

# Fetal and neonatal toxicities of aflatoxins and fumonisins

Studies of early-life effects of exposure to mycotoxins in experimental animals may provide insight into relevant mechanisms of action that could contribute to short- and long-term toxicities in exposed infants in human populations. Some relevant observations are summarized here.

## Aflatoxins

Fetal toxicity of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) has been reported in rats and mice. Toxic effects include decreased fetal weight, external and skeletal malformations, and neural tube defects (NTDs) (IARC, 1993). Developmental NTDs are relatively common birth defects that result from the failure of the neural tube to close properly (Wilde et al., 2014). In humans, the neural tube closes within the first 30 days of gestation, and in mice within the first 9 days.

NTDs have been reported in rat embryos exposed to AFB<sub>1</sub> in vitro (IARC, 1993). Treatment of pregnant rats with AFB<sub>1</sub> resulted in the formation of benign and malignant tumours in the liver, stomach, intestine, endocrine organs, and central and peripheral nervous system in the offspring.

Intraperitoneal AFB<sub>1</sub> treatment of mice during pregnancy produced retardation in fetal development, including cleft palate and diaphragmatic malformation. Carcinogenesis in adult mice mainly targeted the lungs, whereas in infant mice high incidences of liver cell tumours were produced (IARC, 2002). The 20-fold lower level of DNA adducts in adult mice compared with neonatal mice is reflected in the lower incidence of hepatocellular carcinoma (HCC), primarily related to differences in AFB metabolism and

the resulting generation of DNA-reactive intermediates (IARC, 2002; Shupe and Sell, 2004).

Studies in newborn male and female transgenic mice that examined mutations in target genes showed no differences in mutation level between sexes (Woo et al., 2011; Wattanawaraporn et al., 2012). As female mice have a much lower incidence of HCC, sex differences related to inflammatory responses, cytokine expression, and sex hormones could be responsible for the differences in tumour outcome. Sex differences in the occurrence of human HCC exist, and the effect of host responsive factors related to AFB and hepatitis B virus interactions and the differential role of metabolism, oxidative, and inflammatory parameters have been suggested as possible explanatory reasons (Wild and Montesano, 2009).

## Fumonisin

Dietary folate sufficiency plays a crucial role in reducing NTD incidence in humans (Wilde et al., 2014). In areas of the world where maize is a dietary staple and where there is chronic fumonisin exposure, NTD rates are often very high compared with countries where maize consumption is low (Marasas et al., 2004; Gelineau-van Waes et al., 2009). Wilde et al. (2014) noted that in blocking folate transport, fumonisin was a plausible risk factor for NTDs. In 2012, the Joint WHO/FAO Expert Committee on Food Additives (JECFA) evaluated the existing human epidemiological studies linking fumonisin exposure to NTDs and concluded that the results, in combination with what is known about the toxicology of fumonisin, “indicate that fumonisin exposure in pregnant women may be a contributing factor to increased NTD risk in their babies” (Bulder et al., 2012).

Mechanistically, a case can be made for fumonisin intake as a risk factor since fumonisin inhibition of ceramide synthases disrupts the function of sphingolipid-dependent processes and signalling pathways necessary for normal neural tube closure. For example, studies in fumonisin-treated cells, mouse embryos, and mice *in vivo* show that folate transport is inhibited as a result of alterations in the membrane biophysical properties induced by inhibition of the biosynthesis of complex sphingolipids (Sadler et al., 2002; Marasas et al., 2004). In mice, NTD incidence induced by intraperitoneal exposure on gestation days 7.5 and 8.5 was significantly reduced by folate supplementation and almost completely prevented by restoration of lipid raft function through the administration of ganglioside GM1 (Gelineau-van Waes et al., 2005). At this gestational time

point, the chorion (from the maternal side) and allantois (from the embryonic side) are still in the process of fusing, initiating formation of the mature placenta. Radiolabelled fumonisin B<sub>1</sub> (FB<sub>1</sub>) crossed the developing placenta, resulting in accumulation of free sphingoid bases in the placenta and embryos, a finding indicative of fumonisin inhibition of ceramide synthase in the developing embryo. Both the NTD incidence in the mice and the degree of disruption of sphingolipid metabolism were strain-dependent, indicating a possible genetic linkage between NTD induction and disruption of sphingolipid metabolism (Gelineau-van Waes et al., 2005).

Subsequent studies showed that elevated levels of sphingoid base 1-phosphates could also be detected in the livers of fetuses from pregnant mice fed diets containing FB<sub>1</sub> (Riley et al., 2006). The levels of sphinganine 1-phosphate in the fetuses from the NTD-susceptible mouse strain were significantly higher than in the resistant strain. More recent studies in mice have shown that the sphingoid base analogue FTY720 can also induce high incidences of NTD in the susceptible mouse strain after oral exposure during the window of neural tube closure (gestation day 6.5–8.5). Both free sphinganine and FTY720 are phosphorylated by sphingosine kinase to form sphinganine 1-phosphate and FTY720 1-phosphate, which can accumulate to very high levels in maternal blood and placenta of mice of the susceptible strain treated with FB<sub>1</sub> and FTY720, respectively (Gelineau-van Waes et al., 2012). In pregnant mice dosed with FTY720, both FTY720 and FTY720 1-phosphate were shown to accumulate in exencephalic embryos examined on gestation day 9.5 (Gelineau-van Waes et al., 2012). The results provide proof in

principle that, in addition to inhibition of folate transport, sphingoid base 1-phosphates also play an important role in NTD induction in fumonisin-treated mice.

