MINIREVIEW

⌘ *Author's Choice*

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1-Deoxysphingolipids Encountered Exogenously and Made *de Novo*: Dangerous Mysteries inside an Enigma*

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The traditional backbones of mammalian sphingolipids are 2-amino, 1,3-diols made by serine palmitoyltransferase (SPT). Many organisms additionally produce non-traditional, cytotoxic 1-deoxysphingoid bases and, surprisingly, mammalian SPT biosynthesizes some of them, too (*e.g.* 1-deoxysphinganine from L-alanine). These are rapidly *N*-acylated to 1-deoxy-"ceramides" with very uncommon biophysical properties. The functions of 1-deoxysphingolipids are not known, but they are certainly dangerous as contributors to sensory and autonomic neuropathies when elevated by inherited SPT mutations, and they are noticeable in diabetes, non-alcoholic steatohepatitis, serine deficiencies, and other diseases. As components of food as well as endogenously produced, these substances are mysteries within an enigma.

The 2-amino, 1,3-diol moieties of sphingosine (Fig. 1*A*) were first described in a letter to the editors of *The Journal of Biological Chemistry* from H. E. Carter and colleagues in 1942 and then in a full manuscript (1). The term "sphingolipid" was also proposed (2) for this category of compounds, building on the "sphingo-" morpheme chosen by J. L. W. Thudichum in naming "sphingosin" for " . . . the many enigmas which it presented to the inquirer . . . " (3).

Sphingosine is the prevalent member of a family of traditional sphingoid bases (Fig. 1*A*) found in complex sphingolipids such as ceramide (Cer),[2] sphingomyelin (SM), glycosphingolipids, etc. that are important for cell structure and signaling (4). Many organisms, such as fungi, bivalves, and sponges, *inter alia*, additionally have 1-deoxysphingolipids (1-deoxySL) (Fig. 1, *B* and *C*) (5), and this minireview provides an introduction to these fascinating compounds, and especially ones now known to be made by mammals (6–8).

Examples of 1-Deoxy-sphingoid Bases

Sphinganine Analog Mycotoxins

The most extensively studied 1-deoxy-sphingoid bases are represented by fumonisin B_1 (FB_1, Fig. 1*C*). These sphinganine analog mycotoxins are produced by *Fusarium verticillioides* and related fungi (9) that infest maize and cause diseases in plants (10) and in animals that consume contaminated food (11–14). Their major biochemical targets in both plants (10) and animals (11–13, 15) are ceramide synthases (CerS), enzymes responsible for *N*-acylation of sphingoid bases (16, 17). In addition to being inhibitors of CerS, fumonisins are *N*-acylated by CerS (18), as is the aminopentol backbone released from fumonisins when corn is treated with lye in preparation of masa (19). *N*-Acyl-aminopentols also inhibit CerS.

Disruption of sphingolipid metabolism by fumonisins and related AAL toxins (from *Alternaria alternata*) (10) induces plant programmed cell death pathways associated with defense and disease (20, 21). This is thought to be a major reason that these mycotoxins are produced, but they might additionally provide protection against other inhabitants of the ecological niche of these fungi (22).

Fumonisin consumption causes a wide spectrum of animal disease: hepatotoxicity and hepatocarcinogenicity, renal toxicity, neurotoxicity, pulmonary edema (9, 23), and in humans, esophageal cancer (9, 11–14) and probably birth defects (13, 24, 25). It is not surprising that they produce so many disorders because CerS inhibition causes buildup of highly bioactive compounds (sphinganine, sphinganine 1-phosphate, *N*-acetylsphinganine, and others) and suppresses biosynthesis of Cer and complex sphingolipids, depending on the length of exposure and dosage (11, 12). FB_1 is often used as a tool to block Cer production and study Cer functions; however, the results must be interpreted with caution because this alters many other bioactive sphingolipids.

Oceanin, Calyxin, and Other Complex 1-Deoxy-sphingoid Bases

Perhaps the most structurally amazing 1-deoxy analogs are "two-headed", *i.e.* appearing as if two sphingoid bases are connected tail-to-tail (see oceanapiside from *Oceanapia phillipensis* (26, 27), Fig. 1*C*). These compounds often display antibacterial or antifungal activity, which might be their biologic function; many are cytotoxic for cancer cells (27–32). These compounds illustrate only a fraction of the sphingoid base biodiversity (5).

Simple 1-Deoxysphingoid Bases, e.g. 1-Deoxysphinganines and Related Compounds

Many organisms have been known to produce simple 1-deoxy- and 1-deoxymethyl-sphingoid bases (5) (Fig. 1, *B* and *C*)

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[2] The abbreviations used are: Cer, ceramide; 1-deoxyDHCer, 1-deoxydihydroceramides (*N*-acyl-1-deoxysphinganine); 1-deoxy(DH)Cer, 1-deoxyceramides and 1-deoxydihydroceramides; 1-deoxySL, 1-deoxysphingo-lipids (all compounds lacking 1-hydroxyl on sphingoid base); CerS, ceramide synthase; DHCer, dihydroceramide; (DH)Cer, ceramides and dihydroceramides; FB_1, fumonisin B_1; HSAN1, hereditary sensory and autonomic neuropathy type I disease; PLP: pyridoxal 5′-phosphate; S1P, sphingosine 1-phosphate; SM, sphingomyelin; SPT, serine palmitoyltransferase; NASH, non-alcoholic steatohepatitis; C_6-NBD, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)aminohexanoyl; ssSPT, small subunit SPT; h, human.

