Short Communication

Lyso-sphingomyelin is elevated in dried blood spots of Niemann–Pick B patients

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Niemann–Pick disease type B (NPD-B) is caused by a partial deficiency of acid sphingomyelinase activity and results in the accumulation of lysosomal sphingomyelin (SPM) predominantly in macrophages. Notably, SPM is not significantly elevated in the plasma, whole blood, or urine of NPD-B patients. Here, we show that the de-acylated form of sphingomyelin, lyso-SPM, is elevated approximately 5-fold in dried blood spots (DBS) from NPD-B patients and has no overlap with normal controls, making it a potentially useful biomarker.

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1. Introduction

Niemann–Pick disease type B (NPD-B; OMIM #607616) is a rare, autosomal recessive, lysosomal storage disorder caused by a deficiency in acid sphingomyelinase (ASM) activity. Sphingomyelin (SPM), the primary substrate of ASM, accumulates predominantly within macrophages, but also within hepatocytes and other cell types, resulting in marked hepatosplenomegaly, thrombocytopenia, interstitial lung disease, and coronary artery disease [1–3]. NPD-B patients have an attherosclerotic lipid profile characterized by low HDL, high LDL, and hypertri-glyceridemia, and exhibit secondary accumulation of acidic and neutral glycosphingolipids (GSL) in the spleen [4]. Notably, SPM is not significantly elevated in the plasma, whole blood, or urine of NPD-B patients, rendering it of limited use as a non-invasive biomarker.

Lyso-glycosphingolipids (lyso-GSL), the deacylated forms of GSL, are elevated in many lysosomal storage disorders. Galactosylsphingosine (psychosine) is elevated in the brains of Krabbe disease patients [5]. Recently, psychosine was also reported to be increased in dried blood spots (DBS) from patients with Krabbe disease [6]. Aerts et al. detected increased plasma globotriaosylsphingosine (lyso-GL-3) in males with Fabry disease [7]. Elevated plasma glucosylsphingosine (lyso-GL-1) was detected in Gaucher disease patients by the same group [8]. The de-acylated form of sphingomyelin, lyso-SPM (also known as D-erythro-sphingosyl-phosphocholine, SPC), has been shown to be elevated in the spleens and livers of NPD-B patients, as well as in the brains of NPD type A patients who have little to no ASM activity and manifest severe neuronopathic disease [3]. We developed a quantitative and sensitive assay to determine whether lyso-SPM is elevated in DBS from NPD-B patients as compared to normal controls.

2. Materials and methods

Whole blood was obtained from 27 NPD-B patients with previously confirmed clinical diagnosis. The patients provided written informed consent. Control blood samples from 20 healthy adults were purchased (ProMedDx, LLC, Norton, MA). Venous blood was drawn into Vacutainer® tubes containing EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ), shipped on cold packs overnight, and held at 4 °C upon arrival. Within 48 h of collection, the blood was mixed by inverting the tubes several times, and 75 μL of blood per spot was applied onto Whatman 903® (Whatman, Inc. Sanford, ME) specimen collection paper and dried at room temperature for at least 4 h.

ASM activity was measured according to a previously published method [9]. To quantify lyso-SPM, 1-O-hexadecyl-(7,7,8,8-d4)-2-O-acetyl-sn-glyceryl-3-phosphorylcholine (platelet-activating factor (PAF) C16-D4, Cayman Chemical Company, Ann Arbor, MI) was used as the internal standard (IS). A 3.2 mm punch of DBS was extracted by vortexing for 30 min and sonicing for 10 min in 200 μL methanol/acetoni-trile/water (80/15/5) containing 0.8 ng IS. The solution was centrifuged at 16,200 g for 5 min, and then 30 μL of the solution was injected into an Agilent 1100 HPLC system (Agilent, Palo Alto, CA) interfaced with an API Qtrap 4000 mass spectrometer (AB Sciex, Toronto, Canada) system (LC/MS/MS). The chromatographic separation was achieved with a normal-phase silica column run in isocratic mode using a mixture of methanol/acetoni-trile/water as the mobile phase.

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Mass spectrometry (MS) was performed in selected ion monitoring mode with the following transitions: m/z 465.4 > 184.1 for lyso-SPM and m/z 528.5 > 184.1 for PAF C16-D4. To quantitate SPM, C12-SPM (Avanti Polar Lipids, Alabaster, AL) was used as the IS. The extraction and LC/MS/MS procedures were similar to those used for lyso-SPM except that the eluent was diluted 320-fold before injection. A total of 12 isoforms of SPM were identified and summed.

3. Results and discussion

Although SPM levels have been shown to be elevated more than 10-fold in the livers and spleens of NPD-B patients, SPM levels in plasma have been found to overlap with those of normal controls [3,10,11]. Similar results were obtained in this DBS study. The mean SPM concentration in DBS from NPD-B patients (n = 27) was 962 μg/mL (range: 711–1431 μg/mL) compared to 858 μg/mL (range: 706–1025 μg/mL) from normal controls (n = 20) (P = 0.0171) (Fig. 1A). As a major component of the plasma membranes of circulating cells and lipoproteins, SPM is normally present at high levels in blood and undergoes rapid turnover. The slight elevation of SPM observed in DBS from NPD-B patients may be related to its accumulation in monocytes, from which macrophages are derived, or a change in its turnover.

In contrast, we determined that lyso-SPM levels in DBS from NPD-B controls were substantially elevated when compared to normal controls, with no overlap in values. The mean lyso-SPM concentration in DBS from NPD-B patients was 2464 ng/mL (range: 910–3836 ng/mL) compared to 525 ng/mL (range: 425–574 ng/mL) in normal controls (P < 0.0001) (Fig. 1B). The lyso-SPM level did not correlate with the amount of residual ASM activity in DBS or with patient age at collection (data not shown). Incomplete clinical information of the patients in the study prevents from correlation of lyso-SPM levels with clinical presentation and disease severity. The approximately 5-fold increase in mean lyso-SPM concentration observed in NPD-B patients is modest when compared to the 100-fold increase in lyso-GL-3 in Fabry disease [7], 200-fold increase in lyso-GL-1 in Gaucher disease [8], and 50-fold increase in psychosine in Krabbe disease [6].

The mechanism by which lyso-SPM and other lyso-GSLs (e.g., lyso-GL-3, lyso-GL-1, and psychosine) are produced has not been elucidated. The deacylation of the corresponding sphingolipid is one of the likely routes of generation [7,8,12]. However, the only sphingomyelin deacylase identified to date is from the stratum corneum of an atopic dermatitis patient [13]. The expression of this deacylase appears to be limited to selected cell types under certain physiological conditions [14]. Conversely, the synthesis of lyso-SPM from sphingosine or the free sphingoid base has only been demonstrated by reacting sphingosine with CDP-choline in the presence of phosphorylcholine-sphingosine transferase from chicken liver [15]. Lyso-GL-3 and lyso-GL-1 can be hydrolyzed by recombinant alpha-galactosidase and glucocerebrosidase administered as enzyme replacement therapies for Fabry disease and Gaucher disease, respectively [7,8]. However, purified ASM from placenta, brain, and urine failed to hydrolyze lyso-SPM [16–18]. Lyso-SPM has a short half-life in blood in vitro because of its rapid metabolism to sphingosine–1-phosphate through autotaxin, an exoenzyme with lysophospholipase D activity [19,20].

Other than spleen and liver, there are no reports on the levels of lyso-SPM in other organs of NPD-B patients, even though it should be as widespread as lyso-GL-3 in Fabry disease and lyso-GL-1 in Gaucher disease [7,21,22]. Lyso-SPM, and the structurally related sphingosine-1-phosphate, play important roles as lipid mediators in the cellular functioning of the heart, blood vessels, skin, brain, and immune systems [12,23]. Studies are needed to better understand the physiological impact of elevated lyso-SPM in NPD-B patients, its correlation with disease status, and its response to treatment.

In conclusion, lyso-SPM is significantly increased in DBS from NPD-B patients at levels that are clearly distinguishable from normal controls. It has the potential to be an NPD-B biomarker for diagnosis, disease monitoring or therapeutic efficacy after systematic and longitudinal study of lyso-SPM in clinic.

References


Fig. 1. Sphingomyelin (A) and lyso-sphingomyelin (B) concentrations in dried blood spots from normal controls and NPD-B patients.