Human embryonic stem cell-derived neural epithelial progenitor cells treated with FB<sub>1</sub> *in vitro* accumulate both free sphingoid bases and sphinganine 1-phosphate, which were shown to disrupt signalling pathways in these human cells (Callihan et al., 2012). Fumonisin inhibition of ceramide synthase has been shown in other human cells in primary culture (human umbilical vein endothelial cells and epidermal keratinocytes). Thus, fumonisin is an inhibitor of ceramide synthase in human cells *in vitro*, and (as in mouse *in vivo*) the accumulated sphinganine can be metabolized to the highly bioactive sphinganine 1-phosphate.

Taken together, the data from the mouse studies *in vivo* and studies with human cells *in vitro* support the hypothesis that if the fumonisin enters the developing embryo it will inhibit ceramide synthase and has the potential to disrupt sphingolipid metabolism. Alternatively, bioactive sphingoid bases and sphingoid base 1-phosphates, which are present at very high concentrations in the blood, could cross the placenta or act indirectly on the vasculature to cause changes in the developing embryo.

Many feeding studies in farm and laboratory animals have documented the dose-dependent relationship between fumonisin exposure and the tissue and blood levels of key sphingolipids known to regulate physiological processes and signalling systems that are essential for the animals' health (Marasas et al., 2004). Many of the processes potentially affected by altered levels of bioactive sphingolipids are also critical for the health of the mother,

developing fetus, neonate, and litter. For example, complex sphingolipids and sphingolipid metabolites are critical for intestinal nutrient uptake (Jennemann and Gröne, 2013), insulin and insulin-like growth factor 1 receptor (IGF-1R) signalling (Martin et al., 2009; Park et al., 2014), lymphocyte trafficking (Pappu et al., 2007), blood–brain barrier and vascular endothelial integrity (Cannon et al., 2012; Cruz-Orengo et al., 2014), and histone acetylation (Hait et al., 2009), among others.

### **In utero exposure to fumonisin in humans: scientific gaps and research needs**

As noted above, in areas of the world where maize is a dietary staple and where there is chronic fumonisin exposure, NTD rates are often very high. For example, in South Africa, high NTD incidence has been reported in parts of rural Transkei (61/10 000) and in rural areas in Limpopo Province (35/10 000). In contrast, the incidence is much lower in urban communities such as Cape Town (1.06/10 000), Pretoria (0.99/10 000), and Johannesburg (1.18/10 000) (Marasas et al., 2004). The difficulties of accurately capturing population-based rates for NTDs, particularly in low-income countries, make these assessments complicated in regions where there is high fumonisin exposure.

There are many gaps in the understanding of the in utero exposure to fumonisins and possible

effects on child health. Studies in mice have revealed that NTDs result from exposure very early in fetal development. At the moment there are no human data demonstrating the ability of fumonisin to cross the emerging human placenta as is the case in mice. It is unlikely that fumonisin would be detectable in umbilical cord blood, given the very small amount of fumonisin that has been detected in animal blood after exposure to relatively high levels of fumonisin (Riley and Voss, 2006; Bulder et al., 2012) and the rapidity with which FB<sub>1</sub> is cleared from human urine (Riley et al., 2012), suggesting that the half-life in the human body is very short.

Although it provides evidence for fumonisin inhibition of ceramide synthase, a shortcoming of using elevated sphinganine 1-phosphate in blood as a biomarker is that it will work well only when high and low fumonisin exposure groups are compared, based on concurrent comparison with the urinary FB<sub>1</sub> levels.

Progress in developing a better understanding of the potential for in utero exposure to either fumonisin or bioactive sphingolipid metabolites in humans is dependent on the discovery of new biomarkers that have a longer half-life or reflect long-term exposure. The half-life of sphingoid base 1-phosphates in human blood is likely to be short, based on the half-life of FTY720 1-phosphate and sphingoid base 1-phosphates in mouse blood (Gelineau-van Waes et al., 2012; Riley et al., 2015).

Studies in rats and mice show that the half-life of elevated free sphinganine and sphinganine 1-phosphate is longer than that of FB<sub>1</sub> in the blood or urine; however, it is still elevated for only a few days to a week before returning to control levels (Bulder et al., 2012; Riley et al., 2015).

Fumonisin has been shown to inhibit folate transport in animal models. Folate supplementation has been shown repeatedly to reduce NTD incidence in humans. Thus, studies to assess folate, vitamin, and micronutrient sufficiency in populations consuming maize as a dietary staple are needed to better inform the design of educational approaches to improve the nutritional status of women. This information will also be useful for designing approaches to allow supplementation at either an individual or a community level.

Equally unknown are the possible consequences of fumonisin exposure or exposure to elevated sphingoid base 1-phosphates in utero on child health in early infancy or later in life. Studies on the susceptible mouse model have identified several molecular markers and targets in embryos from fumonisin-treated mice, but their relevance to human exposure is unknown.

In areas where maize is a dietary staple, future studies intended to reveal any possible linkage between maternal fumonisin exposure and reproductive toxicity and growth impairment in children will need to consider the possibility of co-exposure to other mycotoxins, and in particular aflatoxin.