Mycotoxin Fumonisin B₁ Increases Intestinal Colonization by Pathogenic Escherichia coli in Pigs

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Fumonisin B₁ (FB₁) is a mycotoxin that commonly occurs in maize. FB₁ causes a variety of toxic effects in different animal species and has been implicated as a contributing factor of esophageal cancers in humans. In the present study, we examined the effect of dietary exposure to FB₁ on intestinal colonization by pathogenic Escherichia coli associated with extraintestinal infection. Three-week-old weaned pigs were given FB₁ by gavage as a crude extract or as a purified toxin at a dose of 0.5 mg/kg of body weight daily for 6 days. On the last day of the toxin treatment, the pigs were orally inoculated with an extraintestinal pathogenic E. coli strain. All animals were euthanized 24 h later, necropsies were performed, and tissues were taken for bacterial counts and light microscopic examination. Ingestion of FB₁ had only a minimal effect on animal weight gain, did not cause any macroscopic or microscopic lesions, and did not change the plasma biochemical profile. However, colonization of the small and large intestines by an extraintestinal pathogenic E. coli strain was significantly increased. Our results show that FB₁ is a predisposing factor to infectious disease and that the pig can be used as a model for the study of the consequences of ingesting mycotoxin-contaminated food.

Mycotoxins are secondary metabolites of fungi which may contaminate animal and human feeds at all stages of the food chain. Their global occurrence is considered an important risk factor for human and animal health, as up to 25% of the world crop production may be contaminated with mycotoxins (16, 31).

Fumonisin B₁ (FB₁) belongs to the fumonisin family of toxins (4) which are produced by Fusarium verticillioides and Fusarium proliferatum, fungi that commonly contaminate maize. Recent surveys of fumonisins in food and feed throughout the world, including the United States and most European countries, raised concerns about the extent of FB₁ contamination of maize and its implications for food safety (13, 52, 53). FB₁ was found in up to 50% of maize samples collected between 1988 and 1991 from the midwestern United States (41). In this survey, up to 10% of the samples had toxin levels between 10 and 50 ppm (41). Similarly, another survey of fumonisins in maize gluten and other maize products in the United Kingdom found these mycotoxins in almost every sample at concentrations of up to 32 ppm (52).

At high concentrations (50 to 500 ppm), FB₁ causes a variety of species-specific toxicological effects in domestic and laboratory animals. It induces leukoencephalomalacia in horses, pulmonary edema in pigs, and nephrotoxicity in rats, rabbits, lambs, and calves (3, 14, 21, 22, 32, 54). In all species studied, both acute and chronic exposure to FB₁ are associated with alteration of sphingolipid metabolism and hepatotoxicity (9, 20, 21, 26, 44, 46, 48). FB₁ also has been implicated as a contributing factor in human esophageal cancers (45) and is a renal and hepatic carcinogen in male and female rats, respectively (22). The mechanism(s) of toxicity for fumonisins is complex and may involve several molecular sites (47). The primary biochemical effect of fumonisin is to inhibit ceramide synthase activity, leading to the accumulation of sphingoid bases and sphingoid base metabolites and the depletion of more complex sphingolipids (36).

Although Escherichia coli is a normal inhabitant of the intestinal flora, it is frequently associated with both intestinal and extraintestinal infections. Extraintestinal pathogenic E. coli (ExPEC) strains usually possess virulence determinants that allow them to persist in the intestine, cross epithelial barriers, resist nonspecific host defense mechanisms, establish specifically in extraintestinal tissues, and potentially cause damage at these sites (50, 55). For instance, ExPEC strains with similar virulence determinants have been associated with urinary tract diseases in humans and septicemia in pigs (7, 15, 23). We have established a septicemia model involving oral inoculation of porcine ExPEC strains in newborn, colostrum-deprived, germ-free pigs to study the pathogenic mechanisms of these bacteria in the natural host when it is highly susceptible to bacterial infection (17). These bacteria are also opportunistic pathogens, as they have been found in the intestines of healthy older pigs (19), dogs (23), and humans (7). Host conditions, therefore, are of critical importance in the ability of bacteria to infect and colonize the host and cause disease (34, 38, 42, 58).

The intestinal tract is the first barrier to ingested mycotoxins but is also the first line of defense against intestinal infection. Ingestion of some mycotoxins increases susceptibility to experimental or natural mucosal infections (18, 56, 57), but no data...
are available concerning the effect of fumonisin as a predisposing factor to intestinal infections. The objective of the present study was to determine the effect of dietary exposure to low doses of FB1 on intestinal colonization by the pathogenic bacterium E. coli.

