Sphingolipid analogue biomodulators in foods alter sphingolipid metabolism - implications for health and disease

RT Riley[1], DC Liotta[2], A H Merrill, Jr[2]

[1]Toxicology and Mycotoxins Research Unit, USDA/ARS, PO Box 5677, Athens, GA, USA 30604-5677 and [2]Department of Biochemistry, Emory School of Medicine, Atlanta, GA, USA 30322-3050

Summary

Relatively little is known about the importance of sphingolipids as components of the diet. Research on the role of complex sphingolipids, free sphingoid bases, and sphingolipid degradation products in signal transduction pathways has been rapidly expanding our understanding of these lipids. In addition to the newly discovered role of ceramide as an intracellular second messenger for IL1-b, TNF-a and other agonists, sphingosine, sphingosine 1-phosphate and other sphingolipid metabolites have been demonstrated to modulate cellular calcium homeostasis, cell cycle progression, and apoptosis. Alteration of sphingolipid metabolism using synthetic and naturally occurring inhibitors of key enzymes in the biosynthetic pathways is aiding the characterization of the cellular processes regulated by sphingolipids and also is revealing a role for disruption of sphingolipid metabolism in the pathophysiology of disease. Several food-borne fungal metabolites have been shown to inhibit sphingolipid biosynthesis and/or turnover. Activation or inhibition of sphingolipid pathways may be a common mechanism of toxin action.

Structure, de novo biosynthesis and turnover of sphingolipids

Eucaryotic cells synthesize a diverse array (300- 400 distinct molecules) of sphingolipids which serve as important structural components in cell membranes and as regulators of many cell functions (1,2). All sphingolipids contain a long-chain (sphingoid) base backbone. In most mammalian cells the most common sphingoid base is D-*erythro*-sphingosine. Addition of a fatty acid (16-30 carbons) to the amino group of sphingosine results in the formation of ceramides (N-acylsphingosines) and more complex sphingolipids are formed when substitutions are added at the 1-hydroxyl of the long chain base.

Complex sphingolipids are important structural components of cell membranes. They are involved in many vital membrane-dependent cell functions that are part of the normal regulation of cell growth, differentiation, and apoptosis (Figure 1). One of the most prevalent complex sphingolipids in mammalian cell membranes is sphingomyelin. Much of the recent interest in sphingolipids and sphingolipid metabolites is due to the discovery that sphingomyelin participates in a signaling system which controls a myriad of cellular functions in several cell models (3). In this signaling system, activation of a neutral sphingomyelinase and production of ceramide occurs when TNF-α or other agents bind to their membrane receptors. Many of the cellular effects of TNF-α have been reproduced by exogenous ceramide. For example, down regulation of c-*myc* and induction of apoptosis in HL60 cells (3,4). The antiproliferative effects of ceramide and TNF-a have been linked to a ceramide-activated protein phosphatase (4).

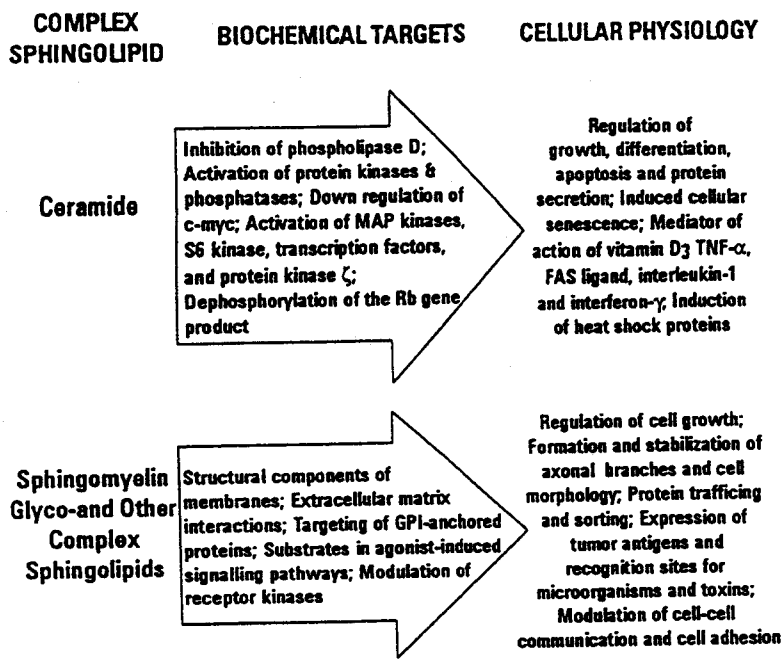

Figure 1. The biochemical targets and cellular physiology associated with ceramide and more complex sphingolipids. The biological activity of dihydroceramide has not been explored as well as ceramide, however, dihydroceramide is not active in the ceramide cycle and does not activate ceramide-activated protein phosphatase or the downstream effects on cellular physiology (eg inhibition of growth and induction of apoptosis).

