB1, highly purified FB1 at 0.01 mol of FB1/g of feed, and FB1 reacted with glucose containing a residual 0.01 mol of FB1/g of feed, respectively. The Sa/So ratio in the FB1-glucose group was the same as in the controls, whereas the FB1 group presented a ratio that indicated an alteration in the synthesis of complex sphingolipids (25).

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RESULTS

In the acute toxicity study, pigs in the FB1 and FB1-G groups received 11 μmol of FB/kg of BW. The dose of free unreacted FB1 in the FB1-G group was 3.3 μmol of FB/kg of BW, representing a 70% free amine FB reduction. No clinical signs were observed in any of the pigs on day 1 of the study. Five pigs in the FB1 group and four in the FB1-G group died from day 2 through day 6. Eight of the nine pigs died acutely with clinical signs typical of PPE with duration of <4 h. The ninth pig, from the FB1 group, was mildly icteric and weak late on day 6 and was found dead early on day 7. From days 7 to 9, the remaining pig in the FB1 group and the two remaining in the FB1-G group were free of clinical signs of PPE except that one of the FB1-G pigs was moderately depressed with evident mild icterus of skin, sclera, and mucous membranes. The control group had the highest weight gain, 1.2 ± 0.1 kg, whereas the FB1 and FB1-G groups gained an average of 0.5 ± 0.14 and 0.7 ± 0.14 kg, respectively. Weight gains of the FB1 and FB1-G pigs as compared to controls, GGT levels were statistically significant differences between the group means at a given time point (p < 0.05). In both studies, average initial body weights (day 0) did not differ significantly among the groups.

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|-----------------|-------|---------|-------|------|
| AST (IU/L)       | GGT (IU/L) | BIL (mg/dL) | CHOL (mg/dL) |
| Control          | Day 0  | Day 3   | Day 0  | Day 3 |
| FB1              | a     | b       | a     | b    |
| FB1-G            | a     | b       | a     | b    |

*Data are expressed as mean ± SEM. n = 6. Different letters indicate statistically significant differences between the group means at a given time point (p < 0.05). FB1 dose: 11 μmol/kg of BW. Free FB1 in FB1-glucose dose: 3.3 μmol/kg of BW.*
In the subacute toxicity study, pigs in the FB and FB1-G groups received 5.5 \mu M of FB1/kg of BW. The dose of free unreacted FB1 in the FB1-G group was 0.6 \mu M of FB1/kg of BW representing an 89% free amine FB1 reduction. The control pigs had no apparent signs of disease throughout the duration of the experiment. Pigs receiving FB1, on the other hand, showed weakness, rough hair, and lateral recumbency by day 4. Their condition continued to worsen on a daily basis as they refused to eat and icterus became evident in the ventral abdominal and inguinal region of the body and in the ocular sclera. Early on day 7, one FB1 pig was found dead. Three of four FB1-G pigs showed rough hair and mild weakness from days 4 to 7, but in general all pigs in this group were considered to be in good health until the end of the experiment. Pigs in the control group had the highest weight gain, 2.02 \pm 0.28 kg, whereas pigs in the FB1 and FB1-G groups gained only 0.2 \pm 0.12 and 0.85 \pm 0.51 kg, respectively. Weight gains of FB1 and FB1-G groups were not statistically different (p < 0.05, Figure 1).

On day 0 of the subacute toxicity study, the serum levels of AST, GGT, BIL, and CHOL were similar among the treatment groups (Table 2). On day 4, the FB1 pigs showed moderately elevated AST and BIL with respect to controls. GGT levels were similar among the treatment groups, and CHOL was lower in the FB1 and FB1-G groups than in the control group. On day 4, Sa concentration and the Sa/So ratio were similar between the treatment groups, and CHOL was lower in the FB1 group but to a lesser extent. CHOL was higher in FB1 pigs followed by FB1-G and controls, with all groups different from each other (p < 0.05).

In the subacute toxicity study, serum levels of AST, GGT, BIL, and CHOL were similar among the treatment groups (Table 3). On day 0, Sa concentration and the Sa/So ratio were similar between the treatment groups. On day 4, Sa concentration in FB1 pigs was greater than in control but similar to that in FB1-G pigs. Control and FB1-G pig results were not different from each other. The Sa/So ratio in FB1 pigs was higher than in control but similar to FB1-G pigs, whereas control and FB1-G pigs had similar ratios. On day 7, the Sa concentration and Sa/So ratio were higher in FB1 pigs as compared to control and FB1-G pigs, which results were not different from each other (p < 0.05). In liver, Sa levels in FB1-G pigs were higher than in FB1 and control pigs (Table 4). FB1 pigs had lower levels of Sa than control and FB1-G pigs. The Sa concentration in control pigs was intermediate between those of FB1 and FB1-G pigs. Levels of Sa were higher in FB1 as compared to controls and FB1-G, which were not different from each other. The Sa/So ratio followed a trend similar to the Sa concentration (p < 0.05).

