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Short Communication

Effect of Ambroxol chaperone therapy on Glucosylsphingosine (Lyso-Gb1) levels in two Canadian patients with type 3 Gaucher disease

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ABSTRACT

Type 3 Gaucher disease (GD3) is characterized by progressive neurological features in addition to the typical systemic manifestations. Enzyme replacement therapy (ERT), the main stay treatment for Gaucher disease (GD), is not efficacious for the neurological manifestations. Ambroxol, in combination with ERT has been suggested to have potential as a promising therapy for patients with GD3. The purpose of this study is to assess the effect of Ambroxol on glucosylsphingosine (Lyso-Gb1) levels, and on the neurological morbidity, in two Canadian patients with GD3.

1. Introduction

Gaucher disease (GD) is an autosomal recessive inborn error of metabolism resulting from a deficient β-glucocerebrosidase enzyme due to mutations in the *GBA* gene, and results in glucosylceramide accumulation. [1].

GD is characterized by three main clinical types (1, 2 and 3) based on the absence or presence of a neurological phenotype in addition to the systemic manifestations (organomegaly, bone involvement, cytopenia). [2].

Prior studies have suggested that Ambroxol; a pharmacological chaperone, increases mutant β-glucocerebrosidase enzyme activity [3–5], and as demonstrated by Narita et al., Ambroxol in combination with ERT, may be a promising therapy for patients with Gaucher disease type 3 (GD3). [6].

Herein, we assess the efficacy of Ambroxol in two Canadian patients with GD3 using levels of glucosylsphingosine (Lyso-Gb1; Lyso-GL1), a downstream metabolic product of glucosylceramide and a biomarker for diagnosis and monitoring of patients with GD [1], as an outcome measure, as well as changes in neurological status clinically before and after Ambroxol supplementation. Lyso-Gb1 levels were measured using high pressure liquid chromatography–tandem mass spectrometry, at Centogene AG, Rostock, Germany, as described previously. [7].

2. Case report

2.1. Patient 1

Patient 1 is a 15-year-old male, diagnosed at 1 year of age with Gaucher disease type 1 (GD1). He is compound heterozygous (G377S/G195E) for a maternally inherited pathogenic variant c.1246G > A, p.(Gly416Ser); and a paternally inherited likely pathogenic variant c.701G > A, p.(Gly234Glu) in the *GBA* gene. β-glucocerebrosidase enzyme activity in Patient 1 was measured at 1.62 μmol/h/mg protein (reference range: 15.2 ± 6.3 nmol/h/mg protein). 60 IU/Kg of imiglucerase biweekly, was started immediately upon diagnosis. At 10 years of age he developed refractory epilepsy and at 11 years age he developed mild unsteadiness on tandem gait. By 12 years of age, his gait progressively worsened such that it was staggering and unsteady and he became non ambulatory, wheelchair dependent, and unable to stand without support, raising the suspicion of GD3 instead of GD1. To assess this worsening in his clinical status from a year ago, a brain MRI was

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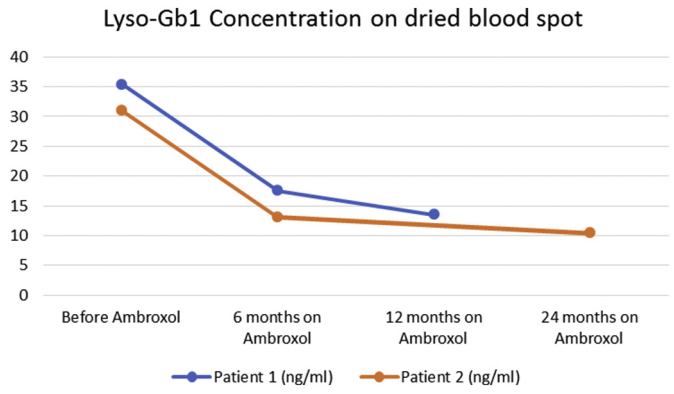

Fig. 1. Lyso-Gb1 concentration on dried blood spot in Patient 1 and Patient 2, before Ambroxol supplementation, and after Ambroxol supplementation.

performed and was normal.

Baseline Lyso-Gb1 level on dried blood spot (DBS) was 35.4 ng/ml (DBS reference range: ≤4.8 ng/ml). Treatment with Ambroxol (25 mg/Kg/day) was initiated a few months after the brain MRI was performed at 12 years of age, and after 6 months on supplementation, the Lyso-Gb1 level dropped to 17.5 ng/ml (50.6% reduction). After another 6 months, the Lyso-Gb1 level dropped further to 10.5 ng/ml, showing a total reduction of 61.9% in a 12-month period of Ambroxol supplementation (Fig. 1). A similar 60.9% reduction in Lyso-Gb1 was noted in CSF. Baseline CSF lyso-Gb1 level was 0.7 ng/ml which dropped to 0.426 ng/ml, after 24 months of Ambroxol supplementation; (CSF reference range: ≤ 0.0029 ng/ml).

