FOOD SAFETY AND PREGNANCY: Investigating the Relationship Between Maize Mycotoxins and Birth Defects

Maize, or corn, is an important source of food in many countries, including Asia, Africa, and the Americas, and is an integral part of the culture and cuisine in Latin America. Fumonisins (FBs) are mycotoxins produced by Fusarium verticillioides, a common fungal contaminant of maize worldwide¹ (FIG. 1). An association between ingestion of fumonisin-contaminated maize during early pregnancy and increased risk for birth defects, specifically neural tube defects (NTDs), has been observed in communities in Guatemala, China, and the Transkei of South Africa, where maize is a dietary staple². In addition, a ‘cluster’ of babies born with NTDs was reported among Mexican-American women along the south Texas border in 1990, following a year in which extremely high levels of fumonisin (>70 ppm) were reported in the local maize crop that was used to make tortillas².³

Neural tube defects (NTDs) are common congenital malformations that occur when the embryonic neural tube, which ultimately forms the brain and spinal cord, fails to close properly during the first few weeks of development. Anencephaly, which is essentially the absence of the brain, is invariably fatal, and results from failure of the anterior neural tube to close properly, while spina bifida refers to incomplete closure of the posterior neural tube (FIG. 2). These defects occur in approximately 1 per 1000 liveborn infants. Including those pregnancies that are electively terminated, approximately 4000 pregnancies per year or 12 pregnancies per day in the U.S. are affected by a NTD. As such, they are among the most common of all human birth defects, yet their etiologic basis remains poorly understood. NTDs appear to be multifactorial in origin, involving complex interactions between environmental factors (i.e. natural toxins, chemicals, pharmaceuticals), genetic susceptibility, and maternal nutrition (i.e. folic acid). Our research focuses on the role of fumonisins as an environmental contaminant that may contribute significantly to NTD risk in populations that consume large amounts of maize-based foods.

The incidence of NTDs in Mexico and Guatemala is higher than in the United States, and NTDs are more frequent among recent Mexican immigrants than among immigrants with longer residency in the U.S. (FIG. 3). This trend suggests that an environmental factor, such as diet, may contribute to the higher incidence of NTDs in Mexico compared to recent and long-term Hispanic residents in the USA. A shift away from maize as a dietary staple among immigrants with longer residency in the U.S. may imply reduced exposure to the mycotoxin because maize is by far the food most likely to be contaminated with high levels of fumonisin. Evidence of dietary exposure to the mycotoxin is documented by a strong correlation between tortilla consumption and urinary fumonisin levels in women cohorts sampled from Morelos County, Mexico⁴. High levels of fumonisin have been found in tortillas, nixtamalized maize products, and Incaparina (high protein food supplement) sampled from various regions in Guatemala⁵,6,7, and, in a recent U.S. study, fumonisin was detected in all samples of corn tortillas and masa flour purchased from various retail sources in Los Angeles, San Diego, and Tijuana, Mexico⁸.

The fumonisin toxin is structurally similar to the natural sphingoid base dihydro-sphingosine that is used as a substrate by ceramide synthase in de novo sphingolipid biosynthesis (FIG. 4). Fumonisins inhibit the enzyme ceramide synthase, resulting in disruption of sphingolipid metabolism⁹,10. Dihydro-sphingosine accumulates upstream of the block, and downstream ceramides, which serve as precursors for more complex sphingolipids found in the nervous system (such as sphingomyelin and gangliosides), are depleted. The excess dihydro-sphingosine that accumulates can be phosphorylated to form dihydro sphingosine-1-phosphate (dHs1P), which then functions as a bioactive lipid ligand for a family of 5 G protein-coupled receptors known as ‘S1P’ receptors (S1P1-5). S1P receptors play important roles in cell signaling events, including cell survival, proliferation, and migration. S1P receptors are also critical mediators of lymphocyte trafficking, and are currently an area of intensified Pharma research as targets for the development of immunosuppressant drugs to treat autoimmune diseases such as multiple sclerosis¹¹.
expression of select S1P receptor isoforms are observed in the mouse neural tube during embryonic development, suggesting that they may play a role in neural tube closure and fetal brain development. In farm animals that consume maize-based feeds with fumonisin concentrations > 5 ppm, it is generally accepted that fumonisin disruption of sphingolipid metabolism is the cause of disease. Altered sphingolipid metabolism leads to hepato- and nephro-toxicity and carcinogenicity in laboratory rodents, leukoencephalomalacia in horses, and pulmonary edema in swine. In every animal, plant, and fungus that has been tested, fumonisins have been shown to inhibit ceramide synthase. It is therefore reasonable to suspect that humans consuming large amounts of maize frequently contaminated with fumonisins are also susceptible to diseases that arise from disrupted sphingolipid signaling, and that the developing fetus might be particularly sensitive. Based on the adverse health effects observed in animals, the FDA released a guidance document in 2001 "Guidance for Industry on Fumonisin Levels in Human Foods and Animal Feeds" stating that human health risks associated with exposure to fumonisins were possible, and, in 2002, the World Health Organization (WHO) released a provisional maximum tolerable daily intake (PMTDI) of 2 μg/kg body weight for fumonisins. However, in areas where maize is consumed in large amounts, such as Mexico and Guatemala, a significant percentage of the population greatly exceeds (>20 fold) the WHO PMTD. A critical barrier to translating what we know from animal studies to humans is that there has not yet been any data collected in humans to determine whether or not consumption of fumonisin-contaminated maize disrupts sphingolipid metabolism, and the critical threshold of fumonisin exposure necessary for sphingolipid disruption to occur.