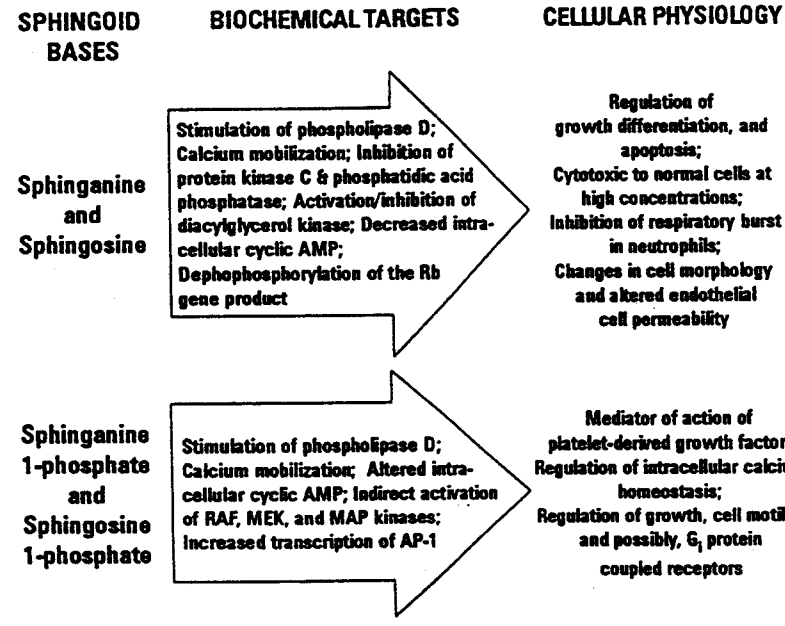

Figure 2. The biochemical targets and cellular physiology associated with free sphingoid bases and sphingoid base 1-phosphates. The biological activity of sphinganine is not as well known as sphingosine, however, where studied they both appear to act in a similar fashion.

Free sphingosine and sphingosine 1-phosphate have also been implicated as components of intracellular signaling systems (2) and have been shown to have many biochemical targets and biological effects (Figure 2). For example, sphingosine is a potent inhibitor of protein kinase C and blocks many protein kinase C-dependent cellular processes (2). Sphingosine and sphingosine 1-phosphate have been shown to induce intracellular calcium release from the endoplasmic reticulum via an inositol 1,4,5-trisphosphate-independent pathway (5). In many cell types exogenous sphingosine is growth inhibitory and at high concentrations, cytotoxic (2). However, in Swiss 3T3 cells exogenous sphingosine is mitogenic, possibly as a result of its conversion to

sphingosine 1-phosphate. Sphingosine and sphingosine 1-phosphate have been reported to be mediators of the mitogenic action of platelet-derived growth factor (6). Both sphingosine and sphingosine 1-phosphate stimulate phospholipase D and phosphatidic acid formation (7). Interestingly, ceramide which has antiproliferative effects (3) inhibits phospholipase D (8).

In mammalian cells, de novo sphingolipid biosynthesis begins in the endoplasmic reticulum and proceeds via the reactions shown in Figure 3 (2). The first, and committed step, is the condensation of serine with palmitoyl-CoA by serine palmitoyltransferase, a pyridoxal 5'-phosphate-dependent enzyme, and the resulting 3-ketosphinganine is reduced to sphinganine using NADPH. Free sphinganine is usually found in very low concentrations in cells because it is rapidly acylated to dihydroceramides (also called N-acylsphinganines) by ceramide synthase (sphinganine N-acyltransferase) using various fatty acyl-CoA's. Headgroups on the 1 hydroxyl (eg, phosphorylcholine, glucose, galactose, and hundreds of more complex polysaccharides) and the 4,5-trans-double bond of sphingosine are subsequently added in the endoplasmic reticulum, Golgi and plasma membranes. Thus, the presence of free sphingosine in cells is believed to be primarily derived from turnover of more complex sphingolipids or from dietary sources, rather than as a biosynthetic intermediate (2).

