Mycotoxins and birth defects

There are parts of the world where the risk of neural tube defects at birth are six to 10 times higher than average. Dr Janee Gelineau-van Waes describes the unique approaches her highly collaborative research project utilises to investigate the causes of this problem.

What are fumonisins and how do they contribute to birth defects – particularly neural tube defects (NTDs)?

Fumonisin B1 (FB1) is a mycotoxin produced by the fungus Fusarium verticillioides, a common contaminant of maize worldwide. An association between ingestion of FB1-contaminated maize during early pregnancy and increased risk of birth defects, specifically neural tube defects (NTDs) – of which the risk is six to 10 times higher than the global average in populations in Guatemala, China, Mexico, and the Transkei of South Africa, where maize is a crucial component of diets. Despite this, the environmental, nutritional, and genetic factors that contribute to the elevated incidence of birth defects in these communities are not known.

Fumonisin inhibits an enzyme called ceramide synthase in de novo sphingolipid biosynthesis. This results in significant elevation of the upstream substrate – sphinganine – and its phosphorylated metabolite, sphinganine-1-phosphate (Sa1P) in blood, tissues, and cells. It is this elevation of Sa1P that may contribute to NTDs.

What differentiates your project from previous studies that investigated the link between fumonisins and NTDs?

Other work in this area has looked at the association between tortilla consumption (assessed by a food frequency questionnaire) and incidence of neural tube defects within a community. Our study is the first to develop and validate internal biomarkers of exposure (detection of fumonisin in urine and elevated Sa1P in blood) and use them in an animal model.

The communities we work with are also unique, ethnically homogeneous and relatively immobile. They consume foods that are likely to contain high levels of FB1 and have diets that do not contain any form of folate fortification, which has shown to protect against FB1-NTDs. Furthermore, we make use of well-developed mouse models that differ in their genetic susceptibility to FB1-NTDs. These have allowed us to develop methods for measuring sphingolipid metabolites in blood spots, and will provide the basis for further in-depth mechanistic studies regarding the role of sphingolipid signalling pathways in neural tube closure.

How important is international collaboration in the advancement of your investigations?

International collaborations are vital for the success of this project. Of particular note is the work with the Centro de Investigaciones en Nutricion y Salud.
The fungal effect

In areas of the world where maize is a dietary staple, consumption of crops contaminated with the fungal toxin, fumonisin, has been linked with increases in the incidence of neural tube defects in newborns. An international project led by the Creighton University School of Medicine is investigating the mechanics of fumonisin toxicology.

What are the barriers in relation to translating existing knowledge into food safety practices and clinical care? How do you plan to overcome these?

The primary barriers will be political hurdles regarding the implementation and enforcement of regulatory policies limiting mycotoxins in foods. Providing scientifically-based evidence of an association between fumonisin exposure and human birth defects will (hopefully) stress the need for government intervention and better education of consumers.

What applications do you hope your research will have in the future?

If we establish that consumption of FB1-contaminated foods during early pregnancy results in increased risk for NTDs in humans, then our ultimate goal will be to reduce this type of birth defect through education, folate supplementation, mycotoxin screening, encouraging greater dietary diversity, and promoting changes in regulatory policy concerning food safety. Understanding the underlying mechanisms through which disruption of sphingolipid metabolism leads to NTDs will also be important for other applications. The development of drugs that target Sa1P receptors is currently an intense area of R&D for pharmaceutical companies, so an understanding of the molecular mechanisms involved in FB1-NTDs will also provide insight into potential risks for pregnant women who need to take prescription medications that impact sphingolipid metabolism.