Our laboratory has developed an in vivo mouse model of maternal fumonisin exposure that reliably produces a high percentage of NTDs in exposed embryos (FIG. 5). In the inbred LM/Bc mouse strain, early gestational exposure to fumonisin B1 (FB1) results in a dose-dependent increase in the percentage of embryos within a litter that have NTDs. Ongoing studies are investigating the role of S1P receptors in mediating placental development and signaling events involved in neural tube closure. In collaboration with the USDA Mycotoxin Research Unit in Athens, GA, we have been able to measure elevated levels of dihydro-sphingosine and dihydrosphingosine-1-phosphate (dhs1P) in blood spots collected from pregnant dams exposed to fumonisin, as well as in placental and embryonic tissues. Based on our initial findings in the mouse studies, a method to quantitate fumonisin in urine and sphingoid bases, sphingoid base 1-phosphates and complex sphingolipids and their sphingoid base backbone in human blood spots has been developed and validated in a pilot study in the U.S.

Preliminary field studies have identified several Guatemalan communities in which fumonisin exposures are likely to be significant. The NTD incidence in some of these communities is often as high as 6-10 per 1000 liveborn infants, yet little information is currently available regarding fumonisin exposures during early pregnancy and biomarkers.
of sphingolipid disruption. Climate stressed environments are those most conducive to fumonisin production in the field, and continuing drought and unfavorable weather events have adversely affected the 2009 and 2010 maize supply in Guatemala. Maize production in Guatemala is currently insufficient to meet demand, resulting in both increased prices and reduced quality in the maize supply sold in local markets. Levels of FB in maize sold in local markets can be dangerously high, and levels far exceeding those allowed in feeds for horses and pigs in the U.S. are quite common. In collaboration with scientists at CIENSA (Centro de Investigaciones en Nutricion y Salud) and the USDA Mycotoxin Research Unit, we will soon begin collecting human blood and urine samples in Guatemalan communities with low vs. high fumonisin levels in the local maize crop. Mass spectrometry will be used to assess human fumonisin exposure levels (urine) and effects on sphingolipid biomarkers of ceramide synthase inhibition (blood spots).

Our objective is to identify high vs. low exposure individuals using fumonisin in urine as an exposure biomarker, and further validate unique and quantifiable sphingolipid biomarkers in human blood spots that will establish the threshold of fumonisin necessary to disrupt sphingolipid metabolism in humans. Parallel studies in mice will establish the threshold of fumonisin exposure and sphingolipid disruption necessary to induce NTDs, and mechanistic studies in mice will further investigate perturbations in downstream signaling pathways that lead to failure of neural tube closure. The potential economic impact of having a child with a NTD is significant, not to mention the emotional burden placed on the family and the child faced with a lifelong disability. Our overall goal is to determine the exposure threshold for fumonisin disruption of sphingolipid metabolism in humans, and identify and validate sphingolipid biomarkers in blood spots that predict NTD risk in humans, such that we can prevent and/or reduce the high incidence of this type of birth defect in maize-based cultures.

REFERENCES

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