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Myriocin prevents fumonisin B_1-induced sphingoid base accumulation in mice liver without ameliorating hepatotoxicity

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Abstract

Fumonisin B_1 (FB_1), a mycotoxin produced by *Fusarium verticillioides* present on corn and corn-based products, causes species- and organ-specific diseases. The hepatotoxic effects of FB_1 in mice have been closely correlated with the accumulation of free sphinganine, a marker for ceramide synthase inhibition, and reduced biosynthesis of more complex sphingolipids. It has been shown that FB_1 modulates expression of many cell signaling factors. In the current study we used myriocin, a specific inhibitor of serine palmitoyltransferase, to investigate the role of free sphinganine accumulation in FB_1-induced hepatotoxicity and increased expression of selected signaling genes in BALB/c mice. The mice were pretreated daily with intraperitoneal injection of 1.0 mg/kg myriocin 30 min before subcutaneous injections of 2.25 mg/kg of FB_1 for 3 days. Results showed that myriocin alone was not hepatotoxic and the combination of myriocin plus FB_1 completely prevented the FB_1-induced elevation of hepatic free sphinganine and prevented the FB_1-induced induction of selected cell signaling genes, suggesting that accumulation of free sphinganine and/or its metabolites contribute to the FB_1-modulation of the cell signaling factors. However, the combination of myriocin and FB_1 did not prevent FB_1-increased concentration of plasma alanine aminotransferase and only slightly attenuated aspartate aminotransferase; it did not affect the FB_1-induced hepatocyte apoptosis or increased cell proliferation. A longer combined treatment of myriocin and FB_1 was highly toxic. The hepatotoxic effects in mice seen in this study are most likely due to a combination of factors including accumulation of free sphinganine, depletion of more complex sphingolipids and sphingomyelin, or other unknown mechanisms.

Keywords: Fumonisin; Myriocin; Cytokine expression; Hepatotoxicity; Sphinganine; Sphingolipids

1. Introduction

Fumonisins were first isolated in 1988 from the fungus *Fusarium verticillioides*, a common endophytic fungus in corn (Gelderblom et al., 1988). Several types of fumonisins have been identified so far as products of *F. verticillioides* in naturally contaminated corn and corn-based products. Fumonisin B_1 (FB_1) is the most abundant and most toxic of all fumonisin isomers investigated so far (WHO, 2000). Fumonisin B_1 induces species-specific toxicity. In horses this toxin is known to cause leukoencephalomalacia (Marasas, 2001); in pigs pulmonary edema and cardiovascular damage (Haschek et al., 2001; Smith et al., 1999). The high incidence of esophageal cancer in people in some areas of South Africa and China was correlated with *F. verticillioides* infection and fumonisin levels in corn (Marasas, 2001; Yoshizawa et al., 1994). It has been demonstrated that FB_1 is hepato- and nephro-carcinogenic in male rats (Gelderblom et al., 1991; Howard et al., 2001), and hepatocarcinogenic in female mice (Howard et al., 2001). Fumonisins are hepatotoxic and nephrotoxic in

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rodents (Sharma et al., 1997; Voss et al., 1998, 2001). The cellular effects of fumonisins consist of a mixture of apoptosis and necrosis and regenerative proliferation (Lemmer et al., 1999; Howard et al., 2001; Sharma et al., 1997).

Fumonisins are structurally similar to free sphingoid bases (sphinganine and sphingosine), and inhibit ceramide synthase (sphingosine *N*-acyltransferase), a critical enzyme in the pathway of de novo sphingolipid synthesis (Merrill et al., 2001; Wang et al., 1991). By inhibiting ceramide synthase, FB_1 increases the level of free sphinganine in tissues, serum, and urine (Riley et al., 1993, 1996, 1997; Wang et al., 1992, 1999), decreases complex sphingolipids (Wang et al., 1992; Yoo et al., 1996), and increases the formation of other lipid metabolites such as sphingoid base-1-phosphates and their downstream metabolites (Merrill et al., 2001; Smith and Merrill, 1995). The hepatotoxicity of FB_1 is closely correlated with the accumulation of free sphinganine in male BALB/c (Tsunoda et al., 1998) and other mouse strains (Riley et al., 2001).

Serine palmitoyltransferase (SPT), the first enzyme in the pathway for de novo biosynthesis of sphingolipids, catalytically incorporates L-serine into palmitoyl-coenzyme A to produce 3-keto-sphinganine, the immediate precursor of sphinganine (Hannun et al., 2001, Fig. 1). Inhibition of SPT to reduce free sphinganine accumulation reversed FB_1 toxicity in mammalian cell cultures (He et al., 2002a; Riley et al., 1999a,b; Schmelz et al., 1998; Tolleson et al., 1999; Yoo et al., 1996). Myriocin, a selective inhibitor of SPT (Miyake et al., 1995), prevented accumulation of free sphinganine in kidney of mice exposed to FB_1, and therefore it was proposed that myriocin might be useful in protecting against FB_1 toxicity in vivo (He et al., 2002a; Riley et al., 1999a).

