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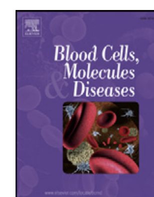

Gaucher disease: Plasmalogen levels in relation to primary lipid abnormalities and oxidative stress

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ABSTRACT

Plasmalogens represent a unique class of phospholipids. Reduced red blood cell plasmalogen levels in Gaucher disease patients were reported, correlating to total disease burden. The relation between plasmalogen abnormalities in Gaucher disease patients and primary glycosphingolipid abnormalities, malonyldialdehyde levels, an indicator of lipid peroxidation, and the total antioxidant status was further investigated.

Significant reduction of C16:0 and C18:0 plasmalogens in red blood cells of Gaucher disease patients was confirmed. In parallel, a significant increase in the glucosylceramide/ceramide ratio in red blood cell membranes, as well as an average 200-fold increase in plasma glucosylsphingosine levels was observed. Red blood cell malonyldialdehyde levels were significantly increased in patients, whereas their total antioxidant status was significantly reduced.

A negative correlation between plasmalogen species and glucosylceramide, ceramide, glucosylceramide/ceramide ratio, glucosylsphingosine and malonyldialdehyde, significant for the C16:0 species and all the above parameters with the exception of malonyldialdehyde levels, was found along with a positive non-significant correlation with the total antioxidant status.

Our results indicate that increased lipid peroxidation and reduced total antioxidant status exist in Gaucher disease patients. They demonstrate a clear link between plasmalogen levels and the primary glycolipid abnormalities characterizing the disorder and an association with the increased oxidative stress observed in Gaucher disease patients.

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Introduction

Gaucher disease (GD) is an autosomal recessive lysosomal storage disease (MIM #230800, 230900 and 231000). It is caused by the deficient activity of the lysosomal enzyme β-glucocerebrosidase (GBA) that, in most instances, is associated with mutations in the GBA gene (MIM #606463, GenBank accession no. J03059.1). It is a highly heterogeneous disease exhibiting a wide range of phenotypes. The deficient GBA activity results in the accumulation of glucosylceramide (GlcCer), which in the non-neuronopathic type I of GD exclusively occurs in tissue macrophages, transforming them into the characteristic Gaucher cells [1]. In addition to GlcCer accumulation, several other abnormalities, some clearly associated with the dysfunctional lipid-laden macrophages, have been described in GD patients. Their link to the different pathologies characterizing the disorder is still under investigation [2,3]. We recently reported that the red blood cell (RBC) levels of a unique class of phospholipids, the plasmalogens, are significantly reduced in GD patients. The observed reduction in plasmalogens correlated with total disease burden, as judged by plasma chitotriosidase activity, and normalized following treatment [4].

Plasmalogens are particularly abundant in the brain, heart, lung, skeletal muscle, growth plates, lens and immune cells [5]. Peroxisomes are essential for their synthesis, since the two enzymes initiating their synthesis, dihydroxyacetonephosphate acyltransferase (DHAPAT; EC 2.3.1.42) and alkyl-dihydroxyacetonephosphate synthase (ADAPS; EC 2.5.1.26) are exclusively localized in these organelles. Their uniqueness stems from the presence of the vinyl-ether bond at the sn-1 position that is combined to the presence of polyunsaturated fatty acids, mainly arachidonic (AA) or docosahexaenoic acid (DHA), at the sn-2 position of the glycerol backbone. They have been assigned several functions, including modulation of membrane dynamics, protection against oxidative stress, participation in signal transduction processes, cholesterol trafficking, processes that are disturbed in sphingolipidoses, including GD [5,6]. In this study we further explored plasmalogens in GD by

Abbreviations: AA, arachidonic acid; ADAPS, alkyl-dihydroxyacetonephosphate synthase; Cer, ceramide; DHA, docosahexaenoic acid; DHAPAT, dihydroxyacetonephosphate acyltransferase; DMA, dimethylacetal; GBA, β-glucocerebrosidase; GD, Gaucher disease; GlcCer, glucosylceramide; GlcSph, glucosylsphingosine; MDA, malonyldialdehyde; NO, nitric oxide; RBC, red blood cells; TAS, total antioxidant status; TBA, thiobarbituric acid.

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examining their relation to GlcCer, Cer and GlcSph. Furthermore, in order to investigate a possible association between plasmalogens and the oxidative stress experienced by GD patients, we estimated the total antioxidant status and lipid peroxidation, the latter by assaying malonyldialdehyde (MDA) levels, in GD patients and evaluated their relation to plasmalogens.

Patients, methods and materials

Patients

Overall 27 patients with Gaucher disease (GD) were studied, including 24 type I (age of diagnosis 4.5-77 years) and 3 type II (age of diagnosis 7 days-7.5 months).

A group of 13 healthy individuals of similar age range served as controls. GlcSph was quantified in a group of 28 healthy individuals that did not include the above group.

Methods and materials

Erythrocytes and plasma were isolated from heparinized blood samples obtained from all studied individuals. In the case of GD patients the samples were obtained on diagnosis and prior to the initiation of any treatment.

Plasmalogen levels were measured as their dimethylacetal derivatives (DMA) by gas chromatography in a lipid extract of red blood cell membranes (RBC) after methyl transesterification, as previously described [4]. They were expressed as the % ratio of the C16:0 DMA to methylpalmitate (C16:0) and C18:0 DMA to methylstearate (C18:0). Malonyldialdehyde (MDA) levels were measured in RBC membrane extracts by the thiobarbituric acid (TBA) method, as previously described [7].