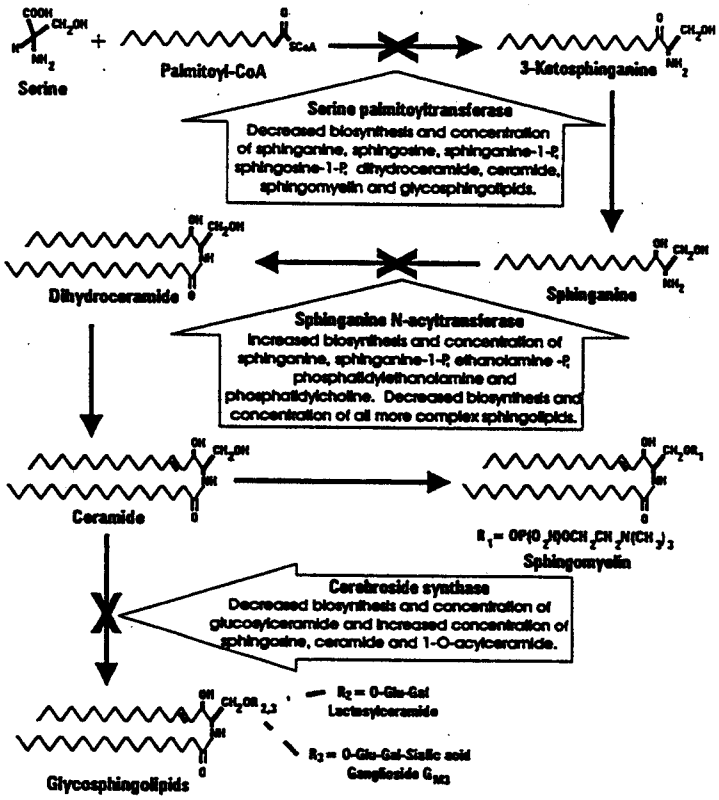

Figure 3. Structures of some long-chain bases and more complex sphingolipids and the pathway of de novo sphingolipid biosynthesis. Key enzymatic steps and the consequences of their inhibition on the biosynthesis and intracellular concentration of intermediates and end-products in the de novo and turnover pathways (Figure 4) are summarized.

Sphingolipid turnover (Figure 4) is thought to begin with the internalization of sphingolipids, followed by their hydrolysis in acidic compartments (lysosomes and endosomes) to ceramides, then to sphingosine. Typically, in tissues, the mass amount of free sphinganine is small (nmol/g). However, in the presence of inhibitors of ceramide synthase (Figure 5) the free sphinganine which is directed into the turnover pathway can be quite large (mmol/g). Other factors have been found to modulate the intracellular concentration of free long-chain bases (eg dexamethazone, phorbol dibutyrate, changing the culture medium) (2). Free sphingosine is generated from hydrolysis of ceramide. Both free sphingoid bases can either be reacylated in the endoplasmic reticulum or

phosphorylated in the cytosol to sphingosine (sphinganine-)-1-phosphate and cleaved to a fatty aldehyde (hexadecenal or hexadecanal) and ethanolamine-phosphate. Ethanolamine-phosphate is incorporated into phosphatidylethanolamine (9) and the fatty aldehyde can be directed back into other lipid products (1,2).

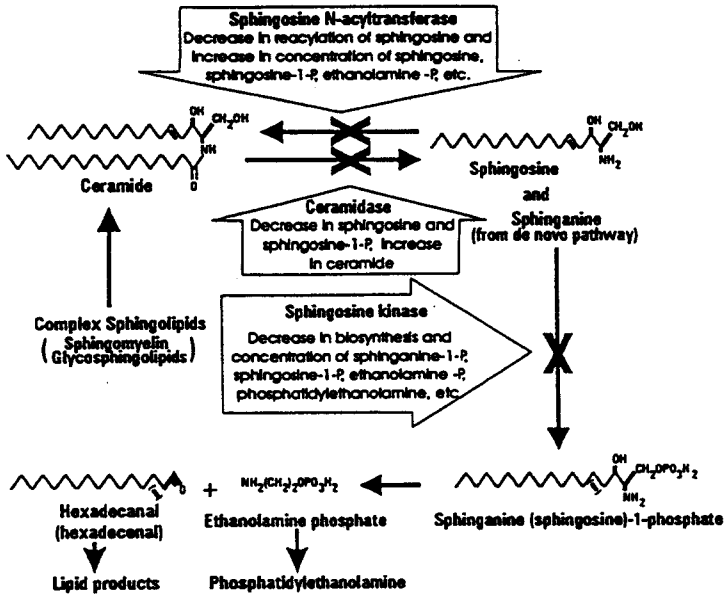

Figure 4. Structures of sphingosine (sphinganine) 1-phosphate and its degradation products and the pathway for sphingolipid turnover. Key enzymatic steps and the consequences of their inhibition on the biosynthesis and intracellular concentration of intermediates and end-products in the turnover and de novo pathways (Figure 3) are summarized.