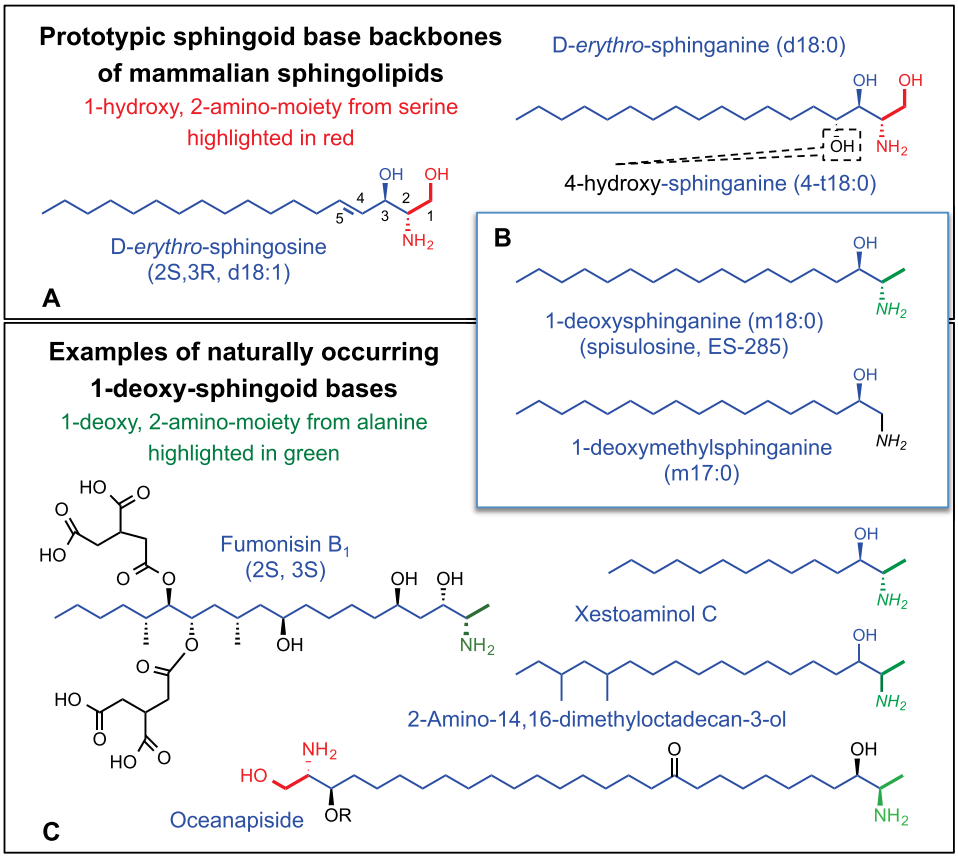

FIGURE 1. **Representative sphingoid bases and 1-deoxysphingoid bases.** *A*, three of the traditional sphingoid bases of mammalian sphingolipids: sphingosine, sphinganine, and 4-hydroxysphinganine (phytosphingosine). There is also some degree of variation in the alkyl chain length, branching, and number of additional double bonds and hydroxyls (not shown). *B*, two simple 1-deoxy-sphingoid bases that are produced by mammals and other organisms; these are also known to vary in chain length and double bonds. *C*, some of the broader structural variation in 1-deoxy-sphingoid bases produced by other organisms. For all of the panels, these structures have been highlighted in *red* to display the portions that are derived biosynthetically from serine, and in *green* for alanine. Some additional information for the compounds has been given in *parentheses* (stereochemistry and alternative names and abbreviations). For more information, see the text and Ref. 5.

such as xestoaminol C from *Xestospongia* sp.) and a methyl-branched 1-deoxysphinganine(2-amino-14,16-dimethyl-octadecan-3-ol, 2-AOD-3-ol), produced by *Fusarium avenaceum*, a fungus found on grains and fruit (33, 34). 1-Deoxysphinganine (Fig. 1*B*) was initially named spisulosine when isolated from the edible Stimpson's surf clam, or Atlantic surf clam (*Spisula polynyma*), during a screen for anticancer compounds (35). Being cytotoxic for cancer cells in culture (36, 37), it has been evaluated in phase I clinical trials, which will be described later in this minireview.

Mammalian Production of 1-Deoxysphingoid Bases

Considering the unusual structural features and cytotoxicity of 1-deoxysphingoid bases, it came as a surprise when mammals, including humans, were found to produce them, as shown by two independent lines of investigation published at approximately the same time (6–8). One study discovered that mutations in the initial enzyme of traditional sphingoid base biosynthesis (serine palmitoyltransferase, SPT) that cause hereditary sensory and autonomic neuropathy type I disease (HSAN1) allow SPT to utilize L-Ala and glycine to make 1-deoxysphinganine and 1-(deoxymethyl)sphinganine, respectively (7), which are neurotoxic when added to dorsal root ganglia neuron cultures (8).