Gross inspection of the FB1 pigs in the subacute toxicity study revealed severe yellow discoloration of subcutaneous, abdominal, and pericardial fat. Lungs had a normal diffuse pink color, were not edematous, and had clear airways and trachea. Pale-yellowish irregular areas could be observed in the liver, which did not appear to be enlarged. The surface of the kidneys appeared pale tan to gray and in some cases had a very congested cortex and very yellow-tinged renal papillae on cut surface. Serosal surfaces of the gastrointestinal tract appeared to be mildly congested and edematous but not hemorrhagic. FB1-G and control pigs showed no obvious icterus and were in general free of lesions. Microscopic liver changes in the FB1 group consisted primarily of mild to moderate, multifocal random necrosis of individual hepatocytes or small groups of hepatocytes with resultant disorganization of hepatic sinuoids. Hepatocytes individualized, became rounded or hyperesinophilic, and had pychnotic, karyorrhexis, or karyolytic nuclei. Mild to moderate karyomegaly was often observed in adjacent...
hepatocytes. A comparison of the three treatment groups is presented in Figure 2. In the FB1-G group, two of four pigs showed large, clear, well-demarcated vacuoles in the distal nephron but were otherwise free of lesions. No microscopic lesions were observed in control pigs. Ratios of organ weight to total body weight for heart, lungs, liver, or kidney showed no significant differences between the treatment groups (data not shown).

**DISCUSSION**

The acute toxicity study did not clearly demonstrate reduction of FB1 toxicity by its reaction with glucose in IP dosing of swine. This was shown by the death from acute PPE of five of six pigs in the FB1 group, dosed at 11 µmol of FB1/kg of BW, and four of six pigs in the FB1-G group, dosed at 3.3 µmol of FB1/kg of BW. On day 3 weight gains were similar among the FB1 and FB1-G groups, and the clinical parameters AST and CHOL were above normal levels (29) and not significantly different in pigs from these two groups. The toxic effects observed in this study were consistent with acute FB1 toxicosis in swine and have been reported by several authors (30–34). The partial failure of the FB1-glucose adduct in protecting the pigs in the acute toxicity study from the effects of FB1 could be explained by the 30% free FB1 in the FB1-G mixture. If this was the case, FB1-G pigs received an ip dose of ∼3.3 µmol of FB1/kg of BW. This dose of FB1 is significantly higher than the intravenous (iv) dose level of 0.55 µmol of FB1/kg of BW used by Harrison et al. (35) to characterize acute PPE. It is worth mentioning that on day 3 of the acute toxicity study, BIL results in FB1-G pigs were within normal range (29), similar to results in the control group and lower than in the FB1 group, providing some evidence of protection of the liver in the FB1-G group (Table 1).