Sphingolipid analogue biomodulators

Fungal metabolites having structural similarity to sphinganine (Figure 5) are now known to be produced by eight separate genera of fungi. It is likely that many more fungal metabolites with some of these structural features will be found. In addition to sphinganine analogue fungal metabolites, there are a number of bioactive long-chain base analogues produced by the sponge *Penares sollasi* (10). The first discovered naturally-occurring inhibitor of sphingolipid biosynthesis was fumonisin B_1 (11). To date, all fumonisins and AAL-toxins which contain a free amino group have been found to inhibit ceramide synthase (12,13). Structural similarity to sphingoid bases is not necessary for a fungal metabolite to disrupt sphingolipid metabolism; for example australifungins (14) bear little obvious similarity to sphingolipids..

Several fungal metabolites have been shown to inhibit serine palmitoyltransferase (15,16,17), the first enzyme in de novo sphingolipid biosynthesis (Figure 5). As with inhibitors of ceramide synthase, structural similarity to a sphingoid base is not necessary for a compound to be an inhibitor of serine palmitoyltransferase; for example haloalanines and cycloserine which inhibit many pyridoxal 5'-phosphate-dependent enzymes are not structurally similar.

There are several other inhibitors which have been used to elucidate the role of sphingolipids in regulating cell function and disease. The synthetic keto amine, D-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) is an irreversible inhibitor of glucosylceramide synthase (cerebroside synthase) probably through covalent binding to the synthase (18). The synthetic fumonisin analog 1-deoxy-5-dehydroxy- *threo*-sphinganine (Figure 5) is both a substrate and an inhibitor of ceramide synthase (13). It is a much poorer substrate than D-erythro-sphinganine; the stereoisomer found in mammalian cells. DL-*threo*-sphinganine has been used to inhibit sphingosine kinase (6) and N-oleoylethanolamine (19) and D-*erythro*-2(N-myristoylamino)-1-phenyl-1-propanol (20) (D-e-MAPP) are inhibitors of ceramidase.

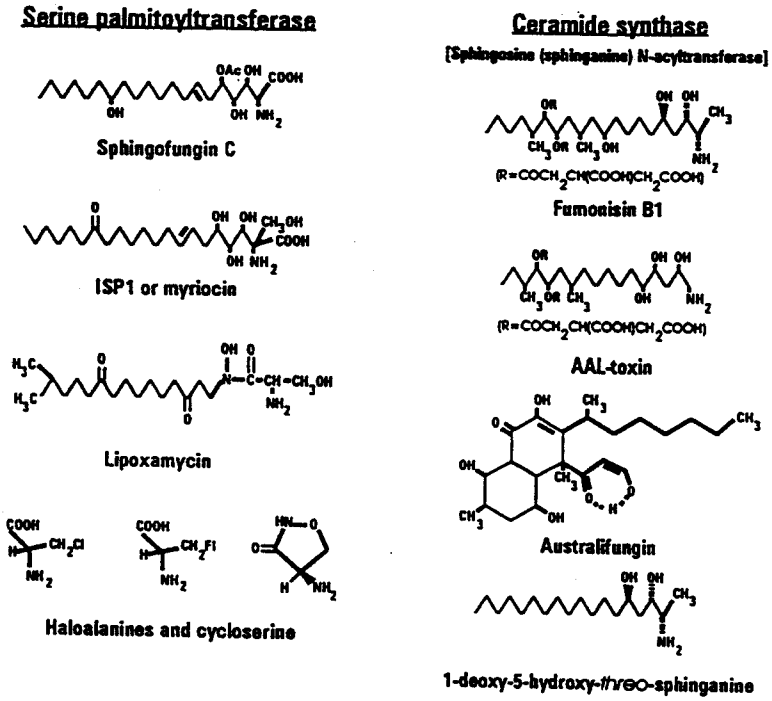

Figure 5. Naturally occurring and synthetic inhibitors of key enzymes in the de novo sphingolipid biosynthetic and turnover pathways.