MAIZE IS A large grain crop that is a staple part of the human diet in many populations throughout Africa, as well as Mexico and, South and Central America. In these areas, the maize crop is susceptible to infection by Fusarium verticillioides, a species of fungus that produces a mycotoxin called fumonisin B1 (FB1). Despite knowledge of animal-based diseases associated with FB1 toxicity, until 1990 there was little published evidence to suggest that this pathogen had an impact on human health. However, in that year, an unusually large number of babies were born with neural tube defects (NTDs) along the Mexican-American border in regions of southern Texas that had recently been plagued with a particularly severe fungus infection in the maize crop. This led scientists to suspect that FB1 could be affecting foetal development in humans. NTDs are one of the most common birth defects, occurring in about one in 1,000 live births in the US. They arise during the third to fourth week of human gestation when there is a failure of the neural tube to fully fold up and close. NTDs result in serious developmental disorders such as spina bifida and anencephaly.

Further to this localised incidence in Texas, increased occurrence of NTDs have also been observed in communities in Guatemala, China, Mexico and South Africa, areas where maize is grown and eaten as a staple food crop. Despite this apparent association between maize intake and foetal development, there is little known about links between prenatal FB1 exposure and increased risk for NTDs. To develop a greater understanding of this interaction, a highly collaborative and international research project, led by Dr Janee Gelineau-van Waes from the Creighton University School of Medicine, Nebraska, is utilising a multi-faceted and multidisciplinary approach to investigate this problem. The project was enabled after the award of a US $2.7 million grant from the National Institutes of Health to investigate a possible link between the ingestion of corn-based food products contaminated with the FB1 fungal toxin and increased risk of birth defects.

A MULTI-FACETED APPROACH

The specific approaches that the different groups in the collaboration are taking to tackle this problem are distinct but complementary. The first unknown under investigation is to determine the threshold level of FB1 consumption in humans that is necessary to have a detrimental effect on the metabolism of sphingolipids – which is thought to influence the incidence of birth defects. The mycotoxin FB1 inhibits an enzyme that is necessary for sphingolipid biosynthesis, causing the accumulation of a sphingolipid substrate (Sa1P) in the blood of animals.

This has two detrimental consequences in the cell which could theoretically induce birth defects. Dr Ronald T Riley and Dr Kenneth Voss from the USDA-ARS Toxicology and Mycotoxin Research Unit at the R B Russell Research Center in Georgia, USA, are validating the use of urinary and blood biomarkers to assess human exposure to fumonisin and fumonisin disruption of sphingolipid metabolism. The biomarkers are also used for investigating the effects of elevated Sa1P in animal and cell models. In these models, Sa1P binds to, and saturates, cell surface G protein-coupled receptors – thus interfering with normal signaling pathways, including those involved in neural tube closure. The second consequence of elevated Sa1P is its interference, inside the cell nucleus, with the action of histone deacetylases (HDACs) that affect the epigenetic regulation of gene expression. This work is being carried out through a collaboration between investigators at Creighton University, and Drs Allison Ashley-Koch and Simon Gregory at the Center for Human Genetics at the Duke University Medical Center in North Carolina. Previous work on HDAC inhibitors has already demonstrated that exposure to these substances during pregnancy can increase the risk of having a baby with an NTD.
To determine:

- Dietary ‘no observed effect’ and ‘lowest observed effect levels’ for fumonisin B1 (FB1) that are necessary for induction of neural tube defects
- FB1 dose-response threshold that results in an elevation in sphingolipid biomarkers in blood spots and correlation with urinary fumonisin levels
- Relationship between FB1 consumption, urinary FB1 (exposure biomarker) and changes in sphingolipids in blood spots (effect biomarker) in human populations consuming corn
- Underlying molecular mechanism(s) through which FB1 disruption of sphingolipid metabolism results in neural tube defects using susceptible and resistant mouse strains and strain-specific cell culture models
- Genetic variants that contribute to increased susceptibility to fumonisin-induced neural tube defects in different mouse strains

**KEY COLLABORATORS**

Allison Ashley-Koch, PhD and Simon Gregory, PhD, Center for Human Genetics, Duke University Medical Center, USA • Ronald T Riley, PhD and Kenneth Voss, PhD, USDA-ARS Toxicology and Mycotoxin Research Unit, R B Russell Research Center, USA • Olga Torres, MS and Jorge Matute, MS, Centro de Investigaciones en Nutrición y Salud (CIENSA), Guatemala

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Janee Gelineau-van Waes earned her doctor of veterinary medicine (DVM) degree in 1983 and her PhD in pharmacology and toxicology in 1996, both from Washington State University. She moved to Creighton University School of Medicine in 2009 where she is currently a tenured Associate Professor in the Department of Pharmacology.

