Sphingoid bases are growth inhibitory and pro-apoptotic for many types of cells when added to cells exogenously, and can be elevated to toxic amounts endogenously when cells are exposed to inhibitors of ceramide synthase. An important category of naturally occurring inhibitors are the fumonisins, which inhibit ceramide synthase through structural similarities with both the sphingoid base and fatty acyl-CoA co-substrates. Fumonisins cause a wide spectrum of disease (liver and renal toxicity and carcinogenesis, neurotoxicity, induction of pulmonary edema, and others), and most—possibly all—of the pathophysiologic effects of fumonisins are attributable to disruption of the sphingolipid metabolism. The products of alkaline hydrolysis of fumonisins (which occurs during the preparation of masa flour for tortillas) are aminopentols that also inhibit ceramide synthase, but more weakly. Nonetheless, the aminopentols (and other 1-deoxy analogs of sphinganine) are acylated to derivatives that inhibit ceramide synthase, perhaps as product analogs, elevate sphinganine, and kill the cells. Somewhat paradoxically, fumonisins sometimes stimulate growth and inhibit apoptosis, possibly due to elevation of sphinganine 1-phosphate, which is known to have these cellular effects. These findings underscore the complexity of sphingolipid metabolism and the difficulty of identifying the pertinent mediators unless a full profile of the potentially bioactive species is evaluated.

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Keywords: Fumonisin; Aminopentol; Ceramide synthase; Sphinganine; Sphinganine 1-phosphate; Toxicity; Apoptosis; Carcinogenesis

Fumonisins are a family of mycotoxins produced by Fusarium verticillioides (formerly F. moniliforme) [1], which are common fungal contaminants of corn and some other grains [2]. As much as 59% of the corn and corn-based products worldwide have been estimated to be contaminated with variable amounts of fumonisin B1 (FB1) (Fig. 1), the most prevalent of the fumonisin subspecies [2–4]. Fumonisins cause an at-first-glance puzzling range of diseases: liver and kidney toxicity and carcinogenicity, pulmonary edema, immunosuppression (and sometimes immunostimulation), neurotoxicity, and probably others [1,5]. Most or all of the toxicities resulting from exposure to these compounds can be explained by their ability to alter sphingolipid metabolism by inhibiting ceramide synthase [5,6].

Fumonisins have also been used extensively to explore the functions of ceramides and complex sphingolipids in cell culture. Quite paradoxically, some of these studies use fumonisins to inhibit apoptosis [7], and in some contexts, fumonisins are growth stimulatory and anti-apoptotic rather than cytotoxic [8]. These reasons for these dichotomous effects are not fully known, but this review will explore the most likely answers as well as discuss other surprises that have surfaced while studying this category of compounds.

1. Inhibition of ceramide synthase by fumonisins

FB1 is comprised of a long-chain aminopentol backbone (AP1) (Fig. 1) with two ester-linked tricarballylic acids [1].

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In the current model for how fumonisins inhibit ceramide synthase, the aminopentol backbone competes for binding of the sphingoid base substrate, whereas the anionic tricarballylic acids interfere with binding of the fatty acyl-CoA [5]. Three lines of evidence support this model: (1) the potency with which FB1 inhibits ceramide synthase is sensitive to the concentrations of both sphingoid bases and fatty acyl-CoA [9]; (2) removal of the tricarballylic acids diminishes the potency of ceramide synthase inhibition [10]; and (3) upon removing the tricarballylic acids, AP1 becomes not only an inhibitor but also a substrate for acylation by ceramide synthase [10]. As will be discussed later, this acylation may explain why aminopentols remain toxic to animals despite the lower potency of these compounds as ceramide synthase inhibitors.