Use of the inhibitors described above results in changes in the biosynthesis (Figure 3) and turnover (Figure 4) of sphingolipids in cells. These changes can be measured either by alterations in uptake and incorporation of radiolabeled precursors, decrease in radiolabeled substrates, or direct quantification of specific lipids. The use of combinations of inhibitors has also proved useful. For example, co-incubation of a ceramide synthase inhibitor and serine palmitoyltransferase inhibitor reduces many of the effects of the ceramide synthase inhibitor on free sphingoid base elevation (21,22) but has an additive effect on the decrease in complex sphingolipid concentration (22).

Sphingolipid analogue biomodulators and disease - an example

There are many ways that toxin-induced alterations in sphingolipid metabolism can result in altered cellular physiology leading to cellular dysfunction (pathophysiology). In order to fully understand the possibilities, it is necessary to consider the multitude of functions of complex sphingolipids and the potent bioactivity of long-chain bases and their metabolites (Figures 1 and 2). Many specific biochemical targets and associated downstream effects have been identified (1,2). Inhibition of de novo biosynthesis and turnover of sphingolipids causes marked increases or decreases in specific sphingolipids or classes of sphingolipids depending on the enzyme that is targeted (Figures 3 and 4). Accurate prediction of which sphingolipids will be affected requires a precise knowledge of the sphingolipid pathways and how these pathways interact with other metabolic pathways (ie phosphoglycerolipid metabolism).

In cultured cells, inhibition of serine palmitoyltransferase with ISP-1 (Figure 5) is associated with reduced cell growth and increased apoptosis (23). The effects on cell growth and apoptosis are prevented by addition of exogenous sphingosine which when supplemented at higher concentrations also inhibits cell growth and induced apoptosis. Studies using Chinese hamster ovary cells with mutated serine palmitoyltransferase (24) and studies with PDMP (9) or D-e-MAPP (20) suggest that the loss of complex sphingolipids leads to increased cell death and altered cell growth. Haloalanines have also been shown to inhibit cell growth and increase cell death in

LLC-PK1 cells (22). There are no natural diseases associated with consumption of serine palmitoyltransferase inhibitors and no animal feeding studies have been reported; feeding studies are clearly warranted.

Fumonisins clearly cause animal disease (25) and the disruption of sphingolipid metabolism is closely correlated with their in vivo toxicity (26,27). In vitro studies with cultured neuronal cells, renal cells, fibroblasts, and keratinocytes have shown that either reduction of free sphinganine with serine palmitoyltransferase inhibitors (haloalanines) or supplementation of growth medium with ceramide completely or partially reverses the effects of fumonisin B_1 on cell growth and/or cell death (21,22,28,29).

The following facts make the case for inhibition of de novo sphingolipid biosynthesis being linked to the diseases associated with exposure to fumonisins (26,27). First, in vivo alterations in free sphingoid bases can be detected before or at the same time as ultrastructural lesions. Second, there is a close dose response relationship between fumonisin levels in naturally contaminated diets or diets containing pure fumonisin B1 and the degree of elevation of free sphingoid bases and depletion of complex sphingolipids. Third, changes in free sphingoid bases are detected prior to elevation of serum biochemical parameters and at lower doses. Fourth, in vitro studies have shown that either reduction of free sphinganine with haloalanines or supplementation of growth medium with ceramide reduces the effects of fumonisin B_1 on cell growth and/or cell death.

Co-administration of a serine palmitoyltransferase inhibitor such as ISP-1 should reduce the fumonisin-induced increase in free sphingoid base concentration in vivo and might reduce the in vivo hepatotoxicity or nephrotoxicity of fumonisins although this experiment has not yet been conducted. If fungal inhibitors of serine palmitoyltransferase prevent the fumonisin-induced increase in free sphingoid bases in vivo but do not reduce the fumonisin nephrotoxicity or hepatotoxicity, then elevation in free sphinganine could be discounted as a direct cause of the toxicity. Likewise if serine palmitoyltransferase inhibitors deplete more complex sphingolipids without inducing nephrotoxicity or hepatoxicity, then the role of more complex sphingolipid depletion in these fumonisin-induced toxicities would be unlikely. Thus, studies of the interaction of fungal serine palmitoyltransferase inhibitors and ceramide synthase inhibitors in vivo will prove interesting.

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