Molecular Genetics and Metabolism Reports 11 (2017) 65–68

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Effectiveness of enzyme replacement therapy in Fabry disease: Long term experience in Argentina

Gustavo Cabrera*, Juan Politei, Norberto Antongiovani, Hernán Amartino, On behalf of GADYTEF (Grupo Argentino de Diagnóstico y Tratamiento de la Enfermedad de Fabry)

GADYTEF (Grupo Argentino de Diagnóstico y Tratamiento de la Enfermedad de Fabry), Argentina

ARTICLE INFO

Keywords:
Fabry disease
Enzyme replacement therapy
Effectiveness

ABSTRACT

Evidence regarding long term effectiveness of enzyme replacement therapy (ERT) in Fabry disease (FD) is needed. The aim of this study was to analyze in a cohort of FD patients in Argentina, the long term effectiveness of ERT on renal, cardiac and cerebrovascular parameters.

Methods: Patients with genetically proven FD were included from GADYTEF (Argentinean group for the treatment of FD) between 2001 and 2014. Renal, cardiac, and cerebral outcomes were prospectively studied in patients treated with ERT. Additionally, the occurrence of major cardiac complications, stroke, end-stage renal disease and death was analyzed during follow up.

Results: During the follow-up 8 major complications occurred in 5 patients ($n = 2$ deaths, $n = 4$ cases of end stage renal disease and $n = 1$ atrial fibrillation), 4 of them males and only 1 female who suffered an atrial fibrillation. Sudden death or stroke did not occur. Four (40%) of 10 males with baseline left ventricular hypertrophy (LVH) reduced left ventricular mass index (LVMI) from 163.1 ± 64.7 to 123.4 ± 49.8 g/m^2, 2 stabilized LVMI and 4 increased LVMI from157.9 ± 32.3 to 261.6 ± 48.6 g/m^2. Estimated glomerular filtration was stable in 30 patients (17 males and 13 females).

Conclusions: We observed a few major complications during the follow up. Future studies are necessary to show the effectiveness of ERT in affected patients.

1. Introduction

Fabry disease (FD) is a rare X-linked lysosomal storage disorder caused by deficiency of the enzyme α galactosidase A (α Gal). The enzymatic deficit results in progressive intracellular accumulation of globotriaosylceramide (GL-3) in almost all cells in different tissues, with profound clinical effects on the heart, kidneys and brain [1,2]. Progressive organ failure leads to early death in hemizygous male patients, typically at the age of 40–50 years, and reduced quality of life in heterozygote patients [3].

In 2001, the European Medicines Agency (EMA) authorized two alfa-galactosidase A preparations for treatment of FD: agalsidase-alfa (Replagal® Shire HGT) and agalsidase-beta (Fabrazyme® Genzyme Corp.) due to their efficacy and safety [4]. Short-term clinical trials, in advanced FD patients in which biopsies of kidney, skin and heart was done, demonstrated that ERT with recombinant α Gal was able to remove the microvascular deposits of globotriaosylceramide (GL-3). This provided proof that ERT can slow the progression towards serious cardiac, renal and cerebrovascular complications [4–6]. Recent studies began to provide evidence about the long term effectiveness of ERT in FD patients regarding progression of the disease, long-term survival and cause-specific death [7,8], however, more evidence regarding long term effectiveness of ERT is needed [7,9,10].

The aim of this study was to analyze in a cohort of FD patients in Argentina, the effectiveness of long term follow up of ERT on renal, cardiac and cerebrovascular parameters.

2. Methods

2.1. Patients and study design

Patients with confirmed diagnosis of FD through enzyme activity and DNA analysis were included from GADYTEF (Argentinean group for the treatment of FD) between 2001 and 2014. Prospective data as well as historical data was obtained from included patients. Patients received agalsidase beta at a dose of 1 mg/kg/2 weeks. After agalsidase beta shortage due to viral contamination at manufacturing, many patients had to reduced doses or switch to agalsidase alpha for an

* Corresponding author at: Dirección Simón Bolivar 487, C1181ACH Buenos Aires, Argentina.
E-mail address: gustavo.h.cabrera@hotmail.com (G. Cabrera).

http://dx.doi.org/10.1016/j.ymgmr.2017.02.005
Received 23 November 2016; Received in revised form 13 February 2017; Accepted 13 February 2017
Available online 04 May 2017

average of 1.5 years. To be included patients must have at least 12 months of follow up since baseline evaluation. Patients were excluded if they had late-onset genetic variants (D313Y, A143T, E66Q, IVS 919G > A), or had end-stage renal disease (dialysis or transplant) before starting agalsidase beta. Only untreated patients meeting treatment criteria according to Argentinean experts Guidelines [11,12] were included to avoid bias.

