Changes in the atherogenic profile of patients with type 1 Gaucher disease after miglustat therapy

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ABSTRACT

Objective: Type 1 Gaucher disease (GD1) is an autosomal recessive lysosomal storage disorder associated with abnormal accumulation of glucocerebrosides. Plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) are decreased in GD1 patients. The effects of substrate reduction therapy (SRT) with miglustat on plasma lipids and atherogenic factors have not yet been examined. Here, we report plasma atherogenic profile data from GD1 patients undergoing long-term SRT.

Methods: Plasma was analysed in 26 GD1 patients treated with miglustat for up to 36 months. Ten patients were therapy-naïve and 16 had switched from enzyme replacement therapy (ERT); the interval between stopping ERT and starting SRT was 2–6 weeks. Plasma TC, triglycerides (TG), LDL-c, HDL-c, apolipoproteins (apoA-I, apoB, and Lp[a]), C-reactive protein (CRP) concentrations, and chitotriosidase activity were measured before SRT (baseline) and at 12, 24, and 36 months follow up.

Results: In therapy-naïve patients, miglustat significantly increased plasma HDL-c and apoA-I, and slightly increased TC; while TG, CRP concentrations, and TC/HDL-c ratios decreased significantly after 24 months. In contrast, there were no changes in HDL-c and apoA-I, or in the TC/HDL-c ratio in switch patients. However, a decrease in CRP was observed after 12 months. LDL-c and apoB were not significantly altered in either patient group.

Conclusions: Miglustat appears to have beneficial effects on plasma lipid, lipoprotein, and CRP concentrations in therapy-naïve GD1 patients, resulting in an improved atherogenic lipid profile. Further studies are required to determine the effect of miglustat on coronary heart disease risk.

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1. Introduction

Gaucher disease (GD) is a progressive lysosomal storage disorder arising from a deficiency of acid β-glucocerebrosidase (GC). It is characterised by intralysosomal storage of glucosylceramide in the mononuclear–macrophage system, which leads to dysfunction in multiple organs. GD is caused by recessively inherited mutations in the glucocerebrosidase (GBA) gene [1]. The predominant clinical manifestations of the disease are hepatosplenomegaly, peripheral blood cytopenias, and skeletal disease.

Although patients with GD are not reported to suffer from a disproportionate number of cerebrovascular or cardiovascular events, reduced plasma concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c) have been described in patients with type 1 Gaucher disease (GD1) [2–5]. In addition, the apolipoproteins that are part of the structure of the HDL and LDL particles (apoA-I and apoB, respectively) were decreased by 40–50% relative to the general population, while apoE was increased by over 30% [2]. The magnitude of the decreases in lipoprotein fractions may be associated with disease severity. Metabolic studies indicate that decreased concentrations of HDL and LDL in patients with GD are due to increased fractional catabolism of apoA-I and apoB, and probably to the increased concentration of apoE [3]. The increase in apoE levels was shown to be due to over-expression of activated macrophages [4]. It is possible that high concentrations of apoE facilitate lipoprotein catabolism, leading to the decreased lipoprotein levels observed in patients with GD. Levels of LDL-c and HDL-c are reportedly inversely correlated with parameters of disease severity in patients with GD [4].
Intravenous enzyme replacement therapy (ERT) with purified or recombinant glucocerebrosidase and substrate reduction therapy (SRT) with 1,5-(butylimino)-1,5-dideoxy-N-butyldexoyojirimycin, NB-DNJ, miglustat, Zavesca®; Actelion Pharmaceuticals Ltd., Allschwil, Switzerland), an iminosugar that reversibly inhibits glucosylceramide synthase, reduces intracellular storage of glucosylceramide in symptomatic patients with GD. Both therapies are effective in improving organ volume, anaemia, thrombocytopenia, bone manifestations, and biomarkers in patients with GD [6–12]. Increased chitotriosidase (ChT) activity is the most relevant biomarker in GD; plasma activities of this enzyme can be increased up to 600 times compared with control (normal volunteer) values [14]. Some individuals do not express any ChT activity due to a 24-bp duplication in the ChT gene that is inherited in an autosomal recessive manner [13].

Previous studies have demonstrated long-term ERT can significantly increase levels of HDL-c, whereas LDL-c and apoB levels remain unchanged and total apoE levels are decreased (reflecting the decrease in lipid-laden macrophages induced by the enzyme infusion) [14,15]. However, a recent study by de Fost et al. concluded that in GD, low HDL-c levels do not lead to an increased risk of coronary heart disease (CHD), as assessed by carotid intima-media thickness (cIMT) measurements—a non-invasive imaging marker of atherosclerosis in large arteries [16].