MATERIALS AND METHODS

Animals. Thirty-five 3-week-old weaned healthy male Yorkshire hybrid pigs were used for the experiments. They were acquired locally at 2 weeks of age, just after weaning, and acclimatized for 1 week in the isolation rooms of the animal care facilities of the Faculté de Médecine Vétérinaire, Université de Montréal, at an ambient temperature of 24°C. The pigs were weighed daily. They had free access to water and were fed a commercial starter diet, free of FB1, throughout the experiment. Animals were cared for in accordance with guidelines of the Canadian Council for Animal Care.

Toxin. In a first experiment, the mycotoxin was administered as a crude extract obtained after in vitro culture. Briefly, sterilized crushed maize (50% water content) was inoculated with the high FB1-producing F. verticillioides strain NRRL 34281 (deposited in the ARS Culture Collection, Peoria, Ill.). The fungal strain was incubated for 4 weeks at 25°C. The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this preparation did not produce detectable amounts of the following fusariotoxins: zearalenone, a detection limit of 50 ng (28). The culture extract used in this prepare

Pigs received 10 ml of 1.2% NaHCO3 through an intragastric tube to neutralize night incubation at 37°C. Bacterial colonies were counted with a minimum of 1 colony per plate. Several colonies from each individual were positively identified as the infecting strain by PCR and agglutination tests.

Histopathology. Tissue samples, fixed in 10% neutral buffered formalin, were embedded in paraffin, sectioned at approximately 5-μm intervals, and stained with hematoxylin and phloxine saffron for examination by light microscopy. Bacterial localization in intestinal and extraintestinal tissues was determined by immunohistochemistry. Sections were stained with Vector red (Vector Laboratories, Burlington, Canada) and examined by light microscopy as previously described (6) by using rabbit polyclonal anti-O75 serogroup serum.

Biochemical analysis. At the time of necropsy, blood was collected on EDTA for plasma biochemical analysis (Biochemistry Laboratory, Ranguel Hospital, Toulouse, France). The analysis included determinations of creatinine, urea nitrogen, total protein, calcium, phosphorus, sodium, potassium, chloride, glucose, cholesterol, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and gamma-glutamyl transferase.

Statistical analysis. Student’s t test and analysis of variance (ANOVA) were used to analyze weight gain and bacterial counts. P values of <0.05 were considered significant.

RESULTS

Effect of FB1 on weight gain. Separate experiments were performed using FB1, as either a crude extract or purified toxin. We first examined the effect of 0.5 mg of FB1/kg of body weight on clinical signs and animal performance. Pigs receiving FB1, either as a crude extract or as the purified toxin, appeared clinically normal throughout the study, and no deaths occurred. Pigs in the FB1-treated groups did not gain as much weight as those in the control group, but the difference was not statistically significant (Table 1). At necropsy, no gross changes were considered to be related to the administration of FB1. Microscopic lesions not usually associated with FB1 toxicity, such as apoptosis, were observed during examination of liver and other tissues following routine hematoxylin and phloxine saffron staining in FB1-treated pigs. Plasma biochemical analysis did not reveal any effect of FB1 (data not shown).

<table>
<thead>
<tr>
<th>Expt and treatment</th>
<th>No. of animals</th>
<th>Initial wt (kg)</th>
<th>Wt gain (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>4.43 ± 0.26</td>
<td>1.32 ± 0.12</td>
</tr>
<tr>
<td>FB1 extract</td>
<td>9</td>
<td>4.66 ± 0.19</td>
<td>1.17 ± 0.12</td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>6.55 ± 0.07</td>
<td>2.41 ± 0.29</td>
</tr>
<tr>
<td>Purified FB1</td>
<td>4</td>
<td>6.23 ± 0.17</td>
<td>2.05 ± 0.10</td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>5.95 ± 0.05</td>
<td>1.98 ± 0.26</td>
</tr>
<tr>
<td>Purified FB1</td>
<td>5</td>
<td>5.42 ± 0.15</td>
<td>1.78 ± 0.20</td>
</tr>
</tbody>
</table>

a Results are expressed as means ± standard errors of the means of the indicated number of animals.

b Two-way ANOVA did not reveal any effect of FB1 administration (FB1 versus control) on weight gain of the animals.