2.2. Baseline and follow up evaluations

Baseline and yearly evaluations were performed in all patients. Baseline was defined as the data closest to the initiation of ERT (prior to ERT or with a maximum of 4 weeks thereafter).

2.3. Cardiac evaluations

Evaluation of cardiac function was conducted at baseline and during follow up by measuring the thickness of the interventricular septum (IVS), the left ventricle posterior wall (LVPW) and left ventricular mass index (LVMI) by using commercially available ultrasound equipment (3.5 MHz variable frequency phased array transducer, ATL, HDI3500 and HDI5000). Normal values for IVS and LVPW were < 11 g/m^2 for males and < 10 g/m2 for females. Normal LVMI was 43–95 g/m^2 in females and 49–115 g/m^2 in males, according to the recommendations of the American Society for Echocardiography (ASE) [13]. Ejection fraction was calculated using the modified Simpson method.

2.4. Kidney evaluation

The evaluation of kidney function was conducted by means of the estimated glomerular filtration rate (eGFR) using the MDRD *epi* formula, measurement of microalbuminuria (> 30 mg/24 h) and proteinuria (> 300 mg/24 h) every 3 months. Kidney disease was broken down into five stages in accordance with the eGFR: (1) between 120 and 90 ml/min; (2) between 90 and 60 ml/min; (3) between 60 and 30 ml/min; (4) between 30 and 15 ml/min; and (5) < 15 ml/min.

2.5. Cerebrovascular evaluation

The presence of cerebrovascular damage was assessed by baseline and yearly brain magnetic resonance imaging (MRI) using a 1.5 T Signa Echo Speed (GE) MRI system with data acquisition techniques standardized for cerebrovascular diseases (conventional FLAIR, T1, T2, diffusion and proton density) and a magnetic resonance angiogram of the intracranial vessels. A cerebral white matter lesion (WML) was diagnosed by a Neuroradiologist as a hyperintense white matter lesion of > 1 mm in diameter on FLAIR- and T2-weighted MRI images.

2.6. Major complications definitions

At least 6 months of follow-up was required for the analysis of renal function, LV mass and cerebral white matter lesions during ERT. Major complications were stroke, cardiac complications, ESRD and death. Cerebrovascular complication was the presence of a stroke diagnosed by a neurologist or the presence of a new lesion on MRI during follow up. Cardiac complications included onset of atrial fibrillation, other arrhythmias necessitating hospitalization, pacemaker or cardiac defibrillator (ICD) implantation and myocardial infarction and myocardial infarction, percutaneous coronary intervention, or coronary artery bypass graft. Another cardiac outcome evaluated was the change in LV wall thickness. ESRD was defined as CKD stage 5 (a GFR ≤ 15 ml/min/1.73 m^2), dialysis or renal transplantation.

2.7. Statistical analysis

Data are presented as mean and standard deviation or percentage, as appropriate. Baseline and follow-up values were compared using paired *t*-test or Fisher's exact test, as appropriate. A *p*-value of $p < 0.05$ was considered statistically significant. Change of renal function and LV mass was assessed using repeated measures with random intercepts for each patient and expressed as mean change per year and standard error (SE). Decline of renal function were calculated per CKD category and LV mass by presence or absence of LVH. Yearly follow-up time intervals ± 2 months from baseline were defined. The appearance of major complication during follow up was described and adjusted by the contribution of age, gender and ERT duration on the development. Multiple logistic regression was applied for the analysis. Analyses were performed with Stata 10.1 version.

Ethical approval was requested from the institutional review board.