The other study (6) characterized 1-deoxysphinganine as a previously noticed (38), but unidentified, compound that accumulates when cells in culture or animals are exposed to FB_1. It was shown to be produced in substantial amounts from L-Ala by wild-type SPT and was probably overlooked previously because it is mainly present as *N*-acyl-metabolites (*e.g.* 1-deoxydihydroceramides, 1-deoxyDHCer) unless CerS is inhibited.

Background Information about SPT

SPT is a family of pyridoxal 5′-phosphate (PLP)-dependent isozymes that catalyze the reaction displayed in Fig. 2 (39, 40). Its proposed mechanism is typical for the α-oxoamine synthase (AOS) family (41), and some of the main features are: formation of a Schiff base between PLP and an active site Lys (called an "internal aldimine"); displacement of Lys when an amino acid substrate is bound (forming the "external aldimine"); orientation of the amino acid-PLP imine in a configuration described as the "Dunathan intermediate" to facilitate abstraction of the amino acid α-proton forming a quinoid intermediate; carbon-carbon bond formation between the amino acid α-carbon and a fatty acyl-CoA, displacing CoASH; decarboxylation of this β-unsaturated intermediate to form a product, external ketimine; protonation of the ketimine to form the external aldimine of the 3-keto-sphingoid base, which is released to regenerate

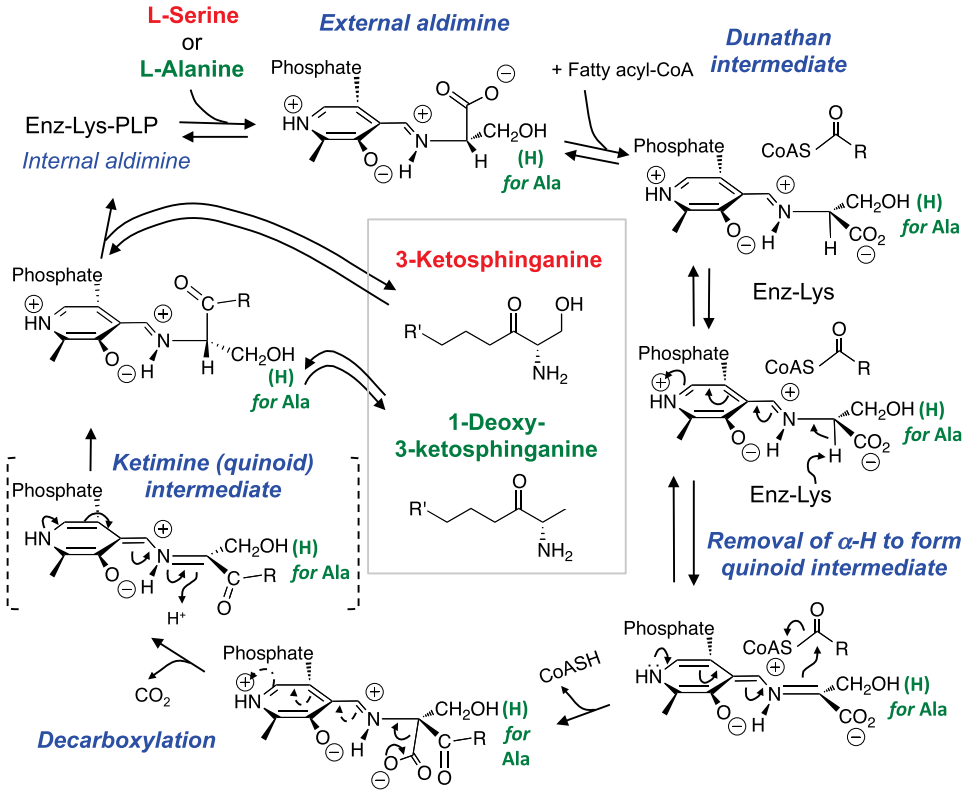

FIGURE 2. **Scheme for the utilization of L-serine or L-alanine for 3-ketosphingoid base biosynthesis by serine palmitoyltransferase.** This diagram has been modified from Ref. 39 to illustrate the proposed catalytic mechanism for this enzyme and how the intermediates involved in the condensation of L-Ser to make 3-ketosphinganine could plausibly be substituted by L-Ala to make 1-deoxy-3-ketosphinganine with minor variations in the active site chemistry. For all of the panels, these structures have been highlighted in *red* to display the portions that are derived biosynthetically from serine, and in *green* for alanine.

the enzyme PLP internal aldimine. The traditional reaction catalyzed by SPT utilizes L-Ser as the substrate to make 3-ketosphinganine (in *red*), the analogous reaction with L-Ala (*green*) produces 1-deoxysphinganine, and the reaction with glycine produces 1-(deoxymethyl)sphinganine (not shown).

