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Lupus-prone NZBWF1/J mice, defective in cytokine signaling, are resistant to fumonisin hepatotoxicity despite accumulation of liver sphinganine

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Abstract

Fumonisin B_1 (FB_1) is a mycotoxin produced by *Fusarium verticillioides*, commonly present in corn and other cereals. Exposure to FB_1 causes organ-specific diseases in various species, e.g., equine leukoencephalomalacia and porcine pulmonary edema; in mice the response is hepatotoxicity. We earlier reported that ceramide synthase inhibition by FB_1, the initial biochemical effect of this mycotoxin, results in modulation of cytokine network in response to accumulated free sphingoid bases. In the current study we used NZB/NZW-F1 (NZBW) mice that have modified cytokine expression and develop lupus beginning at 5 months of age. The NZBW and C57BL/6J (CBL) mice (appropriate control) were given five daily subcutaneous injections of either saline or 2.25 mg FB_1/kg/day and euthanized 24 h after the last treatment. Peripheral leukocyte counts were higher after exposure to FB_1 in CBL but not in NZBW. FB_1 treatment caused increases of plasma alanine aminotransferase and aspartate aminotransferase activity in CBL mice indicating hepatotoxicity; no elevation of circulating liver enzymes was recorded in NZBW mice. Hepatotoxic responses were confirmed by microscopic evaluation of apoptotic cells. The FB_1-induced proliferation of cells observed in CBL strain was abolished in NZBW animals. The sphinganine accumulation in liver after FB_1 was equal in both strains of mice. The NZBW strain lacked the FB_1-induced increases in the expression of liver tumor necrosis factor α, interferon γ, receptor interacting protein (RIP), and tumor necrosis factor α-related apoptosis-inducing ligand (TRAIL), observed in CBL. Results confirmed our hypothesis that initial altered sphingolipid metabolism caused by FB_1 leads to perturbation of liver cytokine network and ultimate cellular injury; the mice deficient in cytokine signaling are refractory to FB_1 hepatotoxicity.

Keywords: Fumonisin B_1; Lupus mice; Sphinganine; Cytokines; Hepatotoxicity

1. Introduction

Interest in fumonisins, mycotoxins produced by *Fusarium verticillioides*, has increased because of

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their widespread prevalence in corn and corn products (WHO, 2000; Bolger et al., 2001). Fumonisin B_1 (FB_1), a congener of sphinganine, is one of the most potent and abundant member of this class and causes a variety of species-specific toxicological effects in domestic and laboratory animals (WHO, 2000; Bolger et al., 2001). It is a known cause of field outbreaks of porcine pulmonary edema (PPE) and equine leukoencephalomalacia (WHO, 2000; Bolger et al., 2001; Marasas, 2001). This mycotoxin is hepatocarcinogenic in male BDIX rats and female $B6C3F_1$ mice and a renal carcinogen in male F344/N rats (IARC, 2002). In all species tested it is hepatotoxic and in many nephrotoxic (Sharma et al., 1997). It has been implicated as a contributing factor in human esophageal cancer (Sydenham et al., 1990) and in primary liver cancer (Ueno et al., 1997).

The structural similarity of fumonisins with sphinganine led to the discovery of the initial biochemical effect of this group of mycotoxins, i.e., inhibition of de novo sphingolipid biosynthesis (Wang et al., 1991). FB_1 inhibition of ceramide synthase (sphinganine and sphingosine-*N*-acetyl transferase) leads to accumulation of sphingoid bases, sphingoid base metabolites, and depletion of more complex sphingolipids (Riley et al., 1996). Although the early effects of FB_1 (increased sphingoid bases and sphingoid base 1-phosphates or decreased complex sphingolipids) have been correlated with toxicity both in vitro and in vivo, how disruption of sphingolipid metabolism leads to cellular death is not clear. The downstream toxic mechanisms involved with fumonisins may be complex and involve several molecular sites. Cells exposed to FB_1 in vitro and in vivo undergo a mixture of necrotic and apoptotic cell death (Tolleson et al., 1996). We have previously demonstrated increased apoptosis in liver and kidney of mice after a short-term treatment with FB_1 (Sharma et al., 1997). The hepatopathy and nephropathy were closely correlated with the disruption of sphingolipid metabolism (Tsunoda et al., 1998).

Cell signaling by small lipid molecules has become a subject of intense research interest recently as different lipid messengers have been reported to possess survival as well as lethal signals (Maceyka et al., 2002). Ceramide, an important sphingolipid, produced by *N*-acylation of sphinganine and subsequent desaturation, or by hydrolysis of sphingomyelin, is known to induce apoptosis in cells (Pettus et al., 2002). However, the involvement of ceramide in the initiation or inhibition of the FB_1-induced apoptotic process is unclear (Tsunoda et al., 1998). The toxic property of ceramide is likely shared by sphingosine or possibly even sphinganine (Le Stunff et al., 2002). Because sphinganine is an intermediate in the de novo sphingolipid biosynthetic pathway, it is present in healthy cells at very low levels unless enzymes downstream, such as ceramide synthase, are blocked. FB_1 inhibition of ceramide synthase causes sphinganine levels to increase from picomolar to nanomolar or micromolar within a few hours (Yoo et al., 1996). Depending on the cell type, sphinganine-dependent cell death or cell proliferation is common outcome in cultured cells (He et al., 2001; Kim et al., 2001; Riley et al., 1999; Schmelz et al., 1998; Schroeder et al., 1994; Tolleson et al., 1999; Yu et al., 2001). The involvement of sphinganine as a cause of increased cell death or proliferation is verified by the fact that inhibitors of the first enzyme in the de novo sphingolipid biosynthetic pathway reduce sphinganine levels and prevent or reduce FB-induced cell death and proliferative effects (He et al., 2001). While free sphinganine is clearly involved in the cellular effects of fumonisins, decreased ceramide biosynthesis, elevated levels of sphingoid base 1-phosphates; decreased levels of glucosyl ceramide and decreased lipid raft associated glycosylphosphotidylinositol anchored membrane proteins are all potential contributors to the cellular consequences of disrupted sphingolipid metabolism (He et al., 2001; Kim et al., 2001; Merrill et al., 2001; Riley et al., 2001).

Our laboratory reported that tumor necrosis factor α (TNFα) is an important intermediary in the toxic responses to FB_1 in mice. In our earlier studies (Dugyala et al., 1998), we showed that (i) peritoneal macrophages derived from mice treated with FB_1 produce higher amounts of TNFα when stimulated by the mitogen, lipopolysaccharide (LPS), compared with controls, (ii) murine macrophage cells (J744A.1) treated in vitro with FB_1 produce TNFα, and (iii) acute in vivo hematological effects of FB_1 in mice are partially reversed by anti-TNFα antibodies. These observations strongly implied that TNFα was a potential mediator of fumonisin toxicity. Later we confirmed that FB_1-treated mice have increased expression of inflammatory cytokines in liver and kidney (Bhandari and Sharma, 2002) and mice lacking either of the TNFα receptors (TNFR-1 or TNFR-2) have decreased hepatotoxic responses to FB_1 (Sharma et al., 2001, 2000b).