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Solving the Riddle of the Sphinx May Provide New Insights Into Diabetes and Polyneuropathy

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COMMENTARY

The biological mechanisms of diabetic polyneuropathy (DPN) are diverse, but only limited clinical benefits are found in therapeutic approaches beyond glucose control where major impact on polyneuropathy development is demonstrated (1). In this issue of *Diabetes*, a new hope emerges with an article by Hornemann and colleagues (2) that reports that L-serine dietary supplementation showed a remarkably favorable effect on neuropathy in a diabetic streptozotocin (STZ) rat model. Not only was the neurotoxic sphingolipid byproduct, 1-deoxysphingolipid (1-deoxySL), reduced in plasma by serine supplementation but also sensory nerve function was improved by measures of *1*) mechanical sensitivity, *2*) nerve conductions, *3*) percentage of large diameter fibers/axons, and *4*) neuronal NA^+/K^+-ATPase activity. The serine-enriched diet did not affect body weight, hyperglycemia, hypertriglyceridemia, or food intake in STZ rats, directly supporting the causal relation of the deoxysphingolipids in the pathogenesis of DPN.

The complex and enigmatic nature of sphingolipids are similar to the sphinx, a mythological creature for which sphingolipids are named. This heterogeneous group of sphingolipids is unique compared to the more abundant phospholipids because their hydrophobic tails are attached to a serine rather than a glycerol molecule. They are ubiquitously expressed in eukaryotic cells and essential in signal transduction, cell metabolism, and channel localization in neural tissues (3). Sphinganine is an abundant sphingolipid intermediate that is formed with the nonessential amino acid serine serving as the substrate. In contrast, toxic 1-deoxySLs are formed when alanine or glycine is used as the substrate under atypical conditions (Fig. 1). The concentrations of 1-deoxySL subclasses have a significant impact on neural cell differentiation and survival. Elevated levels of 1-deoxySLs can lead to cell stress, a process coupled to carbohydrate metabolic pathways such as glycolysis. This has previously been shown in diabetes (4). The increased concentration of deoxysphingoid bases is also found in the LDL and VLDL fractions of plasma and serves as a useful toxic biomarker (5).

The enthusiasm for investigating the neurotoxic effect of deoxysphingolipids comes from an unlikely source, a rare inherited metabolic disorder named hereditary sensory and autonomic neuropathy (HSAN1). This disorder established the neurotoxic effects of deoxysphingolipids through explicit genetic and biochemical studies and provided hope for a rational therapy (6). Both diabetes and HSAN1 have similar components of progressive length-dependent sensory greater than motor polyneuropathy from an axonopathy, including with complications of skin ulcers and infections. Mutations in the genes encoding enzymes catalyzing the de novo sphingolipid synthesis pathway, serine palmitoyltransferase, long chain 1 and 2 (SPTLC), are found causal for HSAN1 and result in elevated ceramide levels and increased neuronal apoptosis (7,8). More important, mutant SPTLCs result in a change of substrate preference, away from canonical substrate L-serine to alanine and glycine, with resultant accumulation of the two atypical deoxysphingoid bases 1-deoxy-sphinganine and 1-deoxymethyl-sphinganine, which are cytotoxic (Fig. 1). After these deoxysphingoid bases are acylated into deoxysphingolipids, they can no longer be further metabolized to complex sphingolipids or degraded by the canonical degradation pathway. In the plasma of HSAN1 patients, deoxysphingoid levels are found at pathological levels of 10–40 nmol/L. In addition, L-serine supplementation in SPTLC mutant rodents led to modestly improved nerve conductions that correlate with reduced deoxysphingolipid concentrations (9). Trials in HSAN1 patients are under way to investigate the effect of L-serine supplementation on neuropathy, and the preliminary results in humans

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See accompanying article, p. 1035.

