TOXICOLOGICAL HIGHLIGHT

A New Perspective on Deoxynivalenol and Growth Suppression

Kenneth A. Voss

Toxicology and Mycotoxin Research Unit, United States Department of Agriculture, Agricultural Research Service, Athens, Georgia 30604-5677

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Deoxynivalenol (DON) is a secondary metabolite of Fusarium culmorum and Fusarium graminearum and is one of the most common mycotoxin contaminants of wheat, barley, maize, and cereal-based food products (Canady et al., 2001; Schothorst and van Egmond, 2004). Like other trichothecenes, DON induces a spectrum of effects in farm and laboratory animals including emesis (DON has the trivial name “vomitoxin” because high doses cause emesis in swine), immunotoxic effects, and suppression of appetite and growth. A dietary no-observed effect level (NOEL) of 1 ppm DON was established in a 2-year mouse feeding study (Iverson et al., 1995). The NOEL corresponded to an average daily intake of 100 µg DON/kg body weight. The critical finding for establishing the NOEL was growth suppression, which occurred in both sexes at dietary concentrations ≥ 5 ppm DON and could not be explained on the basis of decreased feed consumption. A provisional maximum tolerated daily intake (PMTDI) for DON of 1 µg/kg body weight was subsequently established by the World Health Organization Joint Expert Committee on Food Additives on the basis of the NOEL (Canady et al., 2001). Surveys in Europe (Schothorst and van Egmond, 2004; Turner et al., 2008) have revealed that DON intake by cereal consumers, while mostly well below the PMTDI, sometimes approach or exceed the PMTDI in some segments of the population, including young children. It is therefore important to understand the mechanisms by which DON suppresses growth in animal models, particularly at low doses that do not cause inappetence and to determine its relevance to humans. The experimental approach and findings reported by Amuzie et al. (2009) in this issue offers significant new insight about the mechanisms contributing to growth inhibition by DON in a critical animal model. Specifically, they have now shown that DON suppresses growth in mice by reducing growth hormone (GH) signaling through mechanisms mediated by insulin-like growth factor 1 (IGF1) and insulin-like growth factor acid-labile substance (IGFALS). This report is particularly interesting because the findings for the first time provide evidence for a mechanism of growth inhibition that links the well-established role of DON as a modulator of cytokine expression with disruption of GH signaling.

IGFALS AND GROWTH

Regulation of growth is complex, and the interrelated roles of IGFALS, IGF1, insulin-like growth factor binding protein 3, (IGFBP3), and GH have been thoroughly reviewed elsewhere (Domene et al., 2009). Briefly, GH upregulates IGFALS and other genes involved in growth signaling according to the following sequence of events: GH binding to hepatic GH receptors; recruitment of Janus-family tyrosine kinase 2, activation of mitogen-activated protein kinases (MAPKs), and other kinases; and recruitment and activation of signal transducer and activator of transcription (STATs) proteins. STATs are translocated to the nucleus and upregulate gene expression including the expression of genes governing the synthesis of IGFALS, IGF1, and IGFBP3 proteins. IGF1 and IGFBP3 circulate in plasma as a binary complex to which IGFALS binds to form a more stable IGF1-IGFBP3-IGFALS ternary complex. The end result of IGFALS binding is promotion of growth by prolongation of the circulating half-life of the IGF1-IGFBP3 (Domene et al., 2009; Ueki et al., 2000). Negative regulation of the sequence occurs upstream at the level of the GH receptor complex and is mediated by members of the suppressors of cytokine signaling (SOCS) family (Flores-Morales et al., 2006).

The importance of IGFALS for growth has been elucidated with the help of transgenic mouse models. No circulating IGFALS was found in mice having an inactivated IGFALS gene (IGFALS−/−); however, compared to wild-type mice (IGFALS+/+), the homozygous IGFALS null animals exhibited mildly to moderately suppressed growth (Ueki et al., 2000). Growth suppression began 3 weeks after birth, and by
10 weeks, body weights of the homozygous null animals were reduced up to 20%. Impaired growth in heterozygous mice (IGFALS$^{+/−}$) was also observed but was of later onset (after 6 weeks postpartum) and of minimal severity (about 4% reduction). IGFALS gene mutations have been found in a limited number of humans, and these cases have been reviewed by Domené et al. (2009). In the pool of patients ($n = 17$), most of them were male, postnatal growth was mildly reduced, the onset of puberty was delayed, and serum IGFALS and IGF1 protein levels were negligible. Together, these data suggest that IGFALS is not required for growth, but its absence leads to slight to modest growth impairment similar to that observed in both DON-exposed animals (Canady et al., 2001; Iverson et al., 1995) and humans (Domené et al., 2009).

