Treatment of Fabry Disease: Current and Emerging Strategies

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Abstract: Fabry disease is an X-linked lysosomal storage disorder (LSD) due to deficiency of the enzyme α-galactosidase A (GLA). Absent or reduced enzyme activity leads to impaired catabolism of neutral glycosphingolipids, particularly globotriaosylceramide (Gb3), resulting in intracellular deposition of such lipids. Clinical manifestations in hemizygote males include angiokeratoma, hypohydrosis, acroparesthesia, abdominal pain, proteinuria, renal insufficiency, left ventricular hypertrophy and cerebrovascular accidents. Heterozygote women may present with mild to severe signs and symptoms. Since year 2001, enzyme replacement therapy (ERT) is the only specific treatment for Fabry disease. The beneficial effect of ERT on different organs/systems has been extensively evaluated, and an improvement in renal function, cardiac mass and quality of life has been reported.

Different treatment approaches are currently on development. One of them implies the use of the active-site-specific chaperone 1-deoxygalactonojirimycin that acts facilitating folding of mutant GLA in the endoplasmic reticulum and increasing its lysosomal residual activity. Reduction of Gb3 deposits has been shown in lymphoblasts from Fabry patients with missense mutations and transgenic mouse model expressing a missense mutation GLA. Gene therapy has been also developed as a potential option for treatment of Fabry disease. This review will discuss these novel therapeutic options along with their advantages and limitations.

Keywords: Agalsidase alfa, alpha-galactosidase A, chaperone treatment, enzyme replacement therapy, Fabry disease, gene therapy.

INTRODUCTION

Fabry disease (MIM 301500) is an X-linked genetic disorder of glycosphingolipid catabolism that results from a deficiency of the lysosomal enzyme α-galactosidase A (α-D-galactoside galactohydrolase, EC 3.2.1.22; GLA) [1, 2]. This defect leads to the accumulation of the enzyme substrates, mainly globotriaosylceramide (Gal α1-4 Gal β1-4Glc β1-1Cer; Gb3; or ceramide trihexoside) in lysosomes of a variety of different cell types throughout the body [3]. The incidence of Fabry disease has been estimated to range from 1/40,000 to 1/117,000 live births for males [4, 5]. Recently, a high incidence of 1/3,000 of the later onset form has been revealed by newborn screening [6].

Fabry disease exhibits a range of clinical phenotypes, a common feature of all lysosomal storage diseases, from the early-onset severe ''classic form'' to the late-onset moderate one called the ''variant form.'' Classic form is observed in patients with no residual GLA activity. Manifestations start in infancy/adolescence, and include acroparesthesia, hypohydrosis, heat/exercise intolerance, angiokeratoma, gastrointestinal pain, diarrhea, fever and proteinuria. The main complications are more prominent after the age of 30 when kidney, heart and/or cerebrovascular disorders appear [7,8]. Patients with the variant form have residual GLA activity and develop milder clinical manifestations, circumscribed mainly to one organ, such as heart (cardiac variant) [9] or kidney (renal variant) [10].

Penetrance of Fabry disease in females is high, 70% of heterozygotes display clinical manifestations. Disease expression in females depends on the chromosome X inactivation pattern [11]. Age at onset of symptoms in females is generally older and the disease is more variable than in males. Severe complications in females are most often associated to heart disease and stroke [12].

Diagnosis of Fabry disease generally starts with clinical suspicion by the physician in a male with angiokeratoma, acroparesthesia, proteinuria, hypohydrosis, and/or renal failure. Confirmation of diagnosis is based on demonstration of absence or reduced GLA enzymatic activity [13]. Detection of a mutation in GLA gene is a complementary confirmatory assay. This test also leads to testing the presence of the same mutation in female relatives who may be heterozygotes, allowing confirmatory diagnosis in females. Enzymatic activity assay in women is inconclusive [14].

ENZYME REPLACEMENT THERAPY

Fabry disease treatment was strictly symptomatic until the introduction of specific therapy consisting of enzyme replacement. Symptomatic treatment aimed to alleviate symptoms: analgesics, anti-inflammatory, anticonvulsants or even opioids for neuropatic pain; antispasmodics and antimotility agents for abdominal pain; angiotensin converting enzyme inhibitors for proteinuria, dialysis and kidney transplant for renal insufficiency; diuretics, cardiotonics, antiarritmics or pacemaker for cardiac involvement.