In contrast to the acute FB1 dose, the subacute toxicity study revealed reduction of FB1 toxicity in pigs. Only one pig died from apparent liver damage in the 5.5 µmol of FB1/kg of BW group, whereas no pigs administered the FB1-glucose products at a dose of 0.6 µmol of FB1/kg of BW group died. We believe that two main factors, dose level and amount of free unreacted FB1 in the FB1-glucose mixture, contributed to the success of the detoxification treatment in protecting the pigs from FB1-induced liver damage. The ip dose of 5.5 µmol of FB1/kg of BW was half the dose used in the acute toxicity study, and the amount of free unreacted FB1 in the FB1-glucose mixture was only 11%, meaning that FB1-G pigs received an FB1 dose of ∼0.6 µmol/kg of BW. The high mortality observed in the FB1-G pigs of the acute toxicity study, which received 3.3 µmol of FB1/kg of BW, as compared to the FB1 pigs of the subacute toxicity study, which received a dose of 5.5 µmol of FB1/kg of BW, could be explained by the toxicity of the FB1-glucose products. In preparation for the acute toxicity study, the FB1-glucose mixture was heated for 48 h, resulting in 70% free amine FB1 reduction by glucose. Additionally, other reaction products that were not chemically characterized and of which toxicity remains unknown may have also been formed. It is likely that these additional uncharacterized products in the acute toxicity study may have inhibited the enzyme ceramide synthase in the pigs treated with FB1-glucose, disrupting the sphingolipid metabolism and leading to left-sided heart failure and ultimately PPE. Although in the subacute toxicity study the FB1-glucose products were prepared following the same method, the mixture was instead heated for 200 h, resulting in 89% free amine FB1 reduction. The additional FB1-glucose reaction products formed in the subacute toxicity study were not chemically characterized. However, we observed that these did not cause any obvious signs of toxicity in the pigs of the FB1-glucose group. Howard et al. (36) fed female B6C3F1 mice 14, 70, and 140 µmol/kg of diet of several fumonisin derivatives, including FB1 and N-carboxymethylfumonisin B1, one of the fumonisin B1-glucose reaction products previously characterized (23, 24). The study found that only FB1 was hepatotoxic, whereas all other fumonisin derivatives did not alter serum analytes, organ weights, or hepatic structure. The high mortality of pigs administered the FB1-glucose products in the acute toxicity study as compared to the pigs administered FB1 in the subacute toxicity study could also be explained by a higher susceptibility to FB1 exposure. The pigs used in the acute toxicity study were obtained from a different source than the pigs used in the subacute toxicity study, possibly having a different genetic background and a higher susceptibility to FB1 at a 3.3 µmol/kg of BW dose. In pilot studies not previously published we have exposed crossbred pigs to levels as high as 145 µmol of FB1/kg of diet and found no evidence of alteration in AST, GGT, or BIL. These findings contrast with the results of several studies (8, 9) that have found liver damage after pigs were fed diets containing <145 µmol FB1/kg, which leads us to believe that susceptibility to FB1 could be different in pigs from different genetic lines. Harrison et al. (35) reported the observation of pulmonary edema in pigs administered iv pure FB1 at a dose of 0.55 µmol/kg of BW for 4 days, a dose much lower than our ip dose of 3.3 µmol/kg of BW. Toxicokinetics of pure FB1 after iv or ip administration appear to be comparable. Shepard et al. (37) dosed rats ip with 10.4 µmol/kg of BW of 14C-labeled FB1 and after 24 h recovered 66% of radioactivity in feces and 32% in urine. Prelusky et al. (38) administered iv 14C-labeled FB1 to pigs at a dose of 0.5 µmol/kg of BW. After 72 h, 58% of radioactivity was recovered in feces and 21% in urine.
Although in different species, the toxicokinetics results from these two studies appear to indicate similar excretions of FB1 after either iv or ip administration.

The results of our subacute toxicity study agree with the FB1 data of Haschek et al. (31), in which pure FB1 administered iv to pigs at 0.0012 and 0.0016 µmol/kg of BW for 9 and 4 days, respectively, caused mild intermittent respiratory signs but not pulmonary edema or severe liver damage. Osweiler et al. (8) also observed subacute hepatotoxicosis in pigs administered iv 0.0011 µmol of FB1/kg of BW for 14 days. Our weight gain results were equivocal. Pigs in the FB1-G group had a weight gain similar to that in pigs in the FB1 group. This observation is because two of four pigs dramatically decreased their feed consumption on days 4–5 of the study, causing the average weight gain to drop, whereas the other two pigs consumed as much feed as the controls (feed consumption data not shown). Although we could not establish a clear explanation for this variability, experiment-related stress events may have contributed to it. We do not believe these two pigs had developed FB1-glucose adduct-induced feed refusal because two of four pigs ate well. In FB1 pigs, the liver enzymes AST and GGT started to show a time-dependent elevation on day 4. By day 7, dramatic differences in the liver enzyme levels were evident between the FB1 and control or FB1-G groups. This indicated that liver cells in the FB1 pigs of the subacute study were severely damaged, allowing the intracellular enzymes to leak into the bloodstream, events that presumably did not occur in the control or FB1-G pigs. These findings are similar to the results of Liu et al. (25), in which rats exposed to diets containing 0.011 or 0.034 µmol of FB1/g for 12 or 20 weeks had significantly higher plasma alanine aminotransferase (ALT) levels than rats fed a basal or FB1-glucose diet for the same period of time. Our results also agree with a study by Lu et al. (20) in which rats fed 0.07 µmol of FB1/g of diet for 4 weeks had a significantly higher ALT level than rats fed 0.07 µmol of FB1/g of diet reacted with fructose for the same time. ALT is a specific enzyme for hepatocellular injury in small species, including the rat (39). Total cholesterol levels in control pigs showed a time-dependent decrease, whereas levels in FB1-G pigs remained practically unchanged during the study. This pattern could explain the significant differences observed between control and FB1-G pigs at days 4 and 7 of the subacute study.

