Critical Review

Fumonisins Disrupt Sphingolipid Metabolism, Folate Transport, and Neural Tube Development in Embryo Culture and In Vivo: A Potential Risk Factor for Human Neural Tube Defects among Populations Consuming Fumonisin-Contaminated Maize


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ABSTRACT Fumonisins are a family of toxic and carcinogenic mycotoxins produced by Fusarium verticillioides (formerly Fusarium moniliforme), a common fungal contaminant of maize. Fumonisins inhibit ceramide synthase, causing accumulation of bioactive intermediates of sphingolipid metabolism (sphinganine and other sphingoid bases and derivatives) as well as depletion of complex sphingolipids, which interferes with the function of some membrane proteins, including the folate-binding protein (human folate receptor α). Fumonisin causes neural tube and craniofacial defects in mouse embryos in culture. Many of these effects are prevented by supplemental folic acid. Recent studies in LMBc mice found that fumonisin exposure in utero increases the frequency of developmental defects and administration of folate or a complex sphingolipid is preventive. High incidences of neural tube defects (NTD) occur in some regions of the world where substantial consumption of fumonisins has been documented or plausibly suggested (Guatemala, South Africa, and China); furthermore, a recent study of NTD in border counties of Texas found a significant association between NTD and consumption of tortillas during the first trimester. Hence, we propose that fumonisins are potential risk factors for NTD, craniofacial anomalies, and other birth defects arising from neural crest cells because of their apparent interference with folate utilization. J. Nutr. 134: 711–716, 2004.

KEY WORDS: • fumonisins • neural tube defects • craniofacial abnormalities • sphingolipids • folate

Fumonisins are a family of mycotoxins that were first isolated in South Africa in 1988 from cultures of Fusarium verticillioides (formerly Fusarium moniliforme) strain MRC 826 (1), followed soon thereafter by elucidation of the structures of the prevalent isoforms fumonisin B₁ (FB₁)3 and B₂ (FB₂) (2). Leukencephalomalacia in horses and pulmonary edema syndrome in pigs (3) were shown to result from administration of FB₁ (4,5), and field outbreaks were associated with fumonisin contamination (6) when analytical methods were developed (7). Fumonisins were also implicated in esophageal cancer when they were found in home-grown maize in a high-inci-

3 Abbreviations used: AP 1, aminopentol or hydrolyzed fumonisin B₁; FB₁, fumonisin B₁; FB₂, fumonisin B₂; FB₃, fumonisin B₃; NTD, neural tube defects; OR, odds ratio.
Inhibition of folate transport

In the early 1990s, following investigation of an NTD cluster that occurred among Mexican-American women in Cameron County, Texas, Kate Hendrickson from the Texas Department of Health recommended that women avoid the possibility that exposure to fumonisin-contaminated food (tortillas) during gestation may have contributed to these birth defects [reviewed in (41)]. NTD are common congenital malformations that occur when the embryonic neural tube, which ultimately forms the brain and spinal cord, fails to properly close during the first few weeks of development. NTD are among the most common of all human birth defects, yet their etiologic basis and embryology remain poorly understood. Empirical risk figures, along with numerous clinical studies, indicate that NTD are of a multifactorial origin, having both genetic and environmental components (41–47). Epidemiological studies indicate that periconceptional vitamin supplements containing folic acid can significantly reduce (50–70%) a woman’s risk for an NTD affected pregnancy (48,49), and data from clinical trials support the hypothesis that this apparent reduction in risk may be specifically attributable to folic acid (50,51), although the mechanisms underlying the protective effects of folic acid are not fully understood.