3. Results

A total of 39 patients with genetically proven FD, (mean age 31.7 ± 12.8 years) were included during the study period with a mean follow up of 68.2 ± 36.9 months for the entire group (84 ± 25.1 for males and 43 ± 21.6 for females). All patients originated from 8 different families with FD, except one patient with a “di novo” mutation. The most frequent mutation observed in the sample was L415P mutation in exon 7 which was shared by 3 non-connected families (Table 1). Baseline characteristics of all patients with FD are presented in Table 2. Twenty four of these 39 patients were males with a mean age at the beginning of treatment of 26.8 ± 8.8 years, and 15 were females with 41.3 ± 10.6 years. The major clinical complain at baseline was neuropathic pain, which was found in 37 patients (males: 100%). At baseline 11 (45.8%) male patients and 9 (60%) females had increased LVMI, 2 males and 3 females CKD (eGFR EPI < 60 ml/min/1.73 m^2) and 17 males and 13 females had proteinuria and/or albuminuria. One young male was on CKD stage 4 (age 27). Only three patients (two females) had asymptomatic white matter lesions at baseline brain MRI.

3.1. Major complications

There were 8 major complications in 5 patients. Three males required dialysis and two died a few years later due to sepsis. One of the patients who died at the age of 26.5 years, started ERT with nephrotic syndrome and advanced CKD (eGFR, 29 ml/min/1.73 m^2) at the beginning of the treatment. Shortly after the initiation of ERT the patient started with dialysis and a progressive worsening was observed. After 2 years of ERT, an episode of sepsis originated by a mesenteric ischemia was developed and the patient died. The second patient who died, was a 37 years old male with advanced CKD, cardiac involvement, heavy smoker with chronic obstructive pulmonary disease in which an episode of sepsis was developed after 25 months of ERT.

One (case no. 2) of these patients needed a coronary revascularization due to an acute coronary syndrome. One patient started ERT at the age of 45.2 years and kidney function worsened to CKD stage 4 was observed. Only 1 female had an event. The patient suffered a non-complicated atrial fibrillation after 40 months of ERT; she had comorbidities including being overweight, hypertension and diabetes which

Table 1
Mutation distribution between genders.

	Males	Females
L415P	13	7
D264Y	2	2
E398X	4	–
A292T	1	4
Y365X	1	2
Cys223Arg	1	–
C202Y	2	–

Table 2
Baseline characteristics of all patients with Fabry disease.

Parameters	Males	Females
n	24 (61.5)	15 (38.5)
Age at diagnosis (years) (mean ± SD)	27 ± 8.7	39.3 ± 11.2
Age at starting ERT (years) (mean ± SD)	27.8 ± 8.4	41.3 ± 10.6
HTN (n, %)	3 (12.5)	5 (33.3)
Current smokers (n, %)	11 (45.8)	4 (26.7)
LVMI (g/m^2) (mean ± SD)	116.7 ± 47.1	106.5 ± 28.8
Patients with increase LVMI (n, %)	11 (45.8)	9 (60)
Atrial fibrillation (n, %)	0	1 (6.7)
Coronary artery disease (n, %)	1 (4.2)	0
Dyspnea (n, %)	4 (16.7)	2 (13.3)
Baseline eGFR (mean ± SD)	96.9 ± 27.6	79 ± 23.3
eGFR < 60 ml/min/1.73 m^2	2 (8.3)	3 (20)
Proteinuria (mg/24 h) (mean ± SD)	0.9 ± 1	1.3 ± 1.3
Increased proteinuria (n, %)	11 (45.8)	7 (46.7)
Microalbuminuria (mg/24 h) (mean ± SD)	87.9 ± 62.6	52.8 ± 44.9
Increased microlbuminuria (n, %)	6 (25)	6 (40)
Neuropathic pain (n, %)	24 (100)	13 (86.7)
Stroke	0	0
Reported ACEi/ARB use, % (n)	16 (66.7)	7 (46.7)

ERT = enzyme replacement therapy, HTN = hypertension, LVMI = left ventricular mass index, eGFR = estimated glomerular filtration rate, ACEi/ARB = angiotensin-converting enzyme inhibitors/angiotensin receptor blockers.

may complicate outcome beyond FD. One patient was withdrew of ERT during 2 years and then re-started. The patient's condition worsened and required dialysis. No sudden deaths or episodes of ventricular tachycardia were seen and no cardiovascular devices were needed (Table 3).

