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Prominent increase in plasma ganglioside GM3 is associated with clinical manifestations of type I Gaucher disease

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Abstract

Background: Patients with Gaucher disease show signs of insulin resistance. The ganglioside GM3 has recently shown to be a negative regulator of insulin sensitivity. In fibroblasts of Gaucher patients, deficient in degradation of glucosylceramide, an increased anabolism of this lipid to gangliosides occurs. The goal of the current study was to establish whether GM3 is elevated in plasma of type I Gaucher disease patients, and is related to disease manifestations.
Methods: Plasma GM3, glucosylceramide, and ceramide were determined and compared to overall severity of disease, hepatomegaly, and plasma chitotriosidase activity.
Results: The ceramide concentration in plasma of untreated Gaucher patients was slightly but not significantly lower than in controls (median: 9.8 μmol/L, range: 5.7–14.7 μmol/L, (n=40) vs. median: 11.0 μmol/L, range: 5.1–18.0 μmol/L, (n=30)). Glucosylceramide was significantly (p<0.0001) elevated. GM3 was also significantly (p<0.0001) increased (median: 10.2 μmol/L, range: 4.3–19.1 μmol/L, (n=40) vs. median: 3.6 μmol/L, range: 2.7–5.4 μmol/L, (n=30)). Plasma GM3 concentrations correlated with those of plasma chitotriosidase activity (ρ=0.45, p=0.0036), overall severity of disease (ρ=0.39, p=0.012), and hepatomegaly (ρ=0.49, p=0.0015).
Conclusions: GM3 is strikingly elevated in plasma of most Gaucher patients. The increase is comparable to that of glucosylceramide, the primary storage lipid. The marked elevations in GM3 may play a role in the insulin resistance of Gaucher patients.

Keywords: Lysosomal storage disorder; Gaucher disease; Glucosylceramide; Ganglioside; Chitotriosidase

1. Introduction

Gaucher disease (OMIM 230800) is the most common lysosomal storage disorder [1]. The disease is due to a recessively inherited deficiency in lysosomal glucocerebrosidase (GBA1; EC:3.2.1.45), catalyzing the hydrolysis of the glycosphingolipid glucosylceramide to glucose and ceramide in lysosomes. Different phenotypes of Gaucher disease (types I, II, and III) are generally recognized, which are differentiated on the basis of the presence or absence of neurological symptoms. The most prevalent Gaucher phenotype is the non-neuronopathic type I variant. Although glucocerebrosidase is present in lysosomes of all cell types, type I Gaucher disease patients develop only pronounced lysosomal storage of glucosylceramide in macrophages. Recently, the protein (GBA2) responsible for the ubiquitous non-lysosomal glucocerebrosidase activity has been identified [2–4]. Most likely this enzyme largely protects most cell types of Gaucher patients from massive glucosylceramide accumulation. Lysosomal storage in macrophages of Gaucher patients can not be prevented due to the fact that large quantities of glycosphingolipids are directly introduced in their lysosomes by phagocytosis of senescent blood cells. The glucosylceramide-loaded macrophages of Gaucher patients show a characteristic morphology and are referred to as Gaucher cells. It has become clear that Gaucher cells are not inert containers of storage material but viable, chronically activated macrophages which contribute to the diverse clinical manifestations of

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Gaucher disease. In tissue lesions of Gaucher patients, mature storage cells, which are alternatively activated macrophages, are surrounded by newly formed, highly inflammatory cells [5,6]. Consistent with these observations, Gaucher patients show increased plasma levels of several pro-inflammatory and anti-inflammatory cytokines, chemokines, and hydrolases [7]. Factors released by Gaucher cells and surrounding macrophages are thought to play a crucial role in the development of common clinical abnormalities in Gaucher patients such as osteopenia, activation of coagulation, and gammopathies. Metabolic abnormalities also occur in Gaucher patients. Patients show a markedly increased resting energy expenditure and in addition an increased hepatic glucose production pointing to insulin resistance [8,9]. Consistent with this, adiponectin levels are reduced in symptomatic Gaucher patients [10]. The precise cause for these metabolic abnormalities is presently unclear. Recent reports implicate gangliosides like GM3 as important negative regulators of insulin sensitivity in liver and peripheral tissues [11–14]. This is of particular interest since there are reports on abnormalities in GM3 in association with Gaucher disease. Firstly, cultured fibroblasts from Gaucher patients were found to show an increased synthesis of gangliosides, among them GM3 [15]. Apparently, the deficiency in lysosomal glucosylceramide catabolism in fibroblasts is partly compensated by increased anabolism of the lipid to gangliosides. Secondly, increased levels of the ganglioside GM3 were noted for spleen, liver, and brain from a relatively small number of Gaucher patients investigated, again suggesting a compensatory increase in ganglioside biosynthesis [16,17]. This set of observations and the recent availability of a sensitive assay for plasma GM3 determination, prompted us to investigate the plasma concentration of this ganglioside in a large cohort of type I Gaucher disease patients. We here firstly report that plasma GM3 is strikingly increased in association with Gaucher disease manifestation. The potential physiological impact of GM3 elevation in Gaucher patients is discussed.

2. Methods

Plasma samples were collected of forty type I Gaucher disease patients who were referred to the Academic Medical Center in Amsterdam for assessment of the severity of their disease or the institution of therapy. The patients took part in an observational study for which approval for regular blood sampling was obtained by the institution's ethical committee. Thirty healthy control subjects also consented to the use of their stored plasma samples for analysis of glycosphingolipids. A diagnosis of Gaucher disease was confirmed by demonstration of deficient activity of glucocerebrosidase in leucocytes and genotyping. The severity of the disease was classified using the modified severity scoring index (SSI) [18]. Thirteen of the patients had been splenectomized prior to the start of therapy. Liver volume was measured by spiral computed axial tomography [19]. Excess liver volume was calculated as described before [20].

2.1. Chitotriosidase activity assay

The fluorogenic substrate 4MU-deoxychitobiose was synthesized as earlier described [21]. For the enzyme activity assay 25 μL plasma, diluted with BSA/PBS (bovine serum albumin/phosphate buffered saline, 1 g/L) and 100 μL substrate mixtures were incubated for 20 min at 37 °C. The substrate mixtures contained 0.11 mM 4MU-deoxychitobiose and 1 g/L BSA in McIlvain buffer, pH 5.2 [22]. Reactions were stopped with 2.0 mL 0.3 M glycine NaOH buffer pH 10.6 and the formed 4MU was detected fluorometrically (excitation at 366 nm; emission at 445 nm). Only less than 10% difference in the duplicates was allowed. One unit (U) of activity is defined as 1 nmol of substrate hydrolyzed per hr. The chitotriosidase genotype of individuals was determined as described earlier [23].

2.2. Lipid measurements

Plasma glucosylceramide, ceramide, and ceramidetrihexoside were determined exactly as previously described [24]. Ceramidetrihexoside was determined to establish alterations in the globoside anabolic pathway. All samples were run in duplicate and in every run 2 reference samples were included. Gangliosides, including GM3, were detected by analysis of the acidic glycolipid fraction obtained by Folch extraction. Gangliosides were desalted on a disposable SPE C18 column (Bakerbond, Mallinckrodt Baker Inc., Phillipsburg, NJ, USA) as described by Kundu [25] and quantified following release of oligosaccharides from glycosphingolipids by ceramide glycanase (Recombinant endoglycoceramidase II, Takara Bio Inc., Otsu, Shiga, Japan) digestion. The enzyme was used according to the manufacturer's instructions. Released oligosaccharides were labeled at their reducing end with the fluorescent compound anthranilic acid (2-aminobenzoic acid), prior to analysis using normal-phase high-performance liquid chromatography [26]. Throughout the procedure monosialoganglioside-GM1 (Sigma, St Louis, Mo, USA) was used as an internal standard. Similar procedures were used to measure the concentrations of ceramide, glucosylceramide, ceramidetrihexoside, and GM3 in homogenates of spleen specimens.

2.3. Statistical analysis

Results are given as median and [range]. To test differences between groups the Mann–Whitney *U*-test was used. Correlations were tested by the rank correlation test (Spearman coefficient, ρ). A p-value <0.05 was considered statistically significant.

3. Results

The median concentration of ceramide in plasma of Gaucher patients (9.8 μmol/L [5.7–14.7 μmol/L]) was comparable to that of control subjects (11.0 μmol/L [5.1–18.0 μmol/L]) ($p=0.19$) (Fig. 1A). The glucosylceramide concentration was significantly higher in Gaucher patients (20.3 μmol/L [7.2–54.2 μmol/L]) compared to control subjects (5.7 μmol/L [3.7–7.6 μmol/L]) ($p<0.0001$) (Fig. 1B). The ceramidetrihexoside concentration in plasma of Gaucher patients (1.7 μmol/L [0.8–3.3 μmol/L]) was not significantly different from the concentration measured in control subjects (1.7 μmol/L [1.2–2.6 μmol/L]) ($p=0.77$) (Fig. 1C). GM3 concentrations were on average up to 3-fold elevated in plasma of Gaucher patients (10.2 μmol/L [4.3–19.1 μmol/L]) compared to control subjects (3.6 μmol/L [2.7–5.4 μmol/L]) ($p<0.0001$) (Fig. 1D). Negroni previously reported values for total ganglioside concentration in human serum from control subjects between 4.0–8.9 μmol/L [27].

Gaucher cells laden with cerebrosides mainly glucosylceramide, can be found predominantly in spleen and liver causing gross enlargement of these organs. Nilsson et al. [17] reported of a 200–500 times higher glucosylceramide concentration and a 2- to 6-fold elevation in GM3 concentration in spleen and liver of types I, II, and III Gaucher patients. They did not find major differences between the three types with respect to elevations in glucosylceramide and GM3 concentrations. To confirm their results and to see if the glycosphingolipid composition in spleen and plasma is comparable, we analyzed spleens from four Gaucher type I