2. Induction of apoptosis by fumonisins

When fumonisins are added to cells in culture, they cause rapid and dramatic elevations (i.e., one to two orders of magnitude) in sphinganine due to inhibition of de novo sphingolipid biosynthesis (Fig. 2). This most often results in growth arrest [11,12] and apoptosis [13]. The growth arrest has been shown to be due to up-regulation of inhibitors of cyclin-dependent protein kinases (Cip1, Kip1 and Kip2) at both transcriptional and post-transcriptional levels [11,12]. The mechanism(s) for the induction of apoptosis are less clear, but probably involve disruption of many cell regulatory pathways, including inhibition of protein kinase C [14], key hallmarks of apoptotic signaling [15], disruption of the endothelial barrier [16] and others. With so many changes, it is possible that fumonisins are acting via more than one initial target, and it appears that they may affect MAPK directly [17], although this is complicated by the involvement of sphingolipids in many MAPK pathways [18]. All in all, the most conclusive evidence to date that elevations in sphinganine mediate at least the earliest toxicity of fumonisins is that an inhibitor...
of upstream enzyme of sphingolipid biosynthesis (inhibition of serine palmitoyltransferase by ISP1) suppresses the toxicity [13].

The results with fumonisins in cell culture are paralleled with findings in vivo. When animals consume fumonisins, the earliest biochemical alteration that is detected is a time- and dose-dependent elevation in tissue, serum and urinary sphinganine, and usually at later times or higher doses (when major tissue damage has occurred) an elevation in sphingosine [19]. On this basis, increases in the ratio of sphinganine to sphingosine has proven to be a useful biomarker for exposure to fumonisins [20]. Hepatotoxicity occurs in response to FB1 consumption in most exposed animals, and is characterized by cell death in the form of both apoptosis and necrosis; additionally, there are indications of spontaneous cell regeneration (mitogenesis), a condition favorable for carcinogenesis [21–24]. According to the recently published study by the National Center for Toxicologic Research [24], there is clear evidence of carcinogenic activity of FB1 in male, but not female, F344/N rats (the variable expression or activity of enzymes that mediate sphingolipid metabolism in cells must be considered when one seeks to understand species and tissue differences in the responses to these mycotoxins. It is possible that a relatively simple relationship may exist: toxicity occurring in cells that accumulate sphingamine (and sphingosine), and mitogenesis in cells that accumulate sphingoid-1-phoshates [8]. One argument against this interpretation is that addition of sphingosine 1-phosphate to cells in culture sometimes induces apoptosis, however, this may not be caused by sphingosine 1-phosphate per se, but to the sphingosine (or its metabolite ceramide) which is released upon removal of the phosphate group, as has been recently shown with mesangial cells [28].

Because the elevation in sphinganine is so rapid and large, it usually dominates over depletion of complex sphingolipids in the effects of fumonisins on cells. Nonetheless, loss of glycosphingolipids from A431 epithelial cells causes a significant increase in diffusible lipids (i.e., the mobile fraction) and this correlates with a loss of epithelial cell morphology, a reduced rate of cell growth and the inhibition of cell–substrate adhesion [29]. Exogenous administration of GM3 gangliosides to cells, thereby restoring cellular glycosphingolipids, blocks elevations in diffusible lipids and reverses the effects of glycosphingolipid depletion on cell morphology and substrate adhesion [29]. Depletion of complex sphingolipids also alters the function of glycosphatidylinositol-anchor proteins, such as the folate receptor [30]. The treatment of Caco-2 cells with FB1, at concentrations that cause 40% depletion of complex sphingolipids, results in a complete halt in the cellular uptake of folate by its receptor; potentially compromising the cellular processes dependent on this vitamin [30]. Most recently, post-transcriptional modifications, occurring at membrane micro-

3. Other consequences of inhibition of ceramide synthase

As sphinganine accumulates, there is increased synthesis of sphinganine 1-phosphate [26] as well as formation of the downstream degradation product(s) (for example, in fumonisin-treated J774 cells, as much as one-third of the ethanolamine in phosphatidylethanolamine was derived from sphingoid bases) [27]. The elevation in sphinganine 1-phosphate is likely to be a factor in cells where fumonisins are growth stimulatory instead of toxic, and may play an important role in the inhibition of apoptosis in studies where fumonisin has that effect.