Mammalian SPT appears to be composed of heterotrimeric isozymes that share an SPTLC1 (sometimes referred to as SPT1 or hLCB1) subunit combined with either SPTLC2 (also called SPT2 or hLCB2a) or SPTLC3 (also called SPT3, LBC3, or hLCB2b) subunit and one of two highly related isoforms of a third "small subunit" (in humans, ssSPTa and ssSPTb) (40). The active site Lys (Fig. 2) resides in the SPTLC2/SPTLC3 subunit.

The SPTLC2/SPTLC3 subunit influences the specificity for the acyl-CoA substrate (42) in a manner that also depends on the ssSPT isoform (43, 44). That is, as shown in studies in which cells were transfected with these isoforms in different combinations (43, 44): SPTLC1/SPTLC2/ssSPTa had a clear preference for palmitoyl-CoA (the precursor for the 18-carbon-chain length sphingoid bases); SPTLC1/SPTLC2/ssSPTb utilized both palmitoyl-CoA and stearoyl-CoA (the latter producing 20-carbon-chain length sphingoid bases); SPTLC1/SPTLC3/ssSPTa utilized myristoyl-CoA and palmitoyl-CoA (the former producing 16-carbon-chain length sphingoid bases); and SPTLC1/SPTLC3/ssSPTb seems to use a wide range of chain length fatty acyl-CoAs. Another level of regulation involves ORMDL family proteins, which have been proposed to help control flux through the pathway (45–48).

Production of 1-Deoxysphingoid Bases by Mutant SPT

HSAN1 neuropathies have been linked to mutations in five different genes, two of which code for SPTLC1 and SPTLC2. These involve missense mutations (49): for SPTLC1, C133W, C133Y, C133R, V144D, A352V, S331F, and S331Y; and for SPTLC2, V359M, G382V, T409M, I504F, A182P, and a more recently reported S384F (50). After the report of elevated 1-deoxySL from L-Ala in studies of the SPTLC1 C133W mutation in humans and transgenic mice (7), 1-deoxySL have been found in other SPT HSAN1 mutations (8, 51).

The kinetic properties of wild-type and mutant SPT have been compared using microsomes from yeast transfected with cDNA for SPTLC1/SPTLC2/ssSPTa *versus* SPTLC1-C133W/SPTLC2/ssSPTa (44), and the major conclusions have been substantiated by studies with CHO-LyB cells, a mammalian cell line with an unstable and inactive SPTLC1 subunit (52). The K_m for L-Ser for mutant SPT was higher than for the wild-type enzyme (~1.4 *versus* 0.75 mM, respectively), and the V_{max} was lower (~0.3 *versus* 1.4 nmol/mg/min); conversely, the mutant SPT utilized L-Ala better than the wild-type. The K_m and V_{max} with L-Ala were ~9.6 mM and ~0.1 nmol/mg/min for mutant SPT, and it was difficult to measure the kinetics with L-Ala using the wild-type enzyme. The K_i for L-Ala inhibition of L-Ser utilization was 5 mM for the mutant SPT and 2 mM for wild type. These results suggest that the major effect of this HSAN1 mutation is not to facilitate L-Ala binding but to allow bound L-Ala to react with the acyl-CoA substrate. Although crystal structures

are not yet available for mammalian SPT, they have been determined for a soluble homodimeric SPT from *Sphingomonas paucimobilis* EY2395 (53) and were used to map Cys-133 of SPTLC1 onto Asn-100 of the bacterial SPT, which is proximal to the PLP binding site and lies at the dimer interface (39).

A similar approach has been used to analyze V359M, G382V, and I504F mutations in SPTLC2 (54), and all decrease enzyme activity somewhat for reasons that can be rationalized by comparisons with alterations in the soluble enzyme. The impact of these mutations on L-Ala utilization was not reported, but 1-deoxySL have been associated with SPTLC2 mutations A182P (55) and S384F (50). The S384F mutation was suggested to implicate phosphorylation of SPTLC2 at this site as a regulator of 1-deoxySL synthesis by wild-type SPT.

To determine whether lowering 1-deoxySL might be clinically beneficial, Garofalo *et al.* (56) fed a 10% L-Ser-enriched diet to mice bearing a transgene expressing C133W SPTLC1, and 1-deoxySL decreased significantly, reaching the levels of mice with wild-type SPT within 2–4 days. Mice on the L-Ser-enriched diet were also protected from neurodegeneration (measured by mechanical sensitivity and motor performance) and retained neurological function up to 15 months of age; untreated mice developed neuropathy by that age. In contrast to these favorable responses, mice fed a 10% L-Ala diet had elevated 1-deoxySL and developed severe peripheral neuropathy. A pilot study with HSAN1 patients also found that L-Ser supplementation reduced 1-deoxySL levels, and a clinical trial is ongoing (https://clinicaltrials.gov).

Production of 1-Deoxysphingoid Bases by Wild-type SPT

In the other early study, 1-deoxySL were identified as products of wild-type SPT by mass spectrometry (6), which characterized both the free 1-deoxysphinganine in cells incubated with FB_1 and the *N*-acyl-derivatives when CerS was not inhibited. This acylation might explain why these compounds have been overlooked previously because they are somewhat difficult to detect in a background of Cer and other neutral lipids.