In the first 3 months following initiation of Ambroxol, Patient 1 was able to ambulate for short periods of time without a wheelchair, due to improvement in his ataxia. However, no changes were noted in his seizure type, frequency or duration. Patient 1 has now been on Ambroxol for 3 years, and his seizures remain unchanged. Long-term EEG monitoring shows multifocal and generalized epileptiform discharges interictally recorded on a normal background. In addition, spontaneous and photosensitive generalized myoclonic and focal to bilateral convulsive seizures were captured ictally. No changes were noted on EEG, with Ambroxol. Antiepileptic therapy, during the Ambroxol trial, includes levetiracetam, clonazepam, felbamate, dilantin and escitalapram.

In the past 12 months, Patient 1 had several new fractures (femur, tibia and calcaneus), which led to loss of any noted improvement in his ambulation, due to pain from these fractures. In addition, he developed anxiety arising from anticipating the pain and deconditioning from the fracture-related hospitalization. There were no fractures prior to the initial loss of ambulation at the age of 12 years.

2.2. Patient 2

Patient 2 is a 21-year-old female diagnosed at 3 years of age with GD1 and started immediately on 60 IU/Kg of imiglucerase biweekly. Her genotype is N188S/R463H and her β-glucocerebrosidase enzyme activity was measured at 0.6 μmol/h/mg protein (reference range: 15.2 ± 6.3 nmol/h/mg protein). Neurological symptoms appeared at 10 years of age including refractory epilepsy, progressive ataxia and tremor, raising suspicion of GD3 instead of GD1. A brain MRI was performed at 10 years of age and was normal. Patient 2 was wheel chair bound at 18 years of age and had frequent hospitalizations due to episodes of status epilepticus.

Baseline Lyso-Gb1 level on DBS was 31 ng/ml (DBS reference range: ≤4.8 ng/ml). Treatment with Ambroxol (25 mg/Kg/day) was initiated at 18 years of age and after 6 months on supplementation her Lyso-Gb1 level dropped to 13 ng/ml (58% reduction). After another 18 months on Ambroxol, her Lyso-Gb1 level dropped further to 10 ng/ml for a total 67.7% reduction in a 24-month period of Ambroxol supplementation (Fig. 1).

In the first 6 months following initiation of Ambroxol, Patient 2 noted a significant improvement in her ataxia and was able to ambulate for longer periods of time unassisted and was not requiring a wheelchair. This improvement persists to this date. Although seizure type and frequency were unchanged, her seizures were shorter in duration and she has not required a hospital admission for status epilepticus in the past 3 years. There has not been any changes made to her antiepileptics, during this time period. Antiepileptic therapy, during the Ambroxol trial, includes stiripentol, clobazam, lamotrigine and dilantin.

Although the patient reported significant improvements in her quality of life, on Ambroxol, due to her improved ambulation and the shorter duration of seizures, she still continues to have seizures. After 2 years on Ambroxol, she elected to add Cannabidiol oil to her existing therapy, in hopes that this might reduce the frequency of her seizures.

Both patients tolerated the Ambroxol well, without any reported side effects or any interruptions in the supplementation. Both patients had a negative comprehensive epilepsy panel at Blueprint Genetics. In addition, Patient 1 had trio whole exome sequencing, which only identified the already known variants in the *GBA* gene.

3. Discussion

Here we report our experience with oral Ambroxol supplementation in combination with ERT in two Canadian patients with GD3. An improvement in Lyso-Gb1 levels on DBS in both Patient 1 and 2 (improvement was also noted in Patient 1 in CSF as well. This measurement was not done in Patient 2) was demonstrated. Clinical improvement in ambulation was also noted in both patients initially and continued in Patient 2 but was lost in Patient 1 due to fractures. Patient 1 did not have any notable changes in his seizures (neither in type, frequency or duration). However, the duration of seizures in Patient 2 was markedly reduced, not necessitating any admissions to hospital for status epilepticus in the past three years, along with improved ambulation, leading to a significant improvement in her quality of life. The differences in the response of Patient 1 and Patient 2 to Ambroxol is likely secondary to their different genotypes. Furthermore, it was clear that in our patients, the addition of Ambroxol to their regimen of ERT and antiepileptics was still not sufficient to alleviate their seizures completely.

Although this study has a number of limitations, our observations in these two Canadian patients with GD3 are in line with the preliminary findings published by Narita et al. suggesting that Ambroxol in combination with ERT may be a potential promising treatment for patients with GD3, depending on the patient's genotype. However, randomized clinical trials are needed, with a larger number of patients, to further evaluate these findings. Having said that, we realize that the likelihood of randomized controlled clinical trials sponsored by industry may be low, given the low cost of Ambroxol and subsequent poor return on investment, which lends to the relevance of case reports as described herein and by Narita et al.

Disclosure statement

Dr. Ari Zimran receives honoraria from Shire and Pfizer; the Gaucher Clinic run by Dr. Zimran receives research grants from Shire, Pfizer, Sanofi/Genzyme and Centogene, and support from Shire, Pfizer and Sanofi/Genzyme for participation in their respective registries (GOS, TALIAS and ICGG). Dr. Zimran also shares a provisional patent on the use of Ambroxol with glucocerebroside for the prevention of Parkinson's disease and related disorders.

All other authors have no conflicts of interest to declare.

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