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Two recombinant enzyme preparations became available for specific treatment of Fabry disease. Agalsidase alfa (Replagal™; Shire HGT, Lexington, MA, USA) is produced in a human cell line by gene activation technology. This technology activates GLA gene in human fibroblasts. Agalsidase beta (Fabrazyme®; Genzyme Corporation, Cambridge, MA, USA) is produced by expression of human GLA gene inserted into CHO cell. Both products were approved by the European Agency for the Evaluation of Medicinal Products in 2001; agalsidase alfa at a recommended dose of 0.2 mg/kg and agalsidase beta at a dose of 1 mg/kg, administered every other week. In the US, due to orphan drug laws dictated that only one of these products could be approved, agalsidase beta has been approved in 2003. Recently, agalsidase alfa has been available to U.S. patients since December 2009 under an FDA-approved treatment protocol. Because both proteins are derived from the human GLA gene, any structural differences are likely to arise from differences in post-translational modifications, particularly glycosylation, which can influence biodistribution, cellular uptake, stability, and potentially immunogenicity. The studies reported demonstrate that biochemically and structurally the two enzyme preparations tested may be very similar [15].

Biweekly intravenous infusion of the enzymes to Fabry patients was associated with decreases in plasma and urinary Gb3, and reduction of accumulation of glycosphingolipids in capillary endothelial cells, renal glomerular cells, and tubular epithelial cells [16-20].

Enzyme replacement therapy was associated with a substantial decrease in neuropathic pain in affected men [17, 21, 22], and a statistically significant improvement in peripheral nerve function [23,24]. Reduction of pain crisis and chronic pain in affected patients after 24 and 36 months with agalsidase alfa was reported [25]. Relief of gastrointestinal symptoms is one of the earliest and most consistently beneficial effects of ERT [26], together with increase of sweating, generating better thermal regulation and heat tolerance [24]. This collection of results is manifested as an improvement in patients´ quality of life [22].

Elevated cerebral blood flow velocities in Fabry disease have been reversed after enzyme replacement. However clinical significance of these findings is not clear [27], since cerebrovascular attacks have occurred or persisted in some patients despite treatment [20,28].

Many reports have described stabilization of renal function in patients receiving enzyme replacement therapy. Glomerular filtration rate (GFR) in Fabry patients naïve of specific treatment declines at a mean rate of -12 ml/min/1.73 m^2/yr [29]. A double blind placebo controlled study have shown the rate of loss of GFR in patients with baseline GFR from 30 to 135 ml/min/1.73 m^2 was numerically less than that seen during the placebo period [30,31]. Reductions of loss of renal function to around -2 ml/min/1.73m^2/yr have been observed [30,32]. Treatment with agalsidase did not affect proteinuria [30]. However, therapy with agalsidase alfa in patients with proteinuria<1g/24hs receiving angiotensin converting enzyme inhibitors, was effective to control the progression of renal insufficiency [32]. A summary of renal data from a large, longitudinal, observational study of patients receiving ERT have concluded that treatment with agalsidase alfa "can significantly improve renal function in patients with Fabry disease, at least in those with a mild decrease in GFR, and may be able to slow down the natural decline in renal function in patients with moderate reduction in GFR" [33].

Recently published results of a randomized, controlled trial of treatment with agalsidase beta showed the rate of progression of a composite clinical outcome of renal, cardiac, and cerebrovascular complications and death in patients with moderately severe renal impairment was slowed [34]. Although the study sample was relatively small, the results suggest that early intervention with ERT may prevent irreversible end-organ damage in Fabry patients.

Although approved dose of agalsidase alfa is 0.2 mg/kg every other week, studies carried out to analyze the effect of different regimens [35] showed similar pharmacokinetic behavior. When the dosing frequency was switched to 0.2 mg/kg weekly, the rate of decline in loss of estimated glomerular filtration rate over 2 years was significantly slowed [36]. A comparative study of reducing dose of agalsidase beta to 0.2 mg/kg instead of the approved dose of 1 mg/kg, failed to show any effect at reducing left ventricular hypertrophy [37].

Studies give the evidence that enzyme replacement therapy is safe and effective in patients with Fabry disease on maintenance dialysis therapy, improving clinical symptoms of the disease and stabilization of cardiac involvement after 2 years of ERT [21,38]. Cardiac disease is responsible for approximately half of all deaths in dialysis patients [39]. Approximately three quarters of patients with end-stage renal disease starting dialysis therapy have LV hypertrophy, and the process of progressive increase in LV mass continues after the institution of dialysis therapy [40]. ERT could be administered during peritoneal dialysis [41] or hemodyalisis [38, 42] without losing the effect. The same principle accounts for kidney transplanted patients, ERT is indicated to control heart affection and improve other general symptoms [43].

Cardiomyopathy is improved in patients after 12 to 18 months of treatment with agalsidase, manifested as a decreased of left ventricular (LV) mass and better myocardial function [44-46]. Fabry patients at an early stage have virtually no myocardial fibrosis [47,48], however systolic function could be affected. These patients with no detectable fibrosis and mild hypertrophy at baseline showed a normalization of LV wall thickness and mass during ERT [49,50]. Parallel to cardiac morphology, LV radial and longitudinal septal function improved to normal. The effect of ERT critically depended on the stage of the disease at baseline. In patients with localized myocardial fibrosis in only one LV segment, a reduction in LV hypertrophy could be achieved during ERT that was associated with a stabilization of LV function and exercise capacity. Patients in a more advanced stage with fibrosis in several LV segments still showed some reduction in hypertrophy but hardly any benefit in terms of LV function during long-term ERT [51].