As a whole group, LVMI in males slightly increased from 113.2 ± 45 to 127.4 ± 64 g/m^2. When we analyzed patients with and without increased LVMI at baseline we saw that in 4 (36.4%) of 11 males baseline LVH was reduced from 163.1 ± 64.7 to 123.4 ± 49.8 g/m^2, 2 patients stabilized LVMI and 4 patients increased LVMI from 157.9 ± 32.3 to 261.6 ± 48.6 g/m^2. Of 9 females with baseline LVH, only 1 patient increased LVMI from 158 to 204 g/m^2; all remaining patients reduced or stabilized their LVMI. All patients without LVH at baseline remained with normal LVMI at the end of the study.

During follow-up, eGFR was stable in 30 patients (17 males and 13 females). Male mean eGFR at baseline was 96.9 ± 28 and at the end was 102 ± 29 ml/min/1.73 m^2; for female patients eGFR was 85.8 ± 23 and 84 ± 23 ml/min/1.73 m^2 respectively. No patient had a stroke during the follow up. Two male patients presented progression in the number of new MRI lesions, all were asymptomatic.

4. Discussion

ERT with agalsidase beta has been shown to change the natural history of FD. In a multicenter, randomized, placebo-controlled, double-blind study of 58 patients who were treated with agalsidase beta at a dose of 1 mg per kilogram every other week, a significant reduction of GL-3 in the renal, cardiac, and dermal capillary endothelium was shown in all patients [5].

A 10-year follow up of 52 patients from the former cohort (50 men) concluded that patients who initiated treatment at a younger age and with less kidney involvement benefited the most from therapy. Overall, mean LPWT and IVST did not show a significant increase during the 10-year (median) treatment interval, which suggests stabilization over time. In patients aged < 40 years receiving agalsidase beta, LPWT and IVST remained stable. In contrast, in patients aged > 40 years at first infusion, LPWT and IVST significantly progressed from baseline to last follow-up. Patients who initiated treatment at older ages and/or had advanced renal disease experienced disease progression [14].

Previously, the Phase IV study has shown that in patients with advanced FD, agalsidase beta treatment was associated with a significant 61% relative risk reduction of renal, cardiac and cerebrovascular life-threatening events and death in a per protocol analysis adjusted for proteinuria. Larger treatment effects were seen in patients with less organ damage at baseline [4].

In our cohort 95% of patients with classic FD on ERT for a mean of 68.2 ± 36.9 months were alive (2 men died) and 87% of them remained free of major complications. This contrasts with severe renal, cardiovascular and cerebrovascular major complications reported during the fourth and fifth decades of life in ERT-naïve male patients, increasing the risk of premature death in their early 50s [2–4].

The current evidence of unsatisfactory results in relation to male patients who started ERT at the average age of 40 years has been one of the main reasons for justifying therapy at an early age. Furthermore, evidence of agents causing cellular hypertrophy in cardiomyocytes and vascular smooth muscle cells, such as markers associated with fibrosis in different tissues, has clearly demonstrated the need for early treatment [9,10].

The positive results from our cohort are probably related to the young age of the patients at initiating ERT (male mean age: 27.8 ± 8.4 years) and are in accordance with the prior publication of 12 years of treatment of 20 patients with the same mutation [14]. Our population included the former 20 patients with L415P mutation and 19 patients with 6 different mutations, all of whom were related to classic phenotypes of FD.

As is already known, LVH and CKD are predictors for cardiac and renal complications in FD [2,15]. Most of our patients stabilize cardiac mass and kidney function. No cerebrovascular involvement was noticed. Microalbuminuria or proteinuria was treated with ACE-inhibitors and/or angiotensin receptor blockers. In addition, anti-coagulants/anti-aggregatory, antiarrhythmic and pain medication were instituted following current Fabry guidelines. In our study, two patients died due to sepsis. It is important to mention that although sepsis has not been described as a typical complication of FD, it has been associated with intestinal ischemic events and other vascular events in the disease [16].

A limitation of our study is the follow up time, despite is almost 6 years, more time would be necessary to identify disease progression or complications associated. Another limitation is the unblended

Table 3
Baseline characteristics of all patients with Fabry disease with a main outcome event.