GlcCer and Cer levels were also measured in RBC membrane extracts using HPLC and the levels of GlcSph were quantified in plasma by LC-CSI-MS/MS, as previously described [8,9].

TAS in plasma was estimated using the Randox Total Antioxidant Status kit according to the manufacturer's instructions.

Statistical analysis

The results were statistically evaluated by nonparametric tests. Specifically the Wilcoxon test was used for paired comparisons and the Mann–Whitney test was used for group comparisons. All correlations were Spearman's correlations.

Results

Red blood cell plasmalogen levels were estimated as their DMA derivatives and their relative amounts were expressed as the ratio between C16:0 DMA and C16:0, as well as C18:0 DMA and C18:0 [4].

According to our results, the GD patients studied showed statistically significant lower levels of both plasmalogen species compared to controls ($p = 0.009$ and $p < 0.001$ for the C16:0 and C18:0 species, respectively), thus confirming our previous observation [4] (Table 1).

A wider range of concentration for GlcCer and Cer was observed in GD patients compared to controls. The highest concentration for GlcCer and the lowest concentration for Cer observed in GD patients were 2.4 fold increased and 3.4 fold decreased, respectively, compared to controls (Fig. 1). However, overall there was no statistically significant difference for either of the above parameters between GD patients and controls. On the other hand, compared to controls, a higher GlcCer/Cer ratio was observed in GD patients, the difference being statistically significant ($p < 0.001$) (Table 1, Fig. 1). A marked elevation of GlcSph concentration was measured in the plasma of GD patients, the median value of which showed more than a 200- fold increase compared to controls.

In order to investigate whether the reductions observed in plasmalogen levels are related to the levels of the other lipids studied, we compared the levels of these parameters.

The analysis revealed a negative correlation between both plasmalogen species and GlcCer, Cer, GlcCer/Cer ratio and GlcSph. It reached statistical significance only for the C16:0 plasmalogen species and GlcCer, Cer, GlcCer/Cer ratio and GlcSph (GlcCer: r_s: -0.513, $p = 0.01$; Cer: r_s: -0.452, $p = 0.027$; GlcCer/Cer: r_s: -0.402, $p = 0.05$; GlcSph: r_s: -0.420, $p = 0.041$).

RBC MDA levels were investigated as an indicator of lipid peroxidation. A statistically significant increase was observed in GD patients compared to controls ($p = 0.019$). Concomitantly a statistically significant reduction in the total antioxidant status (TAS) measured in plasma was observed in the cohort of patients compared to controls ($p = 0.003$).

In GD patients both plasmalogen species showed a negative, albeit not significant, correlation to MDA levels, whereas a positive, again not significant, correlation was observed between the C16:0 plasmalogen species and TAS. A negative, albeit not significant, correlation was observed between TAS levels and GlcCer, Cer, GlcCer/Cer ratio and GlcSph, whereas there was no apparent correlation between MDA levels and the above lipid parameters. Interestingly, a positive not statistically significant correlation was observed between MDA levels and TAS.

When type I ($n = 24$) and type II ($n = 3$) GD patients were evaluated separately, no consistent trend was observed in the parameters studied. However at this stage no valid conclusions can be drawn regarding differences between the different types of GD due to the small number of type II patients available for this study. Statistical analysis of the data obtained for type I patients vs controls, as well as correlation studies, showed the same results as those obtained when both type I and type II patients were analyzed as a group.

In particular the results for the type I patients were: C16:0 DMA/C16:0: range: 0.073-0.106, median: 0.085, $p = 0.023$; C18:0 DMA/C18:0: range: 0.139-0.212, median: 0.162, $p = 0.000$; GlcCer

Table 1
Red blood cell levels of plasmalogens (C16:0 DMA/C16:0 and C18:0 DMA/C18:0), glucosylceramide (GlcCer), ceramide (Cer), glucosylceramide/ceramide (GlcCer/Cer) ratio and malonyldialdehyde (MDA), plasma levels of glucosylsphingosine (GlcSph) and total antioxidant status (TAS) in Gaucher disease patients and controls.

	C16:0 DMA/C16:0	C18:0 DMA/C18:0	GlcCer (pmol/10^8 cells)	Cer (pmol/10^8 cells)	GlcCer/Cer ratio	GlcSph (nM)	MDA (nmol/10^{10} cells)	TAS (mM)
Gaucher								
Range	0.058-0.106	0.116-0.212	6.5-113.4	68-634	0.073-0.231	28.1-812.0	0.7-10.6	0.19-0.95
Median	0.084[b]	0.155[a]	35.2	300	0.116[a]	260.2	4.5[c]	0.51[d]
n	27	27	24	24	24	24	27	21
Control								
Range	0.064-0.127	0.161-0.233	16.7-46.8	228-768	0.059-0.133	0.8-2.7	1.1-5.4	0.41-1.08
Median	0.097[b]	0.211[a]	24.9	308	0.071[a]	1.3	3.0[c]	0.74[d]
n	13	13	12	12	12	28	13	13

Statistically significant differences were: [a]$p < 0.001$, [b]$p = 0.009$, [c]$p = 0.019$, and [d]$p = 0.003$.

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