A potential link among fumonisins, folate deficiency, and increased risk for NTD seemed plausible based on the research findings of Stevens and Tang and colleagues (52,53), who demonstrated that receptor-mediated folate uptake was reduced by up to 50% in Caco-2 cells pretreated with fumonisin. The placental, high-affinity folate transporter [folate binding protein 1, Folbp1(murine); folate receptor α (human)] is a glycosylphosphatidylinositol (GPI)-anchored protein (54) associated with membrane microdomains (rafts) enriched in cholesterol and sphingolipids (55), which was previously shown to be critical for early embryonic development (56). Fumonisin affects the transporter by altering both its endocytic and recycling pathways containing folic acid (57). The elevation in urinary sphinganine is reversible and subsides soon after the complete removal of fumonisins (although a supposedly subtoxic dose will maintain elevated sphinganine in rats and mice following exposure to a higher dose) (14,37,38). Extrapolation of these findings to humans is difficult, but disruption of sphingolipid metabolism has been associated with liver and kidney toxicity in nonhuman primates exposed to fumonisins (39). A recent study in China found that the free sphinganine to free sphingosine ratio was significantly greater in urine of males from households where the estimated daily FB1 intake was >110 μg/kg body weight per day (40).

Induction of developmental abnormalities in mouse models for neural tube defects

Despite this suggestive link between fumonisins and NTD, developmental defects were not reported in studies of rats (57), rabbits (33), and mice (58) exposed to FB1 orally throughout the period of neural tube closure, and the dosages where developmental effects (fetal toxicity) were seen are not known for NTD by disrupting folate utilization via depletion of cellular sphingolipids needed for normal receptor function.
FUMONISINS AND NEURAL TUBE DEFECTS

The incidence of NTD in FB₁ exposed litters following maternal folate supplementation was reduced from 79 to 43% (n = 10 litters). Since FB₁ may act via inhibition of the biosynthesis of sphingolipids that are required for the function of the folate receptor, and previous reports have shown colocalization of gangliosides with GPI-anchored membrane receptors (such as the folate receptor) (68–71), pregnant dams were administered ganglioside GM₁ (10 mg/kg body weight per day, i.p.) the day before, during, and after fumonisin administration (GD 6.5–9.5). This strategy reduced the incidence of NTD to only 5% (n = 11 litters), which is a significant protective intervention.

Incidence of neural tube defects in countries with consumption of fumonisin-contaminated maize

If fumonisin consumption is a risk factor for NTD in humans, this association might be most evident among populations that consume the highest amounts of maize, such as those of Central and South America and portions of southern Africa and Asia. For example, adults in Guatemala regularly consume several hundred grams of maize daily in the form of tortillas. In 1995 a survey of tortilla consumption and fumonisin amounts was conducted in households in Santa Maria de Jesus (Sacatepequez) and Patzicia (Chimaltenango), which are typical rural Kaqchikel-speaking Mayan communities of Guatemala, located in the Central Highlands (72). Tortillas and nixtamal, the base-treated corn flour that is used to prepare masa for tortillas, was obtained from 50 households in each town and analyzed for the major fumonisin species, FB₁ and FB₂.
AP$_1$. The latter is produced when the tricarballylic acids of FB$_1$ are base hydrolyzed and also inhibits ceramide synthase (73), although AP$_2$ is less toxic than FB$_1$ (32,74,75). The average fumonisin content (FB$_1$ + AP$_1$) of tortillas from Santa Maria de Jesús was 27 µg/g dry weight versus 8 µg/g from Patzicia. A high percentage (66%) of the tortillas from Santa Maria de Jesús (and over one third from Patzicia) contained ≥10 µg FB$_1$ + AP$_1$/g dry weight. Follow-up studies found that fumonisin contamination is highly variable: for the years 2000 to 2002, only 6 to 8% of the maize samples had >3.7 µg of total fumonisins/g dry weight (Torres, O. R. and Riley, R. T., unpublished data). Fumonisins were also reported in Mexican tortillas (76).

Little is known about the incidence of NTD in rural areas of Guatemala; however, a recent retrospective study of the prevalence of children born with NTD (77) reviewed clinical files of live newborns in national and regional hospitals of different departments of Guatemala (Geographical and Administrative Divisions of the country) with the following inclusion criterion: living newborns of either gender presenting with neural tube defects during the year 2000. Some regions have strikingly high frequencies (compared to that for the general U.S. population of ≤3/10,000 live births), such as 106 NTD/10,000 live births in Quetzaltenango, which has a mostly indigenous population that consumes high amounts of maize as their staple food (Fig. 3). The most frequent defect was myelomeningocele.