As stated above, heterozygotes women with Fabry disease affected. Enzyme replacement therapy administered in females is safe and efficient [52,53]. Moreover, agalsidase

alfa has been applied in women during pregnancy without any harmful effect on the mother or the newborn [54].

Both products have been used for treatment in children [8,55-57], demonstrating reduction of plasma and skin Gb3, and improvements of autonomic regulation, sweating and heart rate variability.

Agalsidase therapy should be administered throughout the life of the patient. Hospital-based infusion is perceived as inconvenient and home infusion therapy, assisted by a trained nurse, is greatly appreciated by patients and their families. Generally, first infusions are administered at hospital, and after the patient tolerates the treatment, switch to home therapy could be successful [58].

IgG antibodies develop in treated men, being more frequent with agalsidase beta, even at a 0,2 mg/kg dose [37]. IgE antibodies were detected only in patients receiving agalsidase beta [16, 34, 38, 55, 59]. Differences in immunogenicity between both proteins could be attributable to different cell origin and/or glycosilation pattern of each product.

No study has compared effectiveness of both products at each approved dose until now. The Canadian Fabry Disease Initiative has been initiated in order to analyze both preparations [60] head to head.

Recently, a study with the largest number of patients and clinical variables analyzed at 5 years of treatment has been published, confirming reduction of LV hypertrophy, renal function stabilization and pain and quality of life improvements [61].

CHAPERONES

Many mutated GLA enzymes had residual enzymatic activity but are significantly less stable [62], suggesting that enzyme deficiency was caused by abortive exit from the ER. The ER has a system of quality control of newly synthesized proteins, by which misfolded mutant proteins are retained and degraded [63]. Asano *et al.* have evaluated the concept of using competitive inhibitors of GLA as specific chemical chaperones that could rescue mutant enzymes from degradation in ER and target them to lysosomes, where the mutant enzymes could display their residual activity [64]. The compound 1-deoxygalactonojirimycin (DGJ) enhanced the mutant enzyme activity from human Fabry lymphoblasts when used at a lower concentration than that required for inhibition. DGJ could correct the ER trafficking defect of retained mutant GLA to lysosomes, indicating DGJ exerted a chaperone-like effect [65,66]. In human Fabry fibroblasts, DJG treatment resulted in clearance of lysosomal Gb3 inclusions [67]. These studies suggested the therapeutic potential of a pharmacological chaperone for treatment of Fabry disease [68].

The next step consisted of analyzing enhancement effect of DGJ *in vivo*. To accomplish this task, a transgenic mouse expressing a human mutant GLA in a null background was generated [69]. Oral administration of DGJ was safe, elevated enzyme activity in different organs and reversed Gb3 storage [70].

Chaperone treatment has a disadvantage; DGJ would be effective for patients who have missense mutations causing misfolding problems in GLA protein. However, it would not be useful in patients with nonsense mutations, insertions/deletions or rearrangements. Among patients with missense mutations, predictability of efficacy is not precise, and direct experimental evidence is crucial [71].

The following question is regarding the level of enzymatic activity needed to prevent developing of symptoms. Many LSD patients with a significant level of residual activity are asymptomatic. Based on the fact that classical patients' activity is lower than 3% and variant patients´ ones between 5-10%, one would assume that residual activity greater than 10% might be sufficient at improving clinical manifestations [72]. Various studies have evaluated the response of missense-mutated GLA-Fabry cells to DGJ [73,74]. The response to DGJ is variable. A group of mutated cells did not respond to DGJ exposure, and male patients with these mutations would not benefit from chaperone therapy. Responder mutations are those that GLA activity can be increased to twice the affected level and are likely to show a strong therapeutic response. There is also an intermediate response group of patients that may also benefit from this approach, but may be with a lower outcome. Chaperone therapy could be of benefit for heterozygotes.

In clinical phase I trials of DGJ (Amigal, Amicus Therapeutics, Cranberry, NJ, USA) in healthy volunteers, no drug-related adverse event was reported indicating that DGJ is also well tolerated in man. Results from the Phase 2 studies indicated that treatment with Amigal was generally well-tolerated, with no drug-related serious adverse events. In subjects identified as responders, treatment resulted in increased levels of the GLA, as measured in white blood cells and in the kidney, and reduced levels of the Gb3 as measured in renal interstitial capillary cells from kidney biopsies and in urine. Preliminary data indicate that glomerular filtration rate has remained stable and the average annual rate of change in this parameter, excluding hyperfiltrators, was +2.0 mL/min/1.73m^2 (http://www.amicustherapeutics.com).

