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Fumonisin B_1 hepatotoxicity in mice is attenuated by depletion of Kupffer cells by gadolinium chloride

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Abstract

Fumonisin B_1 (FB_1) is a toxic and carcinogenic mycotoxin produced by *Fusarium verticillioides* found on corn worldwide. The biological effects of FB_1 are attributed to sphingolipid metabolism disruption as a result of ceramide synthase inhibition. Tumor necrosis factor α (TNFα) is an important modulator of FB_1 hepatotoxicity. Kupffer cells are major source of cytokine production in liver. In the present study we investigated the effects of Kupffer cell depletion by gadolinium on FB_1 hepatotoxicity in female BALB/c mice. Mice were given saline or 50 mg/kg of gadolinium chloride once via the tail vein; 16 h later they were treated with subcutaneous injections of vehicle or 2.25 mg/kg/day FB_1 in saline for three successive days. Gadolinium significantly attenuated FB_1-induced increases in the activities of circulating alanine aminotransferase and aspartate aminotransferase and reduced the FB_1-induced hepatocyte apoptosis and free sphinganine accumulation in liver. Both gadolinium and FB_1 treatments individually increased the expression of selected cell signal factors; e.g., TNFα, TNF receptor 1, TNF-related apoptosis-inducing ligand, lymphotoxin β, interferon γ, and transforming growth factor β1; gadolinium chloride did not alter FB_1-induced expression of the above genes. Results indicated that Kupffer cells play a role in FB_1 hepatotoxicity. Decreased FB_1-induced sphinganine accumulation and increased protective TNFα signaling by gadolinium chloride may in part account for its ameliorating effect on FB_1 liver damage.

Keywords: Fumonisin; Hepatotoxicity; Tumor necrosis factor α; Sphinganine; Kupffer cells

1. Introduction

Fumonisins, including fumonisin B_1 (FB_1), belong to a group of structurally related mycotoxins produced by *Fusarium verticillioides*, a common endophytic fungus on corn. Fumonisin contamination of animal feeds and human corn-based food has been reported worldwide (WHO, 2000). Fumonisin B_1 is the most abundant and toxic among various types of fumonisins. Fumonisins caused field outbreaks of equine leukoencephalomaracia and porcine pulmonary edema in the Unites States in 1989–1990 (Ross et al., 1991, 1992). Areas

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with high incidence of human esophageal cancer in southern Africa and China have been correlated with high level of FB_1 contamination of human food (Chu and Li, 1994; Marasas, 2001). Feeding studies demonstrated that FB_1 is hepatocarcinogenic in BD IX rats (Gelderblom et al., 1991), in female B6C3F1 mice (Howard et al., 2001), and nephrocarcinogenic in male F344 rats (Howard et al., 2001). Fumonisins produce liver injury in rodents (Sharma et al., 1997; Voss et al., 1998, 2001), horses (Ross et al., 1993; Wang et al., 1992), milk-fed caves (Mathur et al., 2001), and pigs (Haschek et al., 2001).

Fumonisin B_1 induces intracellular accumulation of free sphinganine, and usually of free sphingosine, due to inhibition of ceramide synthase (sphinganine-*N*-acyltransferase), a critical enzyme responsible for conversion of sphinganine to ceramide (Wang et al., 1991; Merrill et al., 1993). Cell death in response to FB_1 exposure has been related to disruption of sphingolipid metabolism both in vivo and in vitro (Riley et al., 2001; Tolleson et al., 1999; Tsunoda et al., 1998; Yoo et al., 1996).

Tumor necrosis factor α (TNFα) signaling pathways are important in modulating FB_1 toxicity. In response to FB_1 exposure, TNFα was increased in mouse liver along with other inflammatory cytokines (Bhandari and Sharma, 2002) and FB_1 hepatotoxicity was reduced in mice lacking either TNFα receptor (TNFR) 1 or TNFR 2 (Sharma et al., 2000a, 2001). On the other hand, mice carrying a human TNFα transgene exhibited less sensitivity to FB_1 hepatotoxicity (Sharma et al., 2000b).

Kupffer cells, usually referred to as fixed hepatic macrophages, have diverse functions including phagocytosis, endocytosis, immunomodulation and synthesis and secretion of numerous biological active mediators (Laskin et al., 2001). Several reports indicate that Kupffer cells are principal source of inducible nitric oxide (NO) synthase (iNOS), NO production, and of cytokines, e.g., TNFα, interleukin (IL)-1β, IL-6, IL-12 (Laskin et al., 2001; Gregory et al., 1998; Ishiyama et al., 2000). Kupffer cells have been linked in the pathogenesis of liver injury induced by various hepatotoxicants such as carbon tetrachloride (Edwards et al., 1993), acetaminophen (Laskin et al., 1995; Ju et al., 2002), ethanol (Wheeler et al., 2001), and cadmium (Yamano et al., 2000). It is believed that the regulatory role of Kupffer cells in chemical-induced liver damage is mediated through their production of superoxides and cytokines (Ju et al., 2002; Michael et al., 1999; Wheeler et al., 2001; Yamano et al., 2000).

In addition to induction of hepatocellular apoptosis, necrosis and mitosis (Howard et al., 2001; Sharma et al., 1997; Voss et al., 2001), histopathological examination by light microscope revealed that FB_1 exposure increased the number of Kupffer cells in rats, leading to and Kupffer cell hyperplasia in pigs and mice (Haschek et al., 2001; Howard et al., 2002; Theumer et al., 2002). We previously demonstrated that cytokines such as TNFα and interferon γ (IFNγ) are important contributors in murine hepatotoxicity by FB_1 (Sharma et al., 2000b, 2002, 2003). Kupffer cells were suggested as a source of TNFα in response to FB_1 treatment (Bhandari et al., 2002). It was proposed that TNFα and IL-12 secreted by Kupffer cells after FB_1 stimulation activated T lymphocytes and natural killer cells in liver to produce IFNγ, which further amplified the production of TNFα through a positive feedback loop (Bhandari et al., 2002). These findings suggested an important role of Kupffer cell in FB_1-induced liver toxicity and that the modulation of Kupffer cell function would alter production of cytokines to modulate FB_1 hepatotoxicity. In the present study, we studied FB_1 hepatotoxicity in mice after eliminating Kupffer cells by gadolinium chloride, a selective Kupffer cell toxicant in liver (Hardonk et al., 1992). Results demonstrated that gadolinium chloride at the dose used in the current study eliminated Kupffer cells in liver. Pretreatment of mice with gadolinium chloride reduced FB_1-induced hepatotoxicity in response to three daily FB_1 treatments, suggesting that Kupffer cells are involved in FB_1 hepatotoxicity.

2. Materials and methods

2.1. Chemicals

Fumonisin B_1 (purity > 98%) was purchased from Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC, Tygerberg, South Africa). Purified rat anti-mouse mac-3 monoclonal antibody (clone M3/84) was obtained from BD Biosciences (San Diego, CA, USA). Gadolinium chloride ($GdCl_3 \cdot 6H_2O$) and all other reagents were purchased from Sigma–Aldrich