In South Africa, high NTD incidences have been found in a rural Transkei district in the Eastern Cape Province (61/10,000) (78) and in rural areas in the Limpopo Province (35/10,000) (79); in contrast, far lower incidence figures are reported in urban regions such as Cape Town (1.06/10,000) (80), Pretoria (0.99/10,000) (81), and Johannesburg (1.18/10,000) (82). High incidence rates (57 to 73/10,000) are also reported in rural areas in the northern provinces of China (83,84). The inhabitants of both the Transkei region and the northern provinces of China are likely to be exposed periodically to high fumonisins levels as a result of the consumption of fungal contaminated maize (85,86).

Neural tube defects along the Texas-Mexico border

Elevated NTD rates have been noted along the Texas-Mexico border (ranging from 27/10,000 live births in 1990–1991 to 15/10,000 live births in a usual year) (41). It is not clear why the NTD rate along the border is 3 to 5 times that observed elsewhere in the United States (41). A number of possibilities have been evaluated in a recent study (Missmer, S. A., Suarez, L., Felkner, M., Wang, E., Merrill, A. H., Rothman, K. J., and Hendricks, K. A., unpublished data) from March 1995 through May 2000, in which 184 Mexican-American women with NTD-affected pregnancies and 225 women with healthy live births were identified from residents delivering or terminating pregnancies in hospitals or birthing centers in any of the 14 Texas-Mexico border counties. Case and control women were interviewed in person about medical, occupational, environmental, and dietary exposures during the periconceptional period and maternal blood samples were collected. In addition, locally made tortillas were collected throughout the region.

After adjusting for body mass index, date of conception, and serum vitamin B-12, intermediate intake of homemade tortillas during the first trimester (301 to 400 tortillas, compared to low, ≤100) was associated with increased odds of an NTD-affected pregnancy (odds ratio, OR = 2.4; 95% CI = 1.1–5.3) and the association was higher (OR = 2.9, CI = 1.4–5.9) for consumption of any homemade tortillas compared to store purchased tortillas. Although the odds ratio was slightly increased (OR = 1.6, 95% CI = 0.5–5.1) for women with a biomarker for fumonisin exposure (the serum sphinganine-sphingosine ratio, Sa:So), 0.31–0.35 compared to those with Sa:So < 0.10, the confidence interval included 1. Paradoxically, the highest Sa:So ratios (>0.35) were not associated with increased NTD (OR = 0.3, 95% CI = 0.1–1.2). A similar bell-shape relation was observed for self-reported absolute number of tortillas eaten during the first trimester of pregnancy and for absolute micrograms per kilogram of woman’s weight per day of fumonisin exposure as estimated from the local tortilla samples. The observed bell-shape relation may reflect an increased prevalence of NTD at intermediate levels of exposure and an increase in fetal death at higher levels of exposure. Further analyses in humans are necessary to determine temporal associations and possible teratogenic thresholds.

The higher association with homemade tortillas is intriguing. The majority of FB$_1$ (up to 80%) is removed during nixtamalization and the commercial manufacture of fried tortilla chips (87,88), but less is known about the fate of fumonisins in homemade tortillas. A study of some of the smaller facilities in Cameron County, Texas, found that the methods used were similar to those in commercial production (88) but varied in process details, such as the use of lower calcium hydroxide in some recipes (De La Campa, R., Miller, J. D. and Hendricks, K., unpublished data).

**SUMMARY**

In summary, we suggest that fumonisin consumption is a risk factor for human neural tube defects (and related birth defects such as craniopharyngeal abnormalities), especially when there are other risk factors such as genetic susceptibility or limited availability of dietary folate. This hypothesis is based on current knowledge regarding the mechanism(s) of action of...
fumonisins as inhibitors of sphingolipid biosynthesis (and thereby of folate transport), recent reports that fumonisins induce developmental abnormalities in mouse embryos in culture and LMBC mice in utero, and the incidence of NTD in regions of the world where substantial consumption of maize and fumonisins has been documented or plausibly suggested. It would be prudent to monitor this possibility.

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LITERATURE CITED

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