Wild-type SPT was proven to be the source because biosynthesis of 1-deoxySL from L-Ala was absent in CHO-LyB cells and reappeared when the normal SPT1 subunit was restored (6). The amounts of 1-deoxysphinganine made by wild-type SPT can be quite substantial. For example, LLC-PK1 cells have about half of the level of sphinganine after ~4 days in culture with FB_1; Vero cells have high basal 1-deoxySL, which might be due to these cells depleting L-Ser in the medium (57); and RAW264.7 cells (58) have essentially equal amounts of 1-deoxyDHCer and Cer after 4 days in culture, and are also known (59) to deplete the culture medium of L-Ser while accumulating L-Ala and glycine.

There has not yet been an explanation for why wild-type SPT is somewhat "promiscuous" (to use a term applied to mutant SPT) (44) in utilizing three amino acids as substrates, nor whether this might occur with other α-oxoamine synthase family members (40). The side chains of L-Ala and Gly are smaller than the hydroxymethyl group of L-Ser and could fit in the same binding pocket. The favoring of L-Ser appears to be due to an interaction between the side-chain hydroxyl of L-Ser and the 5′-phosphate of PLP, both for substrate binding and for optimal catalytic efficiency (60).

Because amino acid availability is an important factor in the amounts of 1-deoxySL that are made, it would be interesting to know more about other factors that are thought to influence L-Ser utilization for lipid synthesis, such as SERINC (61) and, at least for yeast, CHA1, which codes for an L-Ser deamidase/dehydratase that regulates sphingolipid levels by limiting available L-Ser (and perhaps vice versa) (62).

Metabolism and Trafficking

1-Deoxysphingoid Base Metabolism

Most publications on 1-deoxySL have described them as total 1-deoxysphinganines or 1-deoxySL rather than as individual molecular species because they have been quantified after acid hydrolysis to release the free sphingoid bases. As noted above, when specific molecular species are analyzed by LC-MS/MS, the majority of traditional and 1-deoxysphingoid bases are *N*-acyl-derivatives (Fig. 3) (4).

In this pathway, the initially formed 3-keto-intermediates are rapidly reduced and *N*-acylated followed by the addition of a headgroup (dihydroSM, etc.), desaturation to produce the backbone double bond (making Cer), and then the addition of headgroups *or* hydrolyzed to sphingosine, which can be reacylated or converted to sphingosine 1-phosphate (S1P), which is cleaved to ethanolamine phosphate and hexadecenal (hexadecanal from sphinganine 1-phosphate). There are also reports of *N*-methylation of some sphingoid bases (63, 64).

The early steps of this pathway appear to be similar for the 1-deoxysphingoid bases (Fig. 3). The kinetics parameters for *N*-acylation of various sphingoid base variants have been compared using rat liver microsomes (19). The apparent K_m for 1-deoxysphinganine (2 μM) is somewhat higher than for sphinganine (0.5 μM), but the V_{max} values are similar. Individual CerS have not been analyzed, but the *N*-acyl-chain length distributions of 1-deoxy(DH)Cer of different types of cells suggest that most or all of the CerS accommodate these compounds (65). Little is known about desaturation of 1-deoxy(DH)Cer; likewise, the possibility of alternative metabolites, such as *N*-methylated species, has not been explored. Turnover by lyase cleavage (Fig. 3) would appear to be unavailable to 1-deoxySL unless S1P lyase, or another enzyme, can catalyze an analogous reaction with 1-deoxysphingoid bases.

1-Deoxydihydroceramide Trafficking

The intracellular trafficking of Cer has been studied using analogs with an amide-linked fluorescent fatty acid, such as *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)aminohexanoyl- (C_6-NBD-) (66). C_6-NBD-Cer is rapidly taken up by cells in culture, and fluorescence is seen first in multiple intracellular compartments (the plasma membrane, ER, nuclear envelope, and mitochondria), and then the Golgi apparatus becomes intensely fluorescent concomitant with its metabolism to C_6-NBD-SM and C_6-NBD-GlcCer, which appear at the plasma membrane after longer times. In contrast, C_6-NBD-1-deoxyDHCer (67) was neither metabolized nor labeled the Golgi apparatus and plasma membrane, even after prolonged incubation. Thus, the 1-deoxySL do not appear to undergo the typical trafficking of

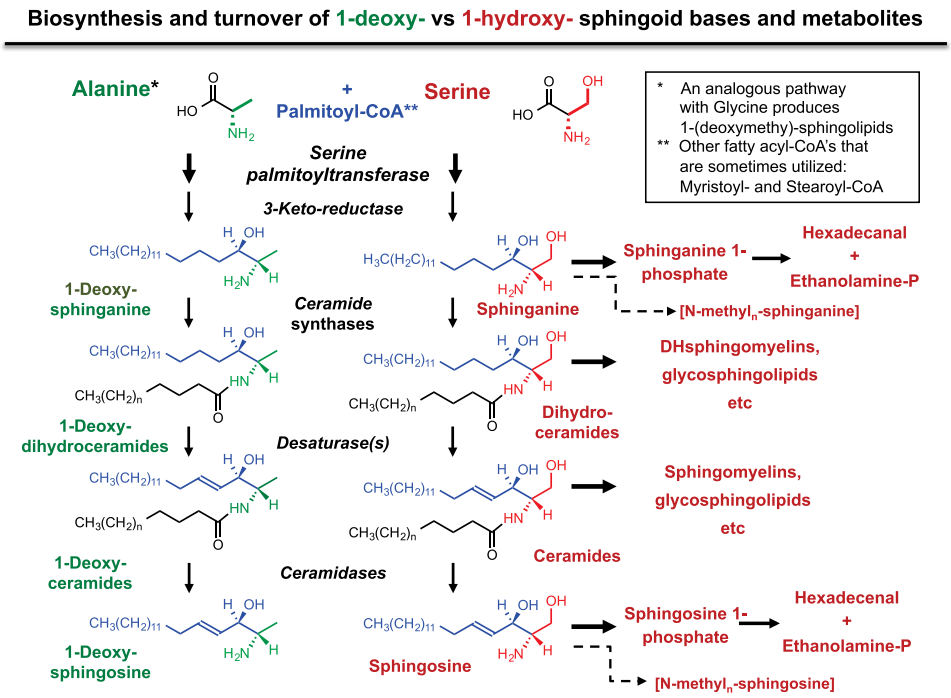

FIGURE 3. **Abbreviated pathway for the biosynthesis and turnover of 1-deoxy-sphingoid bases and traditional sphingoid bases.** This scheme summarizes the steps of *de novo* biosynthesis of traditional sphingoid bases (sphinganine and sphingosine) in *red*, as well as their turnover via phosphorylation and cleavage. The carbons from palmitate are shown in *blue*. The analogous metabolic steps are shown in *green*, as far as they are thought to occur-for 1-deoxy-sphinganine (produced from alanine) and 1-(deoxymethyl)sphinganine (produced from glycine, not shown). The *dashed line* indicates the known *N*-methylation of sphingoid bases, which might also occur for 1-deoxy-sphingoid bases, but this has not yet been established. For more information, see the text and Ref. 4.

traditional Cer, which is in agreement with similar studies with a 1-methoxy analog (68). The 1-deoxySL in plasma (69, 70) appear to be associated mainly with lipoproteins (71), which might be of hepatic origin (70).

Cellular Effects of 1-Deoxy-sphingolipids

Some of the earliest findings with 1-deoxysphinganine were that it has diverse effects on cell growth and survival: sometimes stimulating cell proliferation (for Swiss 3T3 cells at 1 μM) (72); sometimes inhibiting growth (for Vero cells at ~2 μM), possibly due to disruption of actin stress fibers through inactivation of Rho (35), and for the human glioblastoma cell line SHG-44 (73); and often displaying cytotoxicity at low micromolar concentrations for DU145 and LLCPK1 cells (6), MDA MB 468 cells (65), and PC-3 and LNCaP cells (37), as examples. The cytotoxicity has been proposed to have several causes: stimulation of *de novo* synthesis of Cer and PKCζ activation (37); and an atypical cell death program with activation of caspase 3 and 12 and altered phosphorylation of p53 (36). Endoplasmic reticulum stress might also have a role in 1-deoxySL-mediated apoptosis (44, 74). Effects on insulin-producing cells (75) include compromised glucose-stimulated insulin secretion, intracellular accumulation of filamentous actin, activation of Rac1, increased CerS5 expression, and morphologic changes characteristic of senescent, necrotic, and apoptotic cells.

Other reported effects of 1-deoxySL are sphingosine kinase 1 inhibition (and/or its proteasomal degradation) (76) and perturbation of membrane structure because 1-deoxy(DH)Cer are poorly miscible with other lipids (some 1-deoxySL are not even capable of forming monolayers at the air-water interface) (58). The latter might contribute to the formation of lipid bodies in cells accumulating 1-deoxySL (77). Another intriguing finding is that 1-deoxy-(DH)Cer have been reported to be one of the endogenous ligands for human CD1b antigen-presenting molecules (78). As a cautionary note, it is difficult to determine what the normal functions of 1-deoxSL are because studies with cells in culture begin with cells that probably already contain abnormally high 1-deoxySL because they are present in serum and/or produced by the cells themselves, due to the tendency of many cell lines to deplete L-Ser and accumulate L-Ala in the medium.

1-Deoxy-sphingoid Bases and Other Disease

Diabetes

In common with HSAN1, one of the clinical complications of diabetes mellitus is sensory neuropathy; therefore, connections between 1-deoxySL and diabetes have been explored. A case-control study of plasma from healthy and diabetic individuals found that 1-deoxySL levels were higher in the diabetic group, which also displayed lower plasma Ser (71). 1-DeoxySL have been found to be elevated in plasma from subjects with metabolic syndrome (79) and type 2 diabetes (80) (levels in type 1 diabetes did not differ from controls). 1-DeoxySL were also examined as possible predictive biomarkers for type 2 diabetes (81) in a prospective cohort with 339 individuals who were followed for a period of 8 years, and levels were elevated in patients with metabolic syndrome, impaired fasting glucose, and type 2 diabetes and for patients who developed diabetes during the follow-up period. 1-DeoxySL levels were found to be signifi-

cantly elevated in plasma from patients with distal sensorimotor polyneuropathy, a frequent, disabling complication of diabetes mellitus, and were detectable in early disease stages but did not correlate with the clinical course (82).

In analogy to the studies conducted with an animal model for HSAN1, L-Ser supplementation has been tested in streptozotocin-induced diabetic rats (81). This intervention not only lowered plasma 1-deoxySL but also improved mechanical sensitivity, in agreement with the hypothesis that 1-deoxySL are involved in the pathology of diabetic neuropathy and L-Ser supplementation might be clinically beneficial. It is worth mentioning that plasma L-Ala is elevated following glucose ingestion (83), and fructose ingestion has an even greater effect on plasma L-Ala concentration (84).

Non-alcoholic Fatty Liver Disease, Especially Non-alcoholic Steatohepatitis (NASH)

Non-alcoholic fatty liver disease is associated with metabolic syndrome and is becoming one of the most common forms of liver disease worldwide. It is thought to progress from relatively benign stages to steatohepatitis (NASH), which can develop into end-stage liver disease, cirrhosis, and sometimes hepatocellular carcinoma. A recent double-blinded study of plasma, liver biopsies, and urinary lipids from 88 subjects with liver histology categorized as normal, steatotic, NASH, or cirrhotic (70) found that a diverse panel of 20 plasma lipids and aqueous metabolites separated these states by linear discriminant analysis, with the compounds that gave the greatest distinction between NASH and steatosis including the 1-deoxyDHCer. A possible explanation for this association might be L-Ser deficiency that has been reported for NASH (85).

Defective Ser Biosynthesis

L-Ser is made *de novo* by a pathway initiated by D-3-phosphoglycerate dehydrogenase (PHGDH), and mice carrying a brain-specific deletion of *Phgdh* have been used to study the effects of defects in this pathway on 1-deoxySL (77). The mice displayed reductions in both L-Ser and D-Ser and elevation of 1-deoxySL that were associated with mild microcephaly and atrophy of the forebrain, including the cerebral cortex and hippocampus. No significant changes in traditional Cer and SL were noted. Because humans with genetic defects in this enzyme exhibit Ser deficiency and severe neurological symptoms, these results raise the possibility that 1-deoxySL might be involved in the central neurological symptoms (77).

TNF-dependent Toxicity via Caspase Signaling in Dopaminergic Neurons

Dopaminergic neurons in the ventral midbrain selectively degenerate in Parkinson disease, and TNF can increase neuronal cell death. TNF treatment of dopaminergic neurons has been found to increase 1-deoxySL, which reduce cell viability and inhibit neurite outgrowth and branching in primary dopaminergic neurons when added exogenously (86). Therefore, induction of *de novo* biosynthesis of 1-deoxySL might be involved in the neurotoxicity of TNF for dopaminergic neurons.

1-Deoxy-sphingoids as Therapeutic Agents?

Clinical Trials with Atypical Sphingoid Bases

The first structural variant that was evaluated in a phase I clinical trial (87) was Safingol (L-*threo*-sphinganine), which has 2*S*,3*S* stereochemistry as found in fumonisins (Fig. 1*C*). This is not a 1-deoxySL but inhibits sphingosine kinase and affects some of the same targets as 1-deoxysphinganine (88). The maximum tolerated dose was 840 mg/m^2 (~1–2 g based on adult body surface areas of ~1.5–2 m^2) administered intravenously over 120 min, with the dose-limiting toxicity attributed to hepatic enzyme elevation. Plasma S1P was reduced for Safingol doses of 750–930 mg/m^2. Of the 37 patients that were evaluated for response, six were reported to have some degree of disease stabilization, and one patient with adrenal cortical cancer had regression of liver and lung metastases.

Several phase I clinical trials have been conducted with 1-deoxysphinganine (named "ES-285"), and relevant findings from two will be mentioned here. From dose-escalating studies (89, 90), the maximum tolerated dose was ~200 mg/m^2 (*i.e.* ~0.3–0.4 g for body surface areas of 1.5–2 m^2, respectively). The dose-limiting toxicities were relatively consistent for all the studies: hepatic and neurological toxicity as well as injection site reactions. One patient who received eight infusions of ES-285 at 128 mg/m^2 developed numbness of the face, hands, and feet that worsened rapidly to neuropathy, pain, and general weakness that was assessed to contribute to his death (90). Clinical development of ES-285 as a single agent was discontinued due to its questionable safety profile and limited antitumor activity. Noteworthy from these trials was the similarity in the adverse effects and the neuropathies that have been associated with elevations in 1-deoxySL produced *de novo*.