The effects of miglustat on atherogenic profiles in patients with GD have not been explored. To gain more insight into plasma lipid metabolism in GD, we evaluated the effects of long-term SRT with miglustat on plasma lipid and apolipoprotein profiles in a group of GD patients who previously received ERT (imiglucerase) and in another group of therapy-naïve patients.

2. Materials and methods

The study involved 26 patients (11 men and 15 women) diagnosed with GD1 and included in the ZAGAL study [11]. Ten patients had not previously received any treatment (therapy-naïve group) and 16 patients had been switched from ERT (‘switch’ group; mean ± SD) time on ERT, 4.8 ± 3.2 years; range, 1–12 years) The time interval between stopping ERT and starting SRT ranged from 2 to 6 weeks. The overall mean ± SD age of patients was 42.4 ± 13.56 years (range, 22–74 years).

All patients received oral miglustat, 100 mg t.i.d. Prior to therapy, patients were instructed on correct administration of the drug and how to follow a low-carbohydrate diet, as recommended during the first weeks of treatment. No patients were taking any medication known to affect plasma lipid concentrations. Our study protocol was developed according to the ethical standards of the Helsinki declaration of 1975, as revised in 1983, and approved by the Aragon Clinical Ethical Committee (CEICA), Spain. All participants provided written informed consent.

All patients were diagnosed by analysis of acid β-glucosidase activity in leukocytes obtained from peripheral blood samples, and by molecular-genetic mutation analysis as previously described [17]. Patients were evaluated using the disease severity score index (SSI), as described by Zimram [18]. The duration and dose of miglucerase before SRT were recorded from patient files.

Venous blood samples were collected in tubes containing K₂ EDTA after a 12-h fast. Plasma was separated by low-speed centrifugation at 2000 × g for 10 min at 4°C. Triglycerides (TG) and TC were quantified using commercial enzymatic assays (CHOD-PAD and GPO-PAD kits, Boehringer Mannheim, Marburg, Germany). LDL-c was measured after precipitation with magnesium-phosphotungstate (Boehringer Mannheim, Marburg, Germany). LDL-c was estimated using the Friedewald equation.

ApoA-I, apoB100, and Lp(a) were determined by kinetic immunonephelometry with polyclonal antibodies on a Beckman Coulter Image Immunochrometry system. Serum concentrations of high-sensitivity C-reactive protein (hsCRP) were similarly measured by kinetic immunonephelometry. Plasma ChT was measured by the enzyme assay method described by Hollak et al. [13]. All biochemical and lipid parameters were measured before commencing miglucerase (baseline) and after 12, 24, and 36 months of therapy.

The apoE genotype, defined by the common polymorphisms p.Cys130Arg (C112R) and p.Arg176Cys (R158C), was determined by specific pyrosequencing reactions on a PSQ96MA instrument (Biotage AB, Uppsala, Sweden). Determination of the 24-bp duplication in the ChT gene was performed by PCR, followed by electrophoresis of the amplified fragments as previously described [13].

Statistical analyses were based on the Mann–Whitney non-parametric U-test, using the Statistical Package for the Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA). A value of p < 0.05 was considered statistically significant in all analyses.

3. Results

Baseline characteristics of the therapy-naïve and switch patients are summarised in Table 1. No significant differences between therapy-naive and switch patients were observed in terms of body weight, SSI, liver and spleen volumes, haemoglobin levels, platelet counts, GBA, or apoE genotypes. Therapy-naive GD patients were older and had a lower number of wild-type Cht genotypes than switch patients.

Mean (±SD) absolute plasma apolipoprotein, lipoprotein, and lipid concentrations, ChT activities, and percent changes from baseline in these parameters are shown in Table 2 for therapy-naive and switch patients. In addition, data on plasma lipids, lipoprotein concentrations, and ChT activities before ERT in the switch patient group are summarised in Supplementary Table 1. There were no significant differences between therapy-naive and switch patients in terms of plasma TC, HDL-c, LDL-c, TG, TC/HDL-c ratios, apoA-I, and apoB. However, ChT and Lp(a) levels were significantly higher (p < 0.001) in therapy-naive patients than in switch patients. Conversely, hsCRP was significantly higher (p < 0.003) in patients previously on ERT compared to therapy-naive patients. In the case of Lp(a), the increased level in therapy-naive patients could be justified by individual inherited characteristics; five therapy-naive patients had plasma Lp(a) levels up to 20 mg/dL higher than in the switch group.