c In experiment 2, piglet weight was not homogenous, and, thus, the animals were divided into two groups according to their initial weight. In each group, half of the piglets received purified FB1, and the other half were kept as control.
of the intestinal tract and/or the immune system. The low pathogenicity of a given strain may be compromised, e.g., in the absence of colostrum in conventional or germfree newborn pigs (J. M. Fairbrother, unpublished results). It is possible that a similar effect occurs in older animals when other agents, e.g., mycotoxins, affect the intestinal tract and/or the immune system. The low pathogenicity of this strain is also reflected by its poor ability to elicit an inflammatory response in the intestines, as demonstrated by the absence of an inflammatory cell infiltrate (Fig. 1), and of the induction of RNA encoding inflammatory cytokines (data not shown). Since strains of this pathotype are also recovered from patients with urinary tract infections, strain 28CNaI appears to typify opportunistic ExPEC organisms.

The dose of FB1 administered to pigs in our experiments (0.5 mg of FB1/kg of body weight, equivalent to 5 to 8 ppm in the feed) significantly increased the bacterial colonization of the intestine (Table 2); however, this dose did not induce

**TABLE 2. Effect of oral administration of FB1 on bacterial colonization of piglet intestines by E. coli strain 28CNaI**

<table>
<thead>
<tr>
<th>Expt and treatment</th>
<th>Bacterial colonization of sections (log_{10} [CFU/g]) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ileum</td>
</tr>
<tr>
<td>Expt 1c</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.66 ± 0.14d</td>
</tr>
<tr>
<td>FB1 extract</td>
<td>4.26 ± 0.42</td>
</tr>
<tr>
<td>Expt 2</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.74 ± 0.34</td>
</tr>
<tr>
<td>Purified FB1</td>
<td>3.67 ± 0.64</td>
</tr>
</tbody>
</table>

* a Pigs were dosed for 7 days with 0.5 mg of FB1/kg of body weight, administered as a crude extract (experiment 1) or as a purified toxin (experiment 2). 
  b Results are expressed as geometric mean bacterial counts ± standard errors of the means for a group of four to five pigs.
  c Two-way ANOVA did not reveal any effect of the experiment but indicated a significant effect of FB1 treatment on the bacterial count in the different parts of the intestine. For the results for the ileum, cecum, and colon, the effects of the FB1 treatment in both experiments were significant at a P value of 0.0009, 0.0003, and 0.0001, respectively.

**FIG. 1.** In situ visualization of bacteria in colon tissue by immunohistochemistry using an anti-O75 serum and Vector red staining. Piglets were treated with FB1 (A), or left untreated (B), inoculated with E. coli strain 28CNaI, and euthanized 24 h postinoculation. (A) Bacteria, stained red on direct microscopic observation, were found in aggregates closely associated with the colon surface epithelium (arrow) and in the serosa (arrowhead). (B) Similar bacteria were occasionally found individually associated with the colon surface epithelium (arrow) but not in the serosa. Bar size, 100 μm.

To confirm that the increase in susceptibility of the pigs to E. coli infection was due to FB1, the experiment was repeated with purified mycotoxin (Table 2, experiment 2). As expected, greater intestinal colonization was observed in FB1-treated pigs than in the untreated animals. However, in this experiment, the bacteria translocated poorly to extraintestinal organs, and E. coli 28C was recovered only from the mesenteric lymph nodes of two out of five FB1-treated pigs.

Based on immunohistochemistry with Vector red, red-stained bacteria were often observed in the lumen and in close contact with the intestinal mucosal surface and in the serosa, mostly of the cecum and colon, of FB1-treated pigs (Fig. 1A). Similar red-stained bacteria were occasionally observed in the lumen, but only rarely were they in contact with the mucosa or in the serosa of the intestines of untreated animals (Fig. 1B).

**DISCUSSION**

Ingestion of FB1 increased intestinal colonization by E. coli strain 28CNaI. This bacterial strain can persist in the large intestine of pigs under normal conditions and can colonize the gut and translocate to internal organs when the immune system is compromised, e.g., in the absence of colostrum in conventional or germfree newborn pigs (J. M. Fairbrother, unpublished results). It is possible that a similar effect occurs in older animals when other agents, e.g., mycotoxins, affect the intestinal tract and/or the immune system. The low pathogenicity of this strain is also reflected by its poor ability to elicit an inflammatory response in the intestines, as demonstrated by the absence of an inflammatory cell infiltrate (Fig. 1), and of the induction of RNA encoding inflammatory cytokines (data not shown). Since strains of this pathotype are also recovered from patients with urinary tract infections, strain 28CNaI appears to typify opportunistic ExPEC organisms.

