Effects of Fumonisins on Immune Function in Swine


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Introduction

Fumonisins, mycotoxins found in corn and corn-based feed worldwide, can induce liver damage and, at high concentrations, fatal pulmonary edema in swine (Haschek et al., 1992; Motelin et al., 1994; review by Dutton, 1996). However, reports on the immune effects of fumonisins in swine are conflicting. Osweiler et al. (1993) found that pigs fed a diet containing 33 mg fumonisin/kg for 21 days had a decreased titer and lymphocyte blastogenic response to pseudorabies vaccination at 7 but not 14 days. Rotter et al. (1996) reported a dose-dependent decrease in the blastogenic response of lymphocytes isolated from pigs fed diets containing 0, 0.1, 1, or 10 µg fumonisin/kg diet for 8 weeks. However, lymphocytes isolated from sows fed diets containing 100 ppm fumonisin for 17 days did not respond differently from controls in the blastogenesis assay (Becker et al., 1995). We recently demonstrated that fumonisin ingestion by pigs decreases the clearance of particulate material and bacteria from the blood (Smith et al., 1996).

Fumonisins inhibit an enzyme in the biosynthetic pathway of sphingolipids causing dramatic alterations in the concentrations of sphinganine and sphingosine (Wang et al., 1991; Riley et al., 1994). Because sphingoid bases and complex sphingolipids have varied and complex roles in cell signaling pathways and are important components of the cell membrane, inhibition of sphingolipid biosynthesis has been implicated as a mechanism of fumonisin-induced toxicoses (Hannun and Bell, 1989; Merrill et al., 1996). Sphingosine inhibits protein kinase C which activates downstream signaling events that result in the production of the cytokine, tumor necrosis factor (TNF). This cytokine is produced by macrophages and helps to stimulate the immune response.

Therefore, we hypothesized that exposure of swine to fumonisin impairs both specific (B and T lymphocyte) and non specific (T lymphocyte, macrophage) immune function, and that inhibition of sphingolipid biosynthesis is the mechanism of this impairment.

Objectives

1. To examine the effects of fumonisins on phagocyte functions.
2. To determine if fumonisins affect the ability of phagocytes to orchestrate the immune response.
3. To examine the effects of fumonisins on specific immunity.
4. To determine the effect of fumonisins on sphingoid base concentration in immune cells and organs.
Methods and Results

Objective 1. To examine the effects of fumonisins on phagocyte functions. Pulmonary alveolar macrophages (PAMs) were obtained either from untreated pigs or pigs fed fumonisin-containing culture material at a dose of 15 mg fumonisin B₁/kg for 4-5 days or up to 7 mg/kg for 5 weeks. To examine bacterial attachment and killing, PAMs were incubated with opsonized Salmonella at 37°C for up to 2 hours.

Fumonisins exposure consistently inhibited the phagocytic activity of alveolar macrophages after in vivo exposure. Fumonisins inhibited phagocytic activity and bacterial adherence of PAMs, however, the rate of killing did not seem to be affected. The production of superoxide was not affected either. Inhibition of phagocytosis may increase susceptibility to infectious diseases and extend recovery from other diseases.

Objective 2. To determine if fumonisins affect the ability of phagocytes to orchestrate the immune response. Tumor necrosis factor (TNF) production and activity was determined in PAMs obtained either from untreated pigs or pigs fed fumonisin-containing culture material at a dose of 15 mg fumonisin B₁/kg for 4-5 days. PAMs were incubated with and without LPS and PMA for 12-15, 24, and 36 h and TNF production was determined by the standard WEHI killing bioassay (Wollenberg et al., 1993).

PAMs from treated pigs had decreased production of TNF as compared to similarly stimulated PAMs from control pigs. These data indicate that dietary exposure to fumonisins may inhibit the ability of swine to mount an immune response.

Objective 3. To examine the effects of fumonisins on specific immunity.

The response to vaccination was compared between control and fumonisins fed pigs to examine the effects of fumonisins on humoral and/or cell mediated immune responses to specific antigens. Pigs were fed fumonisin-containing culture material at a dose of up to 7 mg/kg for 5 weeks and were vaccinated with a killed pseudorabies virus (PrV) vaccine on weeks 1 and 3. Humoral response was determined by measuring the antibody titer to the vaccine antigen by serum neutralization (SN) at 0, 3, and 5 weeks. The cell mediated response was determined by the blastogenic response to vaccine antigens. [³H]Thymidine uptake after incubation with media, PHA (+ control), and PrV was measured. Changes in cell mediated immunity was further examined by quantifying the number of CD3, CD4, and CD8 positive T lymphocytes in the blood by flow cytometry to determine the numbers of T cells, T helper cells, and cytotoxic T cells.

There were no differences in SN titers to PrV at any time points. A PrV index showing a blastogenic response of PrV over media increased in treated pigs on Week 5. There were no major differences in the relative numbers of the different lymphocytes except at week 5 when there was a slight increase in the numbers of CD3 positive, CD8 positive, and double positive T cells in the treated pigs. These results indicate a potential slightly beneficial effect of fumonisins in enhancing the response to vaccination.
Objective 4. To determine the effect of fumonisins on sphingoid base concentration in immune cells and organs.

Neutrophils, monocytes, and alveolar macrophages were obtained either from untreated pigs or pigs fed fumonisin-containing culture material at a dose of 15 mg fumonisin B1/kg for 4-5 days. The concentrations of sphinganine and sphingosine were determined in these cells as well as the thymus and mesenteric lymph nodes by extraction and HPLC analysis as described by Riley et al. (1994).

Elevations in sphinganine and sphingosine were present in neutrophils, monocytes, and alveolar macrophages from fumonisin-treated pigs. Only monocytes and alveolar macrophages had an increased sphinganine to sphingosine ratio. The thymus and mesenteric lymph node had elevated sphinganine and sphingosine concentrations, and sphinganine to sphingosine ratio. Therefore fumonisin exposure increased the concentrations of sphingoid bases in both immune cells and organs.

Discussion

The effects of fumonisin exposure on specific and non specific aspects of the immune response were examined. Phagocytic activity was decreased in PAMs exposed to fumonisin in vivo. The bacterial killing assay was used because it more completely tests macrophage function, not only adhesion but also uptake and killing of bacteria. Fumonisin did decrease the ability of PAMs to attach and phagocytize opsonized Salmonella sp. in both the high and low dose in vivo studies. The rate of bacterial killing and superoxide production was not altered. This suggests a possible problem with Fc receptor synthesis or expression. Therefore some aspects of the pig’s immune defenses in organs as lungs, liver, gut, spleen, may be compromised with fumonisin exposure.

To mimic a field situation, pigs were exposed to a low dose of fumonisin and PrV vaccination was used to analyze the specific immune responses to this antigen. The blastogenesis studies showed that long term low dose fumonisin ingestion may slightly enhance the pig’s specific immunity in its ability to respond to vaccination. Similarly, flow cytometry showed slightly increased numbers of T lymphocytes, T helper cells, and double positive T cells, indicating that fumonisin treatment may better prepare the pig to mount a cellular immune response. Fumonisin consistently altered levels of sphinganine and sphingosine in neutrophils, monocytes, and alveolar macrophages, as well as in the thymus and lymph nodes. The significance of these alterations in affecting immune function is unclear but changes in the phagocytes may be limited to perturbations at the receptor level on plasma membranes. Any changes in the immune system may affect susceptibility to disease as well as the response to vaccination. These studies need to be repeated with particular emphasis on the ability of PAMs and neutrophils to adhere to and phagocytize bacteria.

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References