Plasma TC increased slightly from baseline after 36 months of miglustat treatment in both groups, but the changes were only statistically significant in the therapy-naive group (p < 0.05). No significant modifications in LDL-c or apoB levels were observed during treatment in either group. There was a significant increase in HDL-c concentrations in therapy-naive patients during the first 24 months of miglustat therapy (p < 0.05), which was maintained for up to 36 months (p < 0.05). Similar changes were observed for HDL-c during miglustat therapy in switch patients, but increases were smaller than in the therapy-naive group.

In therapy-naive patients, TG and the atherogenic index decreased significantly after 24 and 12 months of miglustat therapy, respectively. In contrast, no changes from baseline were observed in these parameters in switch patients throughout the 36 months of miglustat therapy. Similarly, no significant changes from baseline in Lp(a) concentration were observed in either group during miglustat therapy. Plasma apoA-I concentrations increased during the 3 years of treatment with miglustat in therapy-naive patients, but this increase was only statistically significant after 36 months. ApoA-I levels were not significantly elevated in the switch group.

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Switch patients had higher hsCRP levels than therapy-naive patients at baseline ($p<0.03$). Plasma hsCRP concentrations decreased by 52% and 41% in therapy-naive and switch patients, respectively, during the first 12 months of therapy. Levels remained below baseline values up to month 36. The surrogate biomarker, plasma ChT activity, showed a marked decrease after 12, 24, and 36 months of miglustat in therapy-naive patients. However, no significant change from baseline was observed in the switch group.
4. Discussion

Plasma HDL transports approximately 30–40% of cholesterol in human plasma. The main function of HDL is the removal of cholesterol from peripheral tissues, and its transport to the liver for excretion into the bile. In this context, HDL participates in the network of biochemical pathways involved in the strict regulation of intracellular cholesterol content. In addition, HDL has an oxidant and anti-inflammatory effects [19]. Epidemiological studies highlighted the beneficial effect of HDL-c and showed an increase in HDL-c levels is associated with a decreased risk of coronary artery disease (CAD) in men and women [20]. A recent meta-analysis including prospective observational data from HDL-studies highlighted the beneficial effect of HDL-c and showed an association of intracellular cholesterol content. In addition, HDL has been shown to influence the network of biochemical pathways involved in the strict regulation of intracellular cholesterol content. In this context, HDL participates in the network of biochemical pathways involved in the strict regulation of intracellular cholesterol content. In this context, HDL participates in the network of biochemical pathways involved in the strict regulation of intracellular cholesterol content. In this context, HDL participates in the network of biochemical pathways involved in the strict regulation of intracellular cholesterol content.

We previously demonstrated ERT has significant effects on the plasma lipid profile of patients with GD, significantly increasing HDL-c and apoA-I concentrations without affecting LDL-c and apoB levels [14]. As a result, patients with GD1 who received ERT presented with a reduced atherogenic lipid profile [14,15]. Our current comparison of therapy-naïve patients with a group who had previously received ERT supported our earlier findings. Migluristat significantly increased HDL-c and apoA-I without significant increases in LDL-c and apoB. The atherogenic index, TC/HDL-c, was significantly reduced in the therapy-naïve group. In contrast, lipid and lipoproteins were maintained at baseline levels in the switch group, indicating that the effects from ERT can be sustained on migluristat.

Of note, switch patients had a mean of 4.8 years on ERT and, in general, reached a good degree of disease stability. Therefore, our switch patients may have displayed beneficial effects from previous long-term ERT. It was previously reported that GD patients’ disease parameters may not revert to baseline levels for up to 2 years after withdrawal of ERT [23]. Consequently, SRT in our switch patients might have maintained the stable profile of plasma markers established during previous ERT. Our therapy-naïve patients showed mild or moderate disease severity, in accordance with the indication for migluristat [22]. The decreased plasma ChT activity among therapy-naïve patients indicated a decrease in lipid-laden macrophages that was not apparent in the switch group.