The dose of FB1 administered to pigs in our experiments (0.5 mg of FB1/kg of body weight, equivalent to 5 to 8 ppm in the feed) significantly increased the bacterial colonization of the intestine (Table 2); however, this dose did not induce
clinical or pathological changes and had no significant impact on weight gain. Using the same concentration of FB₁, but for a longer period of time (8 weeks), Rotter et al. (49) reported an 11% decrease in daily weight gain of pigs, and a 31% decrease in weight was observed in pigs fed a high dose (20 mg/kg of body weight) of FB₁, for 7 days (54). The toxic dose of FB₁ depends upon the animal species and parameters investigated. In pigs, changes in serum sphingolipids are detected at 5 ppm of FB₁, (46), liver damage occurs at 23 ppm (40), and pulmonary edema occurs at 175 ppm (40). Clinical chemistry profiles indicate that alkaline phosphatase is the most sensitive measure of fumonisin toxicity in pigs (20, 40, 46). The dose and the time exposure used in our study did not induce any change in serum biochemical parameters (data not shown) but did significantly increase bacterial colonization by pathogenic E. coli. Several researchers have described an alteration of biochemical values in pig serum, but these were obtained with higher doses of toxin (54), longer exposure (49, 60), or both (40, 59).

We found that FB₁ increases bacterial colonization in the intestines of piglets. Interestingly, the difference in bacterial colonization between the FB₁-treated and the control (untreated) pigs was greater in the first experiment than in the second one (Table 2). This result may be due to an unidentified compound present in the culture material that was acting synergistically with FB₁. Alternatively, the difference in the initial weights of the pigs might have had an impact. The administration of similar bacterial loads to pigs in the first experiment, who had lower body weights, and consequently smaller intestines than pigs in the second experiment, may have resulted in the presence in the intestinal lumen of a greater number of bacteria relative to the lumen size and thereby may have exacerbated the effect of FB₁ on intestinal colonization.

Several mycotoxins can alter the immune response and increase susceptibility to infectious disease (33, 42, 57), and sublethal concentrations of FB₁ decrease bacterial clearance after intravenous infections (29, 54). However, a recent paper (11) indicates that diets contaminated with 50 or 150 ppm of FB₁ enhance the resistance of mice to parasitic infection. There are a few reports on the influence of mycotoxins on intestinal colonization by pathogenic bacteria; however, none of these reports evaluated fumonisin. Fukata et al. (18) reported increased intestinal colonization by Salmonella enterica serovar Typhimurium in 11-day-old chickens fed high doses of ochratoxin A, although Kubena et al. (27), using the same model, observed no effects attributable to either aflatoxin or T-2 toxin.

FB₁ specifically inhibits ceramide synthase activity, resulting in the disruption of sphingolipid metabolism (35, 48). Sphingolipids and sphingoglycolipids are essential components of eukaryotic cell membranes, and these molecules may act as membrane receptors for bacteria (2, 5, 24) and bacterial toxins (30, 51). Thus, ingestion of FB₁ may induce sphingolipid changes in the gastrointestinal tract and modify bacterial receptors on the surfaces of epithelial cells. These changes may contribute to the increased colonization of the intestinal tract by pathogenic bacteria.

We used pigs in this study for at least three reasons. First, due to their maize-rich diet, pigs are potentially exposed to high levels of fumonisins. From a public health perspective, increased colonization of the pig intestine by potentially pathogenic E. coli following the ingestion of fumonisins may increase animal-to-human transmission of pathogens and/or increased antibiotic concentrations in meat as a consequence of animal treatment. Second, rodents are very resistant to most mycotoxins (25, 37) and are not available as models. Finally, pigs and humans have many biological similarities, especially with regard to the intestinal tract (1, 6, 43), which makes the pig a good model for the study of the consequences of ingestion of mycotoxin-contaminated food.

In conclusion, we found that exposure to FB₁ is a predisposing factor to infectious disease. Considering the high levels of FB₁ that may be present in animal feeds and human food preparations (41, 52, 53), further studies are needed to identify the mechanism(s) by which this mycotoxin acts on the intestinal tract to modulate colonization by opportunistic pathogens. Epidemiological studies are also needed to assess the extent to which fumonisins are involved in the development of infectious diseases in humans and animals.

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