ERT is the only specific treatment available for Fabry disease. However, differential distribution and accessibility of administered enzyme to tissues and organs, including central nervous system, could limit its effectiveness. Considering the route of administration and compared to the intravenous infusion of proteins, DGJ is an oral small molecule drug, and would increase GLA activity in all cell types. Chaperone therapy could be used as a concomitant therapy.

GENE THERAPY

Gene therapy has several advantages over an alternative enzyme replacement approach, as it offers long-term therapeutic effect, eliminates risks associated with repeated parenteral administrations, and is less expensive. Fabry disease is potentially amenable for gene therapy, as target cells are readily accessible, relatively low levels of enzyme would be enough to reduce Gb3 deposits, and metabolic cooperativity effects were also manifested [75]. In gene therapy, expressed GLA could be transferred into uncorrected cells by this cooperativity mechanism.

Biochemical and functional abnormalities of Fabry adult mice can be corrected by gene therapy, although the thera-

peutic efficacy is influenced by various factors including the choice of the vector and promoter, route of vector administration and the sex of mice.

One of the approaches consisted of the application of adeno-associated virus carrying human GLA (rAAV-GLA) delivered via the hepatic portal vein [76]. Two weeks postinjection, GLA activity in the livers of rAAV-GLA-injected Fabry mice was 20-35% of that of the normal mice, which continued as long as 6 months after treatment. In parallel to the elevated enzyme levels, significant reductions in Gb3 levels to near normal at 2 and 5 weeks post treatment were observed. The lower Gb3 levels continued in liver, spleen, and heart, up to 25 weeks with no significant immune response to the virus or GLA [76]. However, the clinical feasibility of the portal-vein delivery seems limited, particularly if it is necessary to perform the procedure repeatedly.

Another via of introduction, an injection into muscle of an AAV vector containing the GLA gene driven by chicken-actin (CAG) promoter and the citomegalovirus enhancer, was investigated. Study from Takahashi *et al.* [77] resulted in a sustained increase of GLA activity in plasma up to 30 weeks along with complete clearance of tissue Gb3 and improvement of cardiac hypertrophy as analyzed by echocardiography. In contrast, results from Park *et al.* failed to show any detectable GLA activity in application of the vector in quadriceps muscle [78], but showed good outcome with intravenous administration. Another depot organ that could function as another portal for the secretion of proteins into the circulation is the lung [79].

The kidney is the one of the main organs affected by Fabry disease. A strategy was developed with the aim to decrease Gb3 level in the kidneys, with an expected concomitant reduction of Gb3 in other organs, based on introduction of a solution containing naked plasmid DNA encoding human GLA by hydrodynamics-based transfection directly into the left kidney of Fabry mice. Expression studies have shown GLA mRNA was present in the left kidney but not in other tissues, and the treated mice showed partial therapeutic effects: increased GLA activity in the injected kidney and by cooperative effect, in the liver, heart, and plasma, and decreased Gb3 in the injected kidney, contralateral kidney, liver, heart, and spleen [80].

Considering early therapeutic intervention may be more effective than delayed treatment of adult patients a study was undergone in order to examine the effects of gene therapy in neonatal period. Neonatal injection of a small amount of AAV [81] or lentiviral vector [82] resulted in sustained GLA expression and prevention of glycosphingolipid accumulation.

CONCLUSIONS

Fabry disease is a chronic, progressive and multisystemic pathology. These features make the disease treatment a real challenge.

Enzyme replacement constitutes the only approved specific treatment until this time. This type of intervention have shown to be effective in slow done the rate of progression or stabilize the disease, especially when it is initiated at a relatively early age, before extensive irreversible damage to tissues develops. It has the inconvenience of the need of a whole life intravenous infusion every other week, however, instauration of home infusion setting makes the treatment more amenable for patients.

Other approaches are on development, such as gene therapy and chaperones. Chaperone treatment could be useful for a certain group of Fabry patients that will benefit from this oral therapy. Moreover, enzyme replacement and chaperone could be use in conjunction. Gene therapy approach would be an option, but still requires demonstration of safety and efficacy, and consensus for use from world wide scientific community.

CONFLICT OF INTEREST

PR received grants for research from Shire HGT.

LIST OF ABBREVIATIONS

GLA	=	Alpha-galactosidase A
LSD	=	Lysosomal storage disorder
Gb3	=	Globotriaosylceramide
ERT	=	Enzyme replacement therapy
GLA	=	α-galactosidase A
GFR	=	Glomerular filtration rate
LV	=	Left ventricular
DGJ	=	1-deoxygalactonojirimycin
AAV	=	Adeno-associated virus

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