Because FB1 disrupts sphingolipid metabolism, results of individual levels of serum Sa and So and their ratio showed protection of the FB1-G pigs in the subacute toxicity study. The levels of Sa, in particular, showed a time-dependent elevation in the FB1 group throughout the study. On day 7, Sa levels and the Sa/So ratio were higher in the FB1 pigs as compared to controls and FB1-G pigs. The higher Sa levels in the FB1 group were what we expected. Several studies had previously found a significant elevation of Sa after swine had been fed pure FB1 at 0.002 µmol/g of diet for 5 days (40), FB1 from culture material at 0.027 µmol/g for 1 day (41), or pure FB1 at 0.013 µmol/g for 8 days (30). In contrast to Riley et al.’s findings (13), the serum Sa levels in the FB1-treated pigs of our study did not plateau before day 4, and the serum So levels did not continue to increase for the duration of the study. The levels of So were not different among the three groups throughout the subacute toxicity study, which agrees with the findings of Riley et al. (13) and Gumprecht et al. (30). Blocking of the primary amine group of FB1 by its reaction with glucose likely prevented the inhibition of the enzyme ceramide synthase in the sphingolipids synthesis pathway. Therefore, accumulation of sphingolipid precursors Sa and So was not observed in the FB1-G group. A similar scenario was observed in liver, where Sa levels were higher in the FB1 group but not different between controls and FB1-G, resulting in Sa/So ratios that followed the same pattern. The results obtained from our FB1-treated pigs were similar to those of previous studies that have found increased liver Sa, and to a lesser extent So, and elevated Sa/So ratios in pigs fed diets containing 0.031 µmol of FB1/g for 14 day (13) or 0.013 µmol of FB1/g for 8 weeks (40). In addition, pharmacokinetic studies have shown a good correlation between the magnitude of sphingolipid alterations and the distribution of FB1, which is highest in liver and kidneys (38).

Gross lesions in the subacute toxicity study agreed with a study by Colvin et al. (33) in which pigs were daily gavaged with 5.5 and 22.2 µmol of FB1/kg of BW for 45 and 7 days, respectively. In that study, pigs were also severely icteric, as was evident from the bright yellow color of the sclera, oral mucosa, and subcutaneous adipose tissue. Livers had a tan coloration. However, the findings of Colvin et al. (33) in kidneys were different from ours in that they found mild to moderate renal tubular necrosis and we did not. These researchers reported that lungs were normal and, although more severe than in our study, their pigs showed gastrointestinal lesions such as ulceration and erosion of the nonglandular mucosa. Microscopic changes consistent with both hepatocyte necrosis and apoptosis were observed in the FB1 pigs of our subacute toxicity study. These changes matched those found in several dietary and iv studies (8, 31, 34). Interestingly, two of our FB1-G pigs had well-demarcated vacuoles in the distal nephrons. We believe this represented a random finding because the remaining two FB1-G pigs did not present such lesions and the other kidney structures were free of lesions.

In conclusion, in contrast to the acute iv dose of 11 µmol of FB1/kg of BW, the subacute iv dose of 5.5 µmol of FB1/kg of BW yielded clear evidence that derivatization of the FB1 amine with glucose can protect the livers of pigs against the hepatotoxic effects of FB1. Further studies are necessary to assess the use of this detoxification strategy in swine feed operations where quality might be adversely affected by FB1 contamination. Its implementation in the field will depend on the use of appropriate processing equipment that would require additional research.

**ABBREVIATIONS USED**

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BIL, total bilirubin; C-20, d,l-erythro-C20-dihydrophosphoglycerine; CHOL, total cholesterol; DEN, diethylnitrosamine; ELEM, equine leukoencephalomalacia; FB1, fumonisin B1; FB1-G, fumonisin B1-glucose; GGT, γ-glutamyltransferase; NEB, nonenzymatic browning reaction; OPA, o-phenaldehyde; PPE, porcine pulmonary edema; Sa, sphinganine; SAP, alkaline phosphatase; So, sphingosine.

**SAFETY**

Fumonisin B1 is a class 2B carcinogen and was handled accordingly.

**LITERATURE CITED**


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