Since it appears that an imbalance between cell death and regeneration primarily contributes to the FB1-induced carcinogenesis [6]; the variable expression or activity of enzymes that mediate sphingolipid metabolism in cells must be considered when one seeks to understand species and tissue differences in the responses to these mycotoxins. It is possible that a relatively simple relationship may exist: toxicity occurring in cells that accumulate sphingamine (and sphingosine), and mitogenesis in cells that accumulate sphingoid-1-phoshates [8]. One argument against this interpretation is that addition of sphingosine 1-phosphate to cells in culture sometimes induces apoptosis, however, this may not be caused by sphingosine 1-phosphate per se, but to the sphingosine (or its metabolite ceramide) which is released upon removal of the phosphate group, as has been recently shown with mesangial cells [28].

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![Diagram](attachment:image.png)

Fig. 3. Schematic representation of the inhibition of the acylation of sphingosine by AP1, which is acylated to PAP1, a more potent inhibitor of ceramide synthase.
domains which transform prions to their infectious form have been linked to complex sphingolipids; the depletion of sphingomyelin in cells, by treatment with FB1, has been found to increase the formation of infectious prions by 3–4-fold [31].

4. Metabolism and cytotoxicity of fumonisins aminopentols

The aminopentols are formed during the treatment of corn with lye, a common practice in regions of Central and South America; furthermore, the intestinal microflora of primates has been reported to hydrolyze FB1 to AP1 [5,32]. As noted above, AP1 is a weak inhibitor of ceramide synthase, but in the presence of palmitoyl-CoA is also acylated to form N-palmitoyl-AP1 (PAP1), which is a more potent inhibitor of ceramide synthase [10] (Fig. 3). Interestingly, PAP1, which is half as effective as FB1 in inhibiting ceramide synthase in vitro, causes significantly greater accumulation of sphinganine and toxicity in HT-29 cells [10]. In recent studies, we have found that other fatty acids (e.g., nervonic acid) are even more rapidly added to AP1, hence, all together, such acylation may explain why AP1 causes liver and kidney lesions and carcinogenicity in rodents that resembles those caused by FB1 [33,34].

5. Studies with 1-deoxy-sphinganine analogs

Structural differences between the fumonisins and sphinganine include the absence of a hydroxyl group at the 1-position, the presence of an additional hydroxyl at position 5, and a threo- versus erythro-stereochemistry at positions 2 and 3. To investigate the contributions of these features to the behavior of AP1, the analogous 1-deoxy 5-hydroxy-sphinganine was prepared by highly stereoselective synthesis [10]. Comparison of this analog with other homologs (i.e., 1-deoxysphinganines) and stereoisomers revealed that all were acylated, but the n-erythro-stereoisomers were the best substrates and inhibitors for ceramide synthase [10].

Since the stereochemistry of the 3- and 5-position hydroxyl groups on AP1 (and FB1) is the same as that of l-threo, 1-deoxy-, 5-hydroxysphinganine, this analog was of particular interest. This compound is significantly more toxic for HT-29 cells than either of the naturally occurring sphingoid bases (sphingosine and sphinganine) or a short-chain (C2-) ceramide (Fig. 4). In preliminary studies, we have observed that this compound is also an inhibitor of sphingosine kinase, which may contribute to its greater toxicity (i.e., by both elevating sphingoid base—which are cytotoxic—and suppressing sphingoid base 1-phosphates, which are often mitogenic and inhibitors of apoptosis). Consistent with this interpretation, another sphingoid base that inhibits sphingosine kinase (N,N-dimethylsphingosine) is also highly toxic (Fig. 4).

6. Perspectives for future research

Much remains to be determined about the signaling pathways that mediate the cellular effects of fumonisins and how they are interwoven with sphingolipid metabolism. Particularly intriguing in this regard are the findings about fumonisins and tumor necrosis factor (TNF) receptors and signaling [15,35,36] since this is one of the most studied pathways involving sphingolipids, and involves both mitogenesis and apoptosis, depending on the types of cells studied and the context. Also somewhat surprising has been the recent identification of UOG1 as a modulator of ceramide synthesis (either as an isoform of ceramide synthase or a regulator of ceramide synthase) from stearoyl-CoA in a FB1-independent manner [37].

It should be evident from the many fumonisin subspecies that may be involved plus the various aspects of sphingolipid metabolism/signaling that are involved, that when these are finally interwoven, it will give a very complex tapestry.

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