Patient (sex)	Type of event	Age at ERT start (years)	Baseline LVMI (g/m^2)	Baseline eGFR (ml/min/1.73 m^2)	Time to event (months)	Mutation	ACEi/ARB use
1 (male)	Dialysis/death	26.5	190	29	14 and 82	Cys223Arg	No
2 (male)	Dialysis/CABG/death	37.3	148.5	63	48	L415P	Yes
3 (male)	Dialysis	39.9	250	27	25	E398X	Yes
4 (male)	CKD stage 4	45.2	190	71.5	88	L415P	No
5 (female)	AF	50.3	139.5	115	52	A292T	Yes

CABG: cardiac bypass graft; CKD: chronic kidney disease; ACEi: angiotensin-converting enzyme inhibitors; ARB: angiotensin receptor blocker; ERT: enzyme replacement therapy; LVMI: left ventricular mass index; eGFR: estimated glomerular filtration rate.

condition of treating physicians, however, objectives measures were done in order to avoid bias.

5. Conclusions

In conclusion, we observed a few major complications favorable of this cohort of FD patients, probably related to age at commencement of ERT. Most of our patients were young at baseline with no advanced FD. These findings reinforced the necessity for the initiation of ERT as early as possible, before the disease progressed to irreversible organ damage and for a control of cardiovascular risk factors. Future studies are necessary to show the effectiveness of ERT in affected patients from real life.

Funding

This research was not funded.

Acknowledgements

The authors would like to thank the many patients and physicians who have agreed to participate in this follow up paper. We acknowledge colleagues at Genzyme, a Sanofi company for medical input (Wytske Kingma) and epidemiological advice (Judy Kempf).

References

[1] A. Ortiz, J.P. Oliveira, S. Waldek, D.G. Warnock, B. Cianciaruso, C. Wanner, et al., Nephropathy in males and females with Fabry disease: cross-sectional description of patients before treatment with enzyme replacement therapy, Nephrol. Dial. Transplant. 23 (2008) 1600–1607.

[2] R. Schiffmann, D.G. Warnock, M. Banikazemi, J. Bultas, G.E. Linthorst, S. Packman, et al., Fabry disease: progression of nephropathy, and prevalence of cardiac and cerebrovascular events before enzyme replacement therapy, Nephrol. Dial. Transplant. 24 (2009) 2102–2111.

[3] S. Waldek, M.R. Patel, M. Banikazemi, R. Lemay, P. Lee, Life expectancy and cause of death in males and females with Fabry disease: findings from the Fabry registry, Genet. Med. 11 (2009) 790–796.

[4] M. Banikazemi, J. Bultas, S. Waldek, W.R. Wilcox, C.B. Whitley, M. McDonald, et al., Agalsidase-beta therapy for advanced Fabry disease: a randomized trial, Ann. Intern. Med. 146 (2007) 77–86.

[5] C.M. Eng, N. Guffon, W.R. Wilcox, D.P. Germain, P. Lee, S. Waldek, et al., Safety and efficacy of recombinant human alpha-galactosidase A—replacement therapy in Fabry's disease, N. Engl. J. Med. 345 (2001) 9–16.

[6] R. Schiffmann, J.B. Kopp, H.A. Austin 3rd, S. Sabnis, D.F. Moore, T. Weibel, et al., Enzyme replacement therapy in Fabry disease: a randomized controlled trial, JAMA 285 (2001) 2743–2749.

[7] S.M. Rombach, B.E. Smid, M.G. Bouwman, G.E. Linthorst, M.G. Dijkgraaf, C.E. Hollak, Long term enzyme replacement therapy for Fabry disease: effectiveness on kidney, heart and brain, Orphanet. J. Rare Dis. 8 (2013) 47.

[8] R. Schiffmann, M. Ries, M. Timmons, J.T. Flaherty, R.O. Brady, Long-term therapy with agalsidase alfa for Fabry disease: safety and effects on renal function in a home infusion setting, Nephrol. Dial. Transplant. 21 (2006) 345–354.

[9] M.A. Engelen, E. Brand, T.B. Baumeister, T. Marquardt, T. Duning, N. Osada, et al., Effects of enzyme replacement therapy in adult patients with Fabry disease on cardiac structure and function: a retrospective cohort study of the Fabry Munster Study (FaMuS) data, BMJ Open 2 (2012).

[10] F. Weidemann, M. Niemann, S. Stork, F. Breunig, M. Beer, C. Sommer, et al., Long-term outcome of enzyme-replacement therapy in advanced Fabry disease: evidence for disease progression towards serious complications, J. Intern. Med. 274 (2013) 331–341.

[11] Amartino H, Politei J, Cabrera G, Raskovsky V. On behalf of Grupo Argentino de Diagnóstico y Tratamiento de la Enfermedad de Fabry (GADYTEF). