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Fenofibrate lowers atypical sphingolipids in plasma of dyslipidemic patients: A novel approach for treating diabetic neuropathy?

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BACKGROUND: The condensation of palmitoyl-CoA and L-Serine is the first step in the de novo formation of sphingolipids and catalyzed by the serine-palmitoyltransferase (SPT). Besides other acyl-CoAs the SPT can also metabolize L-alanine and glycine, which forms an atypical category of neurotoxic 1-deoxy-sphingolipids (1-deoxySL). Several mutations in SPT are associated with pathologically increased 1-deoxySL levels, which cause the inherited sensory neuropathy HSAN1. 1-DeoxySL levels are also elevated in individuals with the metabolic syndrome and diabetes mellitus type II and seem to be involved in the pathology of the diabetic neuropathy.

OBJECTIVE: In previous studies, we observed a strong correlation between plasma 1-deoxySLs and triglycerides (TGs). We were therefore interested whether lowering plasma TG levels also affects plasma sphingolipid and in particular, 1-deoxySL levels.

METHODS: Sixty-six patients with dyslipidemia were treated for 6 wk with the TG-lowering drug fenofibrate (160 mg/d) or extended-release niacin (0.5 g/d for 3 wk, then 1 g/d) with 4 wk of washout between treatments. The sphingoid base profile was analyzed by liquid chromatography–mass spectrometry (LC-MS) before and after each treatment block.

RESULTS: Fenofibrate significantly lowered 1-deoxySLs and other atypical sphingoid bases ($P < .001$) but had no effect on the typical sphingolipids. In contrast, extended-release niacin had no effect on 1-deoxySL levels although both treatments lowered plasma TG levels.

CONCLUSIONS: The lowering of plasma 1-deoxySL levels by fenofibrate in dyslipidemic patients might be a novel therapeutic approach in the prevention and treatment of diabetic neuropathy.

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Introduction

Pathologically altered plasma lipids are one of the major causes for cardiometabolic diseases, and dyslipidemia remains one of the main therapeutic targets for the treatment of cardiovascular diseases and metabolic syndrome (MetS).[1,2] The best characterized lipid-modifying drugs include HMG-CoA reductase inhibitors, (statins), fibric acid derivatives (fibrates), and nicotinic acid derivatives (niacin).[3] Fibrates have been identified as agonists for the peroxisome proliferator–activated receptors α (PPARα),[4] a member of the nuclear hormone receptor superfamily.[5] Three different subclasses of PPARs have been identified: PPARα, β/δ, and γ.[4,6–8] The activation of PPARα by fenofibrate has been shown to increase the expression of lipoprotein lipase, which increases the release of fatty acids from the triglyceride-rich lipoproteins. Activation of PPARα also stimulates fatty acid uptake, activation, and β-oxidation, whereas it decreases fatty acid synthesis by down-regulating acetyl-CoA carboxylase and fatty acid synthase. In addition, fenofibrate increases the expression of the apolipoproteins ApoAI and ApoAII, the 2 major lipoproteins of high-density lipoprotein (HDL), and down-regulates ApoB and ApoCIII. Fibrates, therefore, improve the plasma lipid profile by decreasing plasma triglycerides and increasing HDL-C.[9] Niacin, on the other hand, exerts its functions through both receptor- and nonreceptor-mediated routes. It binds to the membrane receptor GPR109A[10,11] in adipocytes. This leads to the inhibition of lipolysis in adipose tissue by decreasing c-AMP concentration. Low c-AMP levels in adipocytes lead to decreased protein kinase A activity, which decreases the activity of the hormone-sensitive lipase.[12] This, in turn, decreases plasma-free fatty acids and reduces triglyceride synthesis in the liver.[12] Niacin was also shown to lower the activity of cholesterol ester transfer protein (CETP) in ApoE*3 Leiden mice, which were transgenic for the human *CETP* gene.[13] Moreover, nonreceptor-mediated effects of niacin have been reported,[14] but the exact mechanisms leading to the improvements in the lipoprotein profile are not fully elucidated.

Plasma lipids are very heterogeneous in nature with $>$ 600 distinct species. The most abundant classes of plasma lipids are sterols and glycerophospholipids reflecting approx. 50% and 30% of the total plasma lipids, respectively.[15,16] Sphingolipids are present in plasma to a minor extent and represent about 4% of the total plasma lipids.[15,16] However, more than 200 different sphingolipid species have been identified in plasma.[15] This diversity is because of the combination of different sphingoid base backbones with different N-linked fatty acid chains in combination with a great variety of headgroups.[15,17–19] Sphingolipid biosynthesis is typically initiated by the condensation of serine and palmitoy-CoA catalyzed by the enzyme serine palmitoyltransferase (SPT). The product 3-ketosphinganine is reduced to sphinganine (C_{18}SA) and subsequently *N*-acylated to dihydroceramides and finally converted to ceramides. Ceramides are building blocks for complex sphingolipids, which are formed by attaching different headgroups to the C_1 hydroxyl group. In the degradation pathway, ceramides are hydrolyzed to sphingosine (C_{18}SO). C_{18}SA and C_{18}SO are usually referred to as free sphingoid bases. Apart from the canonical substrates (serine and palmitoyl-CoA), SPT can also use other acyl-CoAs and other amino acids, which results in the formation of a diverse class of atypical sphingoid bases. In particular, the use of alanine, instead of serine, forms a group of 1-deoxysphingolipds (1-deoxySL), which lack the C_1 hydroxyl group of the serine-based sphingolipids. Because of the missing C_1-OH group, 1-deoxySLs can neither be converted to complex sphingolipids nor degraded by the canonical sphingolipid catabolism. Pathologically increased 1-deoxy SL levels are found in the rare inherited sensory neuropathy HSAN1, which is caused by missense mutations in SPT. We showed previously that 1-deoxySLs are also significantly elevated in patients with the MetS[17] and type II diabetes mellitus (T2DM)[19] and might be actively involved in the pathology of insulin resistance[20] and the diabetic sensory neuropathy.[21] Principal-component analysis revealed that 1-deoxySLs are among the most significant discriminators to differentiate between MetS and healthy controls. Discriminant analysis revealed that 1-deoxySA and 1-deoxySO together with triglycerides and HDL cholesterol (HDL-C) are the best explanatory variables for nondiabetic MetS.[17] In these previous studies, we also observed a strong positive correlation between 1-deoxySL plasma levels and triglycerides,[17] which was not observed for the serine-based sphingolipids. Such correlation is not obvious on a direct metabolic basis as 1-deoxySLs are not defined by their carbon chain but rather by their alanine moiety. This indicates a metabolic connection between TG, alanine, and 1-deoxySL formation. In the present study, we investigated this relationship in more detail by testing whether the pharmacological lowering of TGs with fenofibrate and extended-release niacin in dyslipidemic patients also affects the plasma sphingolipids and in particular the 1-deoxySL levels.

Materials and methods

Ethical approval

The regulatory approvals to perform this study were obtained in accordance with the applicable regulatory requirements for France, Italy, and the Netherlands. The study was approved in France by the Agence française de sécurité sanitaire des produits de santé (AFSSAPS) (Feb 2, 2007) and the Ethics committee of Ile de France VI (March 7.2007). In Italy, the study was approve by the Ethics Committee board of Ospedale Niguarda Ca'Granda (February 15, 2007) and the Ethics Committee board of Padova (February 14, 2007). In the Netherlands, the study was authorized by the Centrale Commissie Mensgebonden