No changes in Lp(a) levels were observed in GD patients treated with migluristat for up to 36 months. The higher level of Lp(a) observed in therapy-naïve patients could be a chance observation because this lipoprotein is genetically determined by sequence differences linked to the apolipoprotein (a) [apo(a)] locus. High plasma levels of Lp(a) have been associated with an increased prevalence of premature atherosclerotic cardiovascular disease [24]. However, the precise nature of this association is not fully understood. Possible relevant pathways include impaired fibrinolysis (through interference of apo(a) with the physiological functions of plasminogen in the fibrinolytic cascade), increased cholesterol deposition in the arterial wall, and enhanced oxidation of LDL-c [25]. The maintained levels of Lp(a) during migluristat treatment indicate there were no changes in this CAD risk factor.

Serum hsCRP is a biomarker of inflammation that can be a useful predictor of incident myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death among healthy individuals with no history of cardiovascular disease [26]. Recent guidelines from the National Academy of Clinical Biochemistry Laboratory Medicine Practice (NACBLMP) indicate that of the named, emerging biomarkers for monitoring cardiovascular risk, only hsCRP met all of the stated criteria required for acceptance as a biomarker for risk assessment in primary prevention [27]. The guidelines categorise patient cardiovascular risk on the basis of hsCRP levels as follows: low-risk, <1.0 mg/L; average-risk, 1.0–3.0 mg/L; high-risk, >3.0 mg/L; and very high-risk, >10.0 mg/L [27]. Surprisingly, our data show patients previously treated with ERT had higher hsCRP levels at baseline (indicating a greater inflammatory burden) than therapy-naïve patients. CRP is the prototypical acute-phase serum protein that is secreted by the liver in response to a variety of inflammatory cytokines. Levels of CRP increase very rapidly in response to trauma, inflammation, and infection, and decrease just as rapidly with the resolution of these conditions [28]. Therefore, CRP measurements are widely employed to monitor a variety of inflammatory states. Our findings suggest ERT may cause an inflammatory disturbance related to the induction of an aberrant immunological response to this mammalian recombinant enzyme [29]. In contrast, migluristat therapy induced a reduction in plasma hsCRP both in therapy-naïve and switch patients, which could indicate a beneficial effect on cardiovascular risk status. Data from the switch group indicate hsCRP concentration was progressively reduced from high-risk levels (mean 4.2 mg/L at baseline) to average-risk levels (mean 2.5 mg/L at 12 months, 1.5 mg/L at 24 months, and 1.4 mg/L at 36 months). Specific studies are required to further evaluate the clinical relevance of this effect.

Large-scale clinical trials support the importance of lowering LDL-c to decrease the risk of CAD events. However, in trials demonstrating substantial reductions in LDL-c on statin therapy, a significant number of CAD events still occur and the residual risk remains high [30]. This suggests lipid parameters other than LDL-c, such as HDL-c, triglycerides, CRP, and atherogenic index, may influence the risk of CAD. Therefore, other strategies that can alter the lipid profile and, in particular, raise HDL-c, may be beneficial. Our data indicate migluristat has beneficial effects on plasma lipids, lipoprotein, and CRP concentrations in patients with GD1. A study by de Fost et al. [16] recently reported that the inverse relationship between HDL-c levels and coronary vascular disease risk in the general population may not be present in all conditions that involve low HDL-c levels; in GD, low HDL-c levels do not lead to premature atherosclerosis, as assessed by cIMT [16]. However, this study was limited because cIMT is an indirect marker of CAD risk. In addition, most patients (85%) received treatment with imiglucerase (median duration, 13 years). Two patients were treated with migluristat for 9 years, which could have altered HDL-c levels and, subsequently, the risk of coronary vascular disease. Although reduced HDL-c is a well known risk factor for CAD in the general population, other factors could be relevant in patients with GD that protect against CAD.

In conclusion, migluristat appears to improve the atherogenic profile and inflammatory parameters in patients with GD1. Plasma Lp(a) concentration was not modified by either ERT or SRT because its plasma level is largely determined by genetic factors. Our study had several limitations, in particular the lack of cIMT data and insulin resistance parameters. While there was also a moderate difference in age between therapy-naïve and switch patient groups (mean 45 years versus 41 years, respectively), GD patients typically exhibit wide phenotypic heterogeneity; therefore, we consider it unlikely that the observed between-group differences affected our results. Nevertheless, studies with a larger number of patients are necessary to confirm our findings. Long-term data are also required to assess whether the observed treatment effects are maintained. Future studies should ultimately allow a more comprehensive appraisal of the impact of migluristat on the risk of coronary vascular disease.

Disclosure

JP, PA, PI, and JG report no conflicts of interest. PG and MP have received speaker fees from Actelion and Genzyme for participation.
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Appendix A. Supplementary data


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