**DON, CYTOKINES, AND IGFALS**

DON causes the ribotoxic stress response in mice, and as a result, there is activation of MAPKs and upregulation of proinflammatory cytokines. Among the latter are interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), both of which have been shown to exert a negative influence on weight gain when overexpressed (De Benedetti et al., 1997; Probert et al., 1996). In addition, proinflammatory cytokine induction upregulates the expression of SOCS. SOCS proteins negatively modulate cytokine signaling and have been shown to inhibit GH-induced gene expression in liver (reviewed by Flores-Morales et al., 2006). Therefore, SOCS could be upregulated and subsequently have a negative impact growth in DON-exposed animals. Amuzie et al. (2009) recently demonstrated that DON did increase expression of SOCS mRNAs, including those for SOCS1, SOCS2, SOCS3, and cytokine-inducible SH2 (CIS) domain protein in a dose-dependent and transient (although SOCS3 remained elevated longer than the other SOCS proteins) manner. SOCS upregulation occurred together with or shortly after increases in TNF-α and IL-6 mRNA and protein expression were observed. They also established in their earlier study that hepatic IGFALS mRNA expression was suppressed in mice treated with DON, thus providing an important clue suggesting that there is a link between DON-induced upregulation of proinflammatory cytokine signaling and suppressed growth through inhibition of IGF1 and IGFALS.

**ESTABLISHING A CONNECTION: DON, IGFALS, AND GROWTH SUPPRESSION**

The experiments reported in this month’s article advance this concept significantly by demonstrating that as hypothesized, IGF1 and IGFALS are indeed reduced by acute DON exposure. Furthermore, DON suppressed the expression of IGFALS mRNA in the liver of the GH-treated mice but did not suppress mRNA expression of the other ternary complex partners, IGF1 and IGFBP3. SOCS mRNA expression was concurrently upregulated by DON in both GH-pretreated and GH-untreated mice. The authors also report that both growth and plasma IGFALS levels were significantly reduced in mice fed DON over 8 weeks, thus showing the applicability of their model to the more “real-world relevant” situation in which long-term, low-level exposure occurs. Many questions remain and further experiments to fully understand how DON impairs growth are needed. To what extent does decreased plasma IGFALS destabilize the IGF1-IGFBP3 binary complex and impair IGF1 signaling in DON-exposed mice? Is reduction of IGFALS sufficient to impair growth or is the involvement of other mechanisms such as DON perturbation of serotonin-dependent signaling pathways (reviewed in Canady et al., 2001) also required? The findings of Amuzie et al. (2009) are, nevertheless, a novel and significant breakthrough in mycotoxin research in that they establish a plausible cytokine-mediated mechanism for DON growth inhibition that is relevant to both laboratory models and humans.

An equally important implication of this work is IGFALS’ potential as a mechanism-based biomarker. As mentioned, DON intake by consumers of popular cereal products sometimes exceeds the PMTDI, and average exposures in young children in Europe approach the PMTDI (Canady et al., 2001; Schothorst and van Egmond, 2004). The human health implications of this finding remain uncertain as determining if long-term, low-level mycotoxin exposures are unique causes or risk factors for (often) subtle adverse effects in humans, such as mild to moderate growth inhibition, is a difficult task. A coordinated approach involving laboratory animal and epidemiological studies is required to address this challenge, and biomarkers play a central role in this process. In the case of DON, urinary (Turner et al., 2008) or serum mycotoxin concentrations are useful biomarkers of exposure. Development of a companion mechanism-based biomarker of effect for DON will greatly enhance our capability to determine the relevance of suppressed growth in mice (Iverson et al., 1995) as the most relevant adverse effect for risk assessment and, if warranted, to develop a more accurate PMTDI for infants and adults. Plasma IGFALS concentration is now a strong candidate to fill that much needed role.

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**REFERENCES**