Animal Studies with a Synthetic 1-Deoxy-sphingoid Base

A synthetic 1-deoxysphingoid base, named Enigmol (Fig. 4), displayed tumor suppression with little toxicity when administered to mouse models for colon and prostate cancer (91, 92). Enigmol is not phosphorylated and is poorly *N*-acylated (93), and one of the most interesting findings from these *in vivo* studies was a high oral bioavailability *versus* traditional sphingoid bases. The likely explanation for this difference, which might apply to other 1-deoxy-sphingoid bases, is shown in Fig. 4. Traditional sphingoid bases are readily taken up by intestinal cells but mainly phosphorylated and degraded (94), which limits their effectiveness against colon cancer targets, but cleavage reduces the likelihood that the intermediate S1P will promote carcinogenesis (94, 95). Lacking the 1-hydroxyl group, 1-deoxysphingoid bases (at least as exemplified by Enigmol) are absorbed, escape phosphorylation and degradation, and appear in blood and tissues (91). Another factor affecting the absorption of these compounds is efflux via P-glycoprotein (96). All in all, the possible uptake of 1-deoxySL from food highlights the need for a better understanding of their effect(s) on health.

Other Clinical Applications?

Sphingolipids and sphingoid base-like compounds, including derivatives of such compounds, have been suggested to

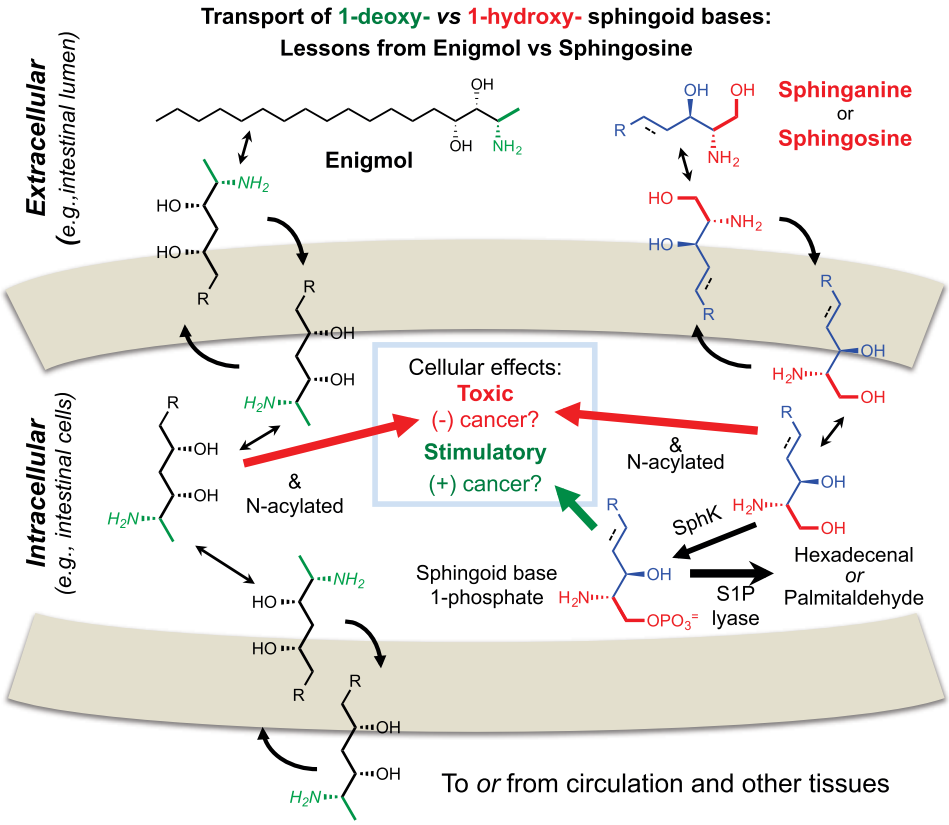

FIGURE 4. **A schematic representation of the intestinal uptake, metabolism, effects on intestinal cells, and transport to blood and tissues of traditional sphingoid bases (sphingosine and sphinganine) and a synthetic 1-deoxy-sphingoid base (Enigmol).** As shown on the *right*, traditional sphingoid bases are absorbed well from the lumen of the intestinal tract, but most are phosphorylated by sphingosine kinase (SphK) and degraded by S1P lyase. Nutritional studies have shown suppression of colon cancer by dietary sphingolipids, probably through the sphingoid base before phosphorylation and cleavage (94); however, if S1P accumulates (for example, due to defective S1P lyase), this can promote cancer (95). Shown on the *left* are findings with the synthetic 1-deoxy-sphingoid base Enigmol, which is absorbed more efficiently and transferred to blood and tissues, presumably because it cannot undergo phosphorylation and cleavage. Enigmol has also been shown to suppress intestinal tumorigenesis prostate cancer in animal models (91, 92). The color scheme is analogous to the one used in Figs. 1 and 3.

offer promise as antibacterial (97) and antifungal (98) drugs and for other diseases (99, 100).

Conclusions and Future Perspectives

The capacity to make 1-deoxy-sphingoid bases was once thought to be the purview of organisms that make bizarre secondary metabolites, but is now clearly established to be a process shared by mammals, including humans. This leads one to wonder whether these compounds are made as accidents of a sloppy *de novo* biosynthesis pathway or to perform biological functions. Also, considering their widespread occurrence, how much is present in food, and to what extent does the diet affect tissue 1-deoxySL? Because these compounds can be toxic (but possibly beneficial), how many ways do they impact health? These are some of the intriguing mysteries to be solved for this branch of a family of compounds long known for their enigmas.

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