To investigate these links, researchers analysed levels of FB1 in urine samples from women in local Guatemalan communities – as well as in the maize crops that form a staple part of their diet – and then compared this to levels of sphingolipid metabolites in blood spots in order to determine the threshold of fumonisin exposure necessary to disrupt sphingolipid metabolism. This work has been greatly facilitated by collaborators Olga Torres and Jorge Matute from the Centro de Investigaciones en Nutrición y Salud (CIENSA) in Guatemala, who recruit volunteers, administer food frequency questionnaires, and collect the urine and blood samples. To date, the studies carried out in Guatemala have shown that increased levels of urinary fumonisin are correlated with changes in the ratio of sphinganine-1-phosphate (Sa1P) to sphingosine-1-phosphate (S1P) in blood, a result consistent with the hypothesis that fumonisin exposure causes disruption of sphingolipid metabolism in humans. Disruption of sphingolipid metabolism in the mouse model is a necessary prerequisite for fumonisin-induced NTDs.

The Duke University group is also working to identify differences between individuals that affect their susceptibility to the occurrence of NTDs; particularly those differences related to gene expression profiles and genetic variation in sphingolipid pathway genes. “We have already begun extracting RNA and DNA from blood samples obtained from Guatemalan subjects to carry out genotyping of single nucleotide polymorphisms (SNPs) and, in the coming year, we will evaluate gene expression levels by measuring RNA,” the group reveal.

**BIOMARKERS FOR NTDs**

The final aim of the project is to identify the mechanism by which changes in sphingolipid metabolism result in a failure of neural tube closure. Gelineau-van Waes explains: “The objective is to use established animal models to identify biomarkers that predict FB1 exposures associated with increased risk of foetal malformations”. These biomarkers are used in conjunction with mouse models of varied susceptibility to FB1-induced NTDs to establish the processes which underlie the failure of neural tube closure. The project has so far succeeded in categorising a number of these biomarkers that are common to the mouse models and are also identifiable in human blood samples. This enables *in vivo* and *in vitro* methods for the identification of changes in sphingolipid metabolism that are uniquely linked to NTDs and present a valuable tool for studies that aim to discover the levels of FB1 needed to have an effect on NTD incidence.

**KNOWLEDGE TRANSLATION**

Once the association between FB1 and birth defects is more fully understood, this knowledge must be translated to the field to prevent contamination of the human food supply with this fungal toxin. The primary focus is the education of local populations about the dangers of consuming contaminated crops and the importance of nixtamalisation, a method of processing corn that reduces the presence of mycotoxins. By building relationships with local communities, workers at CIENSA are well-placed to help with education to inform people about the risks associated with consuming infected corn and the importance of taking folate supplements to help combat levels of the toxin in food products.

**LOOKING FORWARD**

The interdisciplinary and collaborative nature of this project is necessary to fully understand this multifactorial etiology of NTDs. The project has successfully identified and verified appropriate biomarkers of FB1 exposure for mice and humans and, with the help of CIENSA, identified independent communities in Guatemala that are particularly high and low risk of NTDs because of their intake of contaminated maize. Looking to the future, Gelineau-van Waes suggests: “The next logical step would be to conduct a prospective cohort study with around 100,000 women of child-bearing age, and perform longitudinal sampling of urinary FB1 and blood levels of Sa1P at regular intervals in order to ‘capture’ exposure data during early pregnancy and then relate this to pregnancy outcome”.

However, due to insufficient funding and the practicalities and logistics of carrying out this study in developing communities, this is not feasible. As a compromise, the team is designing a case-control study in high and low FB1 exposure areas of Guatemala to carry out monthly analysis of urinary FB1 levels, biomarker surveillance and monitoring of NTD incidence.