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NeuroToxicology 26 (2005) 981–992

NeuroToxicology

# Fumonisin $B_1$ Induces Necrotic Cell Death in BV-2 Cells and Murine Cultured Astrocytes and is Antiproliferative in BV-2 Cells While N2A Cells and Primary Cortical Neurons are Resistant

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Received 22 October 2004; accepted 5 April 2005
Available online 6 July 2005

***Abstract***

*Fumonisin $B_1$ ($FB_1$), a mycotoxin produced by Fusarium verticillioides, causes equine leukoencephalomalacia, impairs myelination, and inhibits neuronal growth in vitro. Intact mice do not show brain damage after systemic administration of $FB_1$. We recently reported that intracerebroventricular administration of $FB_1$ in mice caused neurodegeneration in the cortex and activation of astrocytes in the hippocampal area; results suggested that the neuronal damage may be secondary to activation of immunocompetent non-neuronal cells. Current study investigated effects of $FB_1$ upon murine microglial (BV-2) and neuroblastoma (N2A) cell lines, and primary astrocytes and cortical neurons. BV-2 and N2A cultures and cells prepared from neonatal and postnatal brains of BALB/c mice were exposed to various concentrations of $FB_1$ for 4 (BV-2 and N2A) or 4 and 8 (astrocytes and cortical neurons) days. $FB_1$ at 25 μM decreased viability in BV-2 cells, whereas at 50 μM caused necrotic but not apoptotic cell death in both BV-2 and primary astrocytes (at day 8 only), assessed by lactic dehydrogenase release, and pripidium iodide and annexin V staining. Thymidine incorporation indicated that 2.5 μM $FB_1$ decreased proliferation in BV-2 cells. DNA analysis by flow cytometry showed that the inhibition was not caused by cell cycle arrest. The mitochondrial activity decreased dose-dependently in BV-2 cells and was significantly elevated at 25 μM $FB_1$, but not at 50 μM at days 4 or 8 in astrocytes. In BV-2 cells and primary astrocytes, the expression of TNFα and IL-1β analyzed by real-time polymerase chain reaction was downregulated at 6 or 24 h. In all cell types tested the $FB_1$ treatment caused accumulation of free sphinganine and decrease in free sphingosine levels at selected time points. Results indicated that primary and established murine brain immunocompetent cells are vulnerable to the $FB_1$-dependent cytotoxicity in vitro whereas neuronal cells are not. The toxic effects on the neuronal tissue may therefore be secondary to modulation of astrocyte or glial cell function.*


**Keywords: Fumonisin; BV-2 cells; N2A cells; Astrocytes; Cortical neurons; Necrosis**

## INTRODUCTION

Fumonisins are toxic metabolites synthesized by the fungus *Fusarium verticillioides* (formerly *F. moniliforme*). The occurrence of fumonisin infestation is worldwide and affects various cereals, predominantly corn (Dutton, 1996). The most abundant and metabo-

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0161-813X/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2005.05.001

lically active member of the fumonisin family is fumonisin $B_1$ ($FB_1$). $FB_1$ causes a number of disorders in farm and laboratory animals such as equine leukoencephalomalacia, porcine pulmonary edema, nephrotoxicity in sheep, and hepatotoxicity and nephrotoxicity in rodents (Dutton, 1996). Chronic feeding of animals with $FB_1$ causes liver cancer in female mice and kidney cancer in male rats (Howard et al., 2001). Esophageal cancer in humans has been epidemiologically associated with dietary exposure of $FB_1$ in regions of Southern Africa and China where high levels of this mycotoxin are present in foods (Dutton, 1996).

Equine leukoencephalomalacia is the only naturally occurring brain disorder caused by the environmental exposure to $FB_1$ (Brownie and Cullen, 1987). The disease is specific for equide, shows high mortality (Wilkins et al., 1994; Wilson et al., 1990), and is characterized by focal necrosis of brain primarily in the subcortical white matter. Recently, the consumption of fumonisins was implicated in the etiology of neuronal tube defects (NTD) in children (Marasas et al., 2004). NTD can be induced experimentally in neurulating murine embryos after in vitro exposure to $FB_1$ (Sadler et al., 2002) and this effect was related to decreased folic acid uptake (Stevens and Tang, 1997).

The specific biochemical effect of exposure to $FB_1$ both in vivo and in vitro is inhibition of ceramide synthase, an enzyme in the de novo sphingolipid biosynthesis pathway. The inhibition of ceramide synthase blocks biosynthesis of ceramide and other complex shingolipids resulting in the accumulation of free sphinganine (and later sphingosine). Dynamic balance between free sphingoid bases and their metabolites controls several cellular processes including growth, differentiation, survival and death (Merrill et al., 2001). Long-term inhibition of ceramide synthase results in the depletion of complex sphingolipids such as sphingomyelin, cerebrosides and gangliosides, which maintain the function and structure of neuronal tissue. A number of studies from our laboratory have reported the involvement of proinflammatory cytokines such as tumor necrosis factor α (TNFα) in $FB_1$-induced toxicity in organs such as liver (Bhandari et al., 2002; Sharma et al., 2000) and kidney (Bhandari and Sharma, 2002).

In vitro and in vivo experiments demonstrated a potential for toxic effects upon neuronal tissue by $FB_1$. $FB_1$ treatment altered regional brain neurotransmitter metabolite levels in mice (Tsunoda et al., 1998) and rats (Porter et al., 1993) and modulated electrophysiological activity in the rat neocortex (Banczerowski-Pelyhe et al., 2002). Harel and Futerman (1993) reported $FB_1$-dependent inhibition of neuronal development in culture, whereas Kwon et al. (2000) showed that $FB_1$ decreased the activity of rat primary glia/oligodenrocytes concurrently with the accumulation of sphinganine. The $FB_1$-induced disruption of myelination was demonstrated in vitro (Monnet-Tschudi et al., 1999), and in vivo (Kwon et al., 1997). In cultured rat astrocytes $FB_1$ caused DNA damage but did not alter their viability (Galvano et al., 2002). Oxidative damage after $FB_1$ treatment was recently reported in human U-118MG glioblastoma cells (Stockman-Juvala et al., 2004). $FB_1$ treatment in J774A.1 macrophages deregulated the membrane homeostasis (Ferrante et al., 2002) and increased the LPS-dependent production of nitric oxide and prostaglandin E2 (Meli et al., 2000). In a number of cell lines, $FB_1$ displayed strong antiproliferative properties that were often concurrent with cell cycle arrest (Ciacci-Zanella et al., 1998; Johnson et al., 2003; Mobio et al., 2000; Tolleson et al., 1996b). Apoptosis seems to be an important mode of $FB_1$-induced death in non-neuronal cells in vitro (Tolleson et al., 1996a,b; Yu et al., 2001).

Abundance of studies investigating carcinogenetic properties of $FB_1$ is contrasted by relatively low interest in its neurotoxic effects. There are no laboratory animal models for equine leukoencephalomalacia. Treatment of mice with $FB_1$ via systemic routes causes no neuronal damage; however, we recently reported that cortical neurodegeneration can be induced after intracerebroventricular administration of $FB_1$ (Osuchowski et al., 2005). Activation of hippocampal astrocytes was also observed in this study. It is not known what cells in the neuronal tissue are sensitive to $FB_1$ and are likely target for the initial degenerative effect. We hypothesized that the neuronal damage in brain is secondary to the effects of $FB_1$ on immunocompetent cells of the central nervous system. In the present study we investigated the effect of $FB_1$ upon viability and proliferation of murine neuronal and glial cells and correlated these effects to the sphingolipid accumulation. We also show that both established cell lines and primary cells are equally sensitive to the cytotoxic effects of $FB_1$ upon the sphingolipid metabolism.

## MATERIALS AND METHODS

### BV-2 and N2A Cell Lines

The cultured murine microglial cells were immortalized after infection with a v-raf/v-myc recombinant retrovirus forming a cell line that features the morpho-