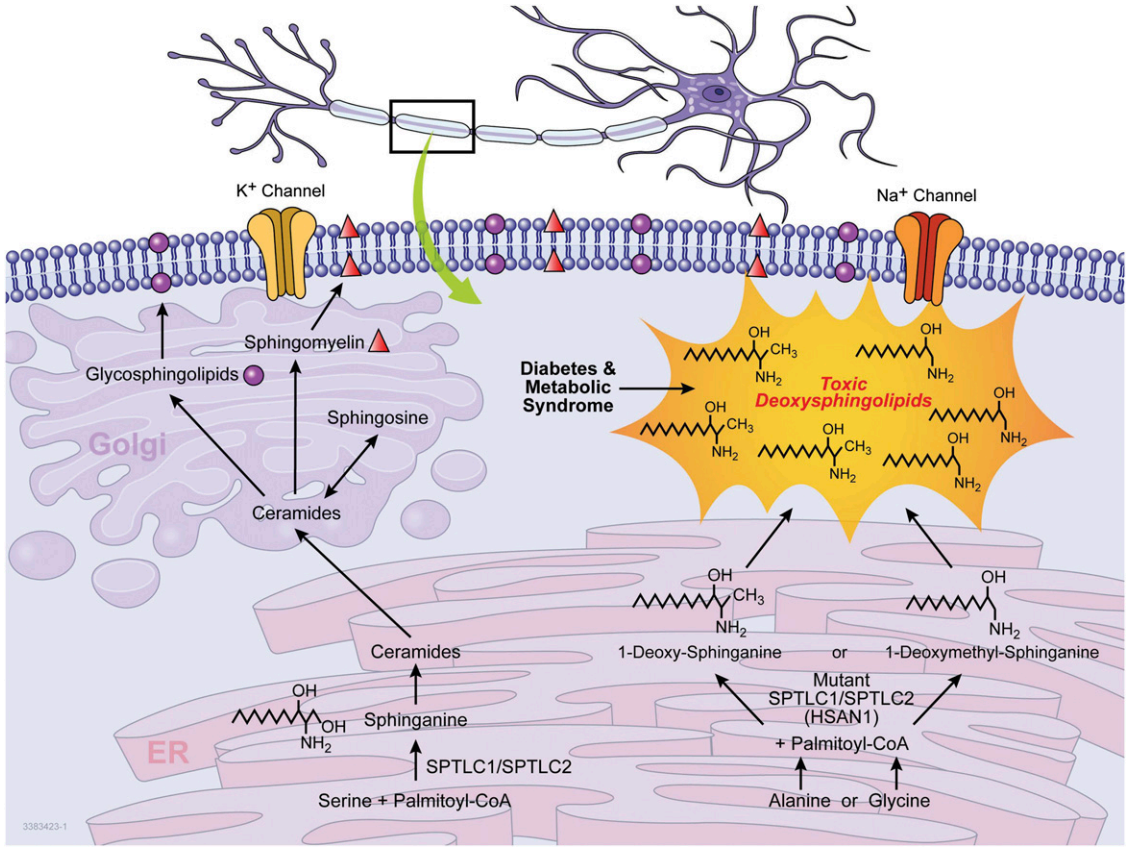

Figure 1—Formation of bioactive sphingolipids and toxic deoxy-sphingolipids in mammalian cells. Sphingolipid de novo synthesis is catalyzed by the enzyme SPTLC through the condensation of palmitoyl-CoA (or other activated fatty acid) and L-serine to form sphinganine. This condensation and ceramide synthesis process takes place in the endoplasmic reticulum (ER). In HSAN1, mutant SPTLCs prefer alanine or glycine instead of serine and form deoxy- or deoxymethyl-sphingolipids, which can no longer be metabolized to complex sphingolipids or degraded by the canonical degradation pathway, thus leading to toxic accumulation. This phenomenon is also seen in diabetes and metabolic syndrome. Under normal conditions, ceramides are transported to the Golgi where the synthesis of sphingomyelin and glycosphingolipids take place. Sphingomyelin and glycosphingolipids are then transported to the plasma membrane.

have demonstrated L-serine supplementation reduced the level of deoxysphingolipids.

The implications of elevated plasma 1-deoxySLs extend to the metabolic syndrome with or without type 2 diabetes (10). Statistical modeling therein showed that 1-deoxySLs bases contribute to the metabolic syndrome state as the second most important factor, just behind hypertriglyceridemia. Elevated triglycerides have also long been linked with varieties of idiopathic neuropathy and glucose impaired neuropathies (11,12). It will be important to investigate the pathological connection of elevated 1-deoxySLs in idiopathic polyneuropathy, especially in those people where hypertriglyceridemia is established. It is intriguing that despite comparable degrees and length of hyperglycemia, not all patients with diabetes will develop neuropathy in the same time or severity (13). Perhaps each individual has varied capability to metabolically resist deoxysphingolipids and neuropathy based on genetic background related to these pathways and diets rich in L-serine such as fish and soybeans.

Because hyperglycemia control has been the major successful intervention in prevention of DPN, it is also important to note that intracellular elevations of 1-deoxy-sphinganine associate with pancreatic islet cell cytotoxicity. Specifically, 1-deoxy-sphinganine treatment of insulin-producing Ins-1 cells led to aberrant insulin secretion, cytotoxicity, altered cytoskeleton dynamics, and upregulated ceramide synthase-5 expression, whereas the inhibition of intracellular 1-deoxy-sphinganine trafficking improved the cell survival (14). These results support that targeting deoxysphingolipids synthesis may be an effective therapeutic approach for maintaining integrity of nerve function and potentially benefiting pancreatic health, preventing downstream hyperglycemia complications.

Although the link between deoxysphingolipids and diabetic neuropathy is an exciting new focus, the pathogenesis of diabetic neuropathy will undoubtedly remain complex. Similar to β-cell pancreatic toxicity, it is clear that increased 1-deoxySLs cannot explain pathogenesis alone (14). The current animal modeling does have inadequacies in addressing

diabetic polyneuropathy. STZ rats are a type 1 diabetic model with relative rapid-onset neuropathy. However, DPN often develops after a period of sustained hyperglycemia and dyslipidemia in patients with type 1 diabetes, whereas in patients with type 2 diabetes, dyslipidemia often precedes the onset of hyperglycemia. These are important issues in disease modeling as hypertriglyceridemia is an independent predictor for developing diabetic neuropathy (15). How triglycerides and deoxysphingolipids interplay in the context of diabetes and metabolic syndromes will need to be carefully addressed as we move forward. This is further emphasized by prospective study of idiopathic polyneuropathy that found a higher prevalence of hyperlipidemia than impaired glucose tolerance or hypertension, indicating dyslipidemia is an independent driving factor underlying nerve injury (16). Future studies are needed to shed light on the cellular and molecular mechanisms that mediate creation and neurotoxic effects of the deoxysphingolipids. As these results are likely to launch clinical L-serine trials in diabetic and possibly idiopathic polyneuropathies, careful study design will be needed in efficacy discovery (17).

As noted in ancient Greek mythology, solving the riddle of the sphinx, in this case its namesake sphingolipids, may provide for a period of new enlightenment.

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