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Validation of fumonisin biomarkers in F344 rats

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Abstract

Fumonisins (FNs) are ubiquitous contaminants of cereal grains. Fumonisin B_1 (FB_1) was linked to several animal and human diseases. To validate FB_1 biomarkers for studying human disease risks, F344 rats were administered by gavage with either a single dose of 0, 10 or 25 mg FB_1/kg body weight (BW) or repeated doses of 0, 1.0, or 2.5 mg FB_1/kg BW/day for 5 weeks. FB_1 excretion and FB_1-induced metabolic alterations of sphingolipids in rat urine, feces and serum were assessed. Dose-dependent urinary and fecal excretion of free FB_1 were found in both single-dose- and repeat-dose-treated rats. In the single-dose study, urinary sphinganine (Sa) to sphingosine (So) ratio (Sa/So) reached a maximum at day 7 for the high-dose group and at day 5 for the low-dose group, whereas serum Sa/So showed only marginal changes. In the repeat-dose study, urinary Sa/So was persistently elevated at 2 weeks, while serum Sa/So was unchanged. Time course changes of sphinganine 1-phosphate (SaP) and sphingosine 1-phosphate (SoP) were also examined. Although serum Sa/So and SaP/SoP ratios showed no signs of time- or dose-dependent changes, a 10-fold increase in urinary SaP/SoP was observed, suggesting that urinary SaP/SoP is a more sensitive biomarker for FB_1 exposure. The accumulation of SaP and SoP was evident in the time course of SaP/Sa and SoP/So, which may reflect activity changes of enzymes closely related to the metabolism and catabolism of SaP and SoP. These results provide concrete evidence towards the practical use of excreted FB_1, Sa/So and SaP/SoP as biomarkers of exposure to FNs.

Keywords: Validation; Biomarkers; Fumonisin B_1 (FB_1); Sphinganine; Sphingosine; Sphinganine 1-phosphate; Sphingosine 1-phosphate; F344 rats

Introduction

Fumonisins (FNs), produced mainly by *Fusarium verticillioides*, are ubiquitous contaminants of cereal grains around the world (Marasas, 1996; WHO, 2000). At least 15 FNs have been isolated, characterized and designated as fumonisin A, B, C and P, although only fumonisin B_1 (FB_1) and B_2 (FB_2) appear to be biologically significant. FB_1, the representative mycotoxin, causes several fatal animal diseases, including leukoencephalomalacia in horses, pulmonary edema in swine and hepatotoxicity in horses, swine and rats (WHO, 2000). FB_1 has been found to induce renal tube adenomas and carcinomas in male F344 rats and hepatocellular carcinomas in female B6C3F1 mice (Howard et al., 2001; Gelderblom et al., 2002). It is also a potent tumor promoter in rats after initiation with diethylnitrosamine and aflatoxin B_1 (Gelderblom et al., 1996). Etiological roles of exposure to FNs through ingestion of moldy corn in human esophageal and liver cancers, as well as neural tube defects, have been suggested by several studies in South Africa, China and the Texas–Mexico border (Marasas et al., 1988; Sydenham et al., 1990; Chu and Li, 1994; Yoshizawa et al., 1994; Ueno et al., 1997; Missmer et al., 2006).

Given the toxicity of FNs in animals and potential worldwide human exposure, the development, validation and application of biomarkers have been a priority for research on these toxins (Turner et al., 1999; WHO, 2000). Toxicokinetic data showed that orally dosed FB_1 was eliminated rapidly from circulation (WHO, 2000). This rapid elimination and low bioavailability, as well as the lack of a major metabolite, indicate that direct measurement of FB_1 in biological fluids may be a possible

Abbreviations: BW, body weight; C17SaP, D-erythro-C17-sphinganine 1-phosphate; C20So, D-erythro-C20-sphingosine; FB_1, fumonisin B_1; FNs, fumonisins; LC/MS, liquid chromatography/mass spectrometry; OPA, *O*-phthaldialdehyde; PBS, phosphate buffered saline; Sa, D-erythro-sphinganine; SaP, D-erythro-sphinganine 1-phosphate; So, D-erythro-sphingosine; SoP, D-erythro-sphingosine 1-phosphate.

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biomarker for studying short-term exposure. To that end, efforts have been made on monitoring free FNs in human urine (Shetty and Bhat, 1998), plasma (Shephard et al., 1992a), feces (Chelule et al., 2001) and hair (Sewram et al., 2003).