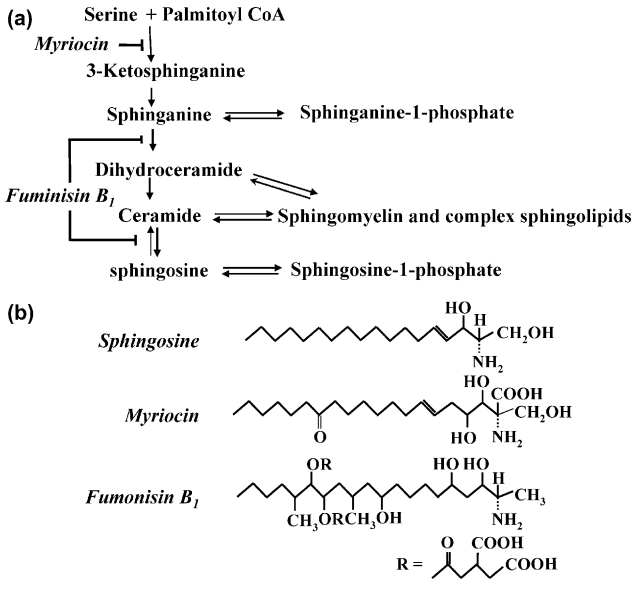

Fig. 1. (a) Sphingolipid pathways indicating inhibition of serine palmitoyltransferase by myriocin and ceramide synthase by fumonisin B_1. Treatment of animals with myriocin decreases synthesis of all sphingolipids, whereas treatment with FB_1 results in the increase of sphinganine, sphingosine and their respective phosphates along with decrease in ceramide and complex sphingolipids. (b) Structural similarity of myriocin and fumonisin B_1 with free sphingoid base sphingosine.

Numerous studies have shown that FB_1 modulates the expression of inflammatory cytokines and other cell signaling factors. For example, FB_1 treatment induced the expression of tumor necrosis factor (TNF) and pro-apoptotic signaling genes in liver and kidney of mice (Bhandari and Sharma, 2002; Bhandari et al., 2002). Peritoneal macrophages from FB_1-treated mice produced higher amount of TNFα than those from saline controls in response to lipopolysaccharide ex vivo (Dugyala et al., 1998). Treatment of LLC-PK_1 cells, a pig renal epithelial cell line, with FB_1 transiently increased the expression of TNFα but this induction of TNFα by FB_1 was unaltered when free sphinganine accumulation was prevented in the cultures by myriocin (He et al., 2001). Expression of TNFα receptor-associated protein (TRAP) 2 was induced in FB_1-sensitive CV-1 cells but repressed in FB_1-resistant COS cells (Zhang et al., 2001). It remains uncertain whether FB_1-induced alterations of gene expression in tissues are due solely to the disruption of sphingolipid metabolism.

Myriocin is able to inhibit the activity of hepatic SPT in mice (He et al., 2004a), and subsequently prevent the accumulation of free sphinganine in response to FB_1 both in vivo and in vitro (Enongene et al., 2002; Riley et al., 1999a; Schmelz et al., 1998). It has been widely used to study the role of sphinganine and de novo generated ceramide in regulation of cell functions under various conditions (He et al., 2001, 2002a,b; Le Stunff et al., 2002; Riley et al., 1999a; Schmelz et al., 1998). In the present study, we investigated the effect of myriocin on FB_1 hepatotoxicity and gene expression of selected cytokines in mice. Myriocin effectively blocked the activity of SPT and prevented the FB_1-accumulation of free sphinganine in both liver and kidney; however, it did not reduce FB_1-induced hepatocyte apoptosis or increased proliferating cell nuclear antigen (PCNA) containing cells. The FB_1-induced plasma alanine aminotransferase (ALT) was unaltered by myriocin pretreatment; the elevation of plasma aspartate aminotransferase (AST) was significantly reduced. In spite of minimal protection against FB_1-induced hepatotoxicity, myriocin reversed the FB_1-induced increases in the expression of *TNFα*; *TNF related apoptosis-inducing ligand* (*TRAIL*), *TNFα receptor* (*TNFR*)1, *lymphotoxin* (*LT*)β, *Interferon* (*IFN*)γ, and *transforming growth factor* (*TGF*)β1. Results suggested that elevation in free sphinganine and/or its metabolites are involved in FB_1-induced alterations in expression of various cell signaling factors. However, with the dosing regime used in