FNs disrupt sphingolipid metabolism by inhibition of sphinganine (sphingosine) *N*-acetyltransferase due to their structural similarity to long-chain sphingoid base backbones (Fig. 1) (Wang et al., 1991; Merrill et al., 1993). FB_1 causes an increase in intracellular free sphinganine (Sa) and, to a lesser extent, sphingosine (So) which precedes the depletion of complex bioactive lipids (Riley et al., 2001; Ogretmen and Hannun, 2004). The FB_1-induced biochemical alterations, particularly elevated Sa levels and Sa to So or Sa 1-phosphate to So 1-phosphate ratios in tissues, urine and blood, have been proposed as potential biomarkers in various animal species including foals (Wang et al., 1992), pigs (Riley et al., 1993), mink (Morgan et al., 1997), rats (Wang et al., 1999), vervet monkeys (van der Westhuizen et al., 2001a) and ducks (Tran et al., 2006). Several studies have also been carried out to explore the potential of the Sa/So biomarker for human dietary exposure to FNs (van der Westhuizen et al., 1999; Abnet et al., 2001; Qiu and Liu, 2001; Solfrizzo et al., 2004; Missmer et al., 2006).

Biomarkers are measurements of the changes that occur in response to an insult and are indicators of exposure, effects or susceptibility. Ideally, putative biomarkers are validated in pilot animal studies where sensitivity, specificity, accuracy and reliability parameters can be established. Data obtained in animal studies can be used to assess intra- or inter-individual variability, background levels, relationship of biomarkers to external dose or to disease status, as well as feasibility for use in

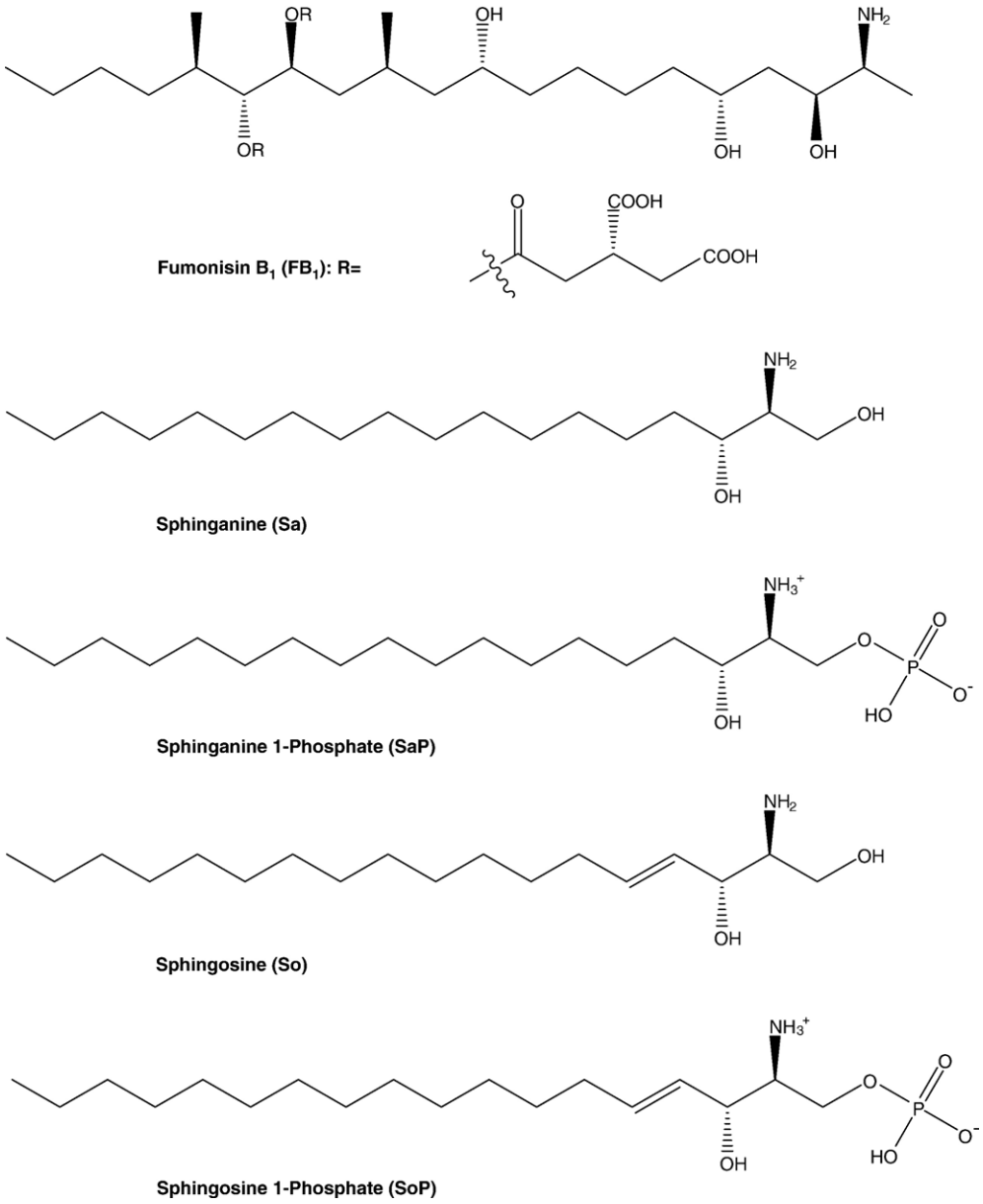

Fig. 1. Chemical structure of FB_1 and the major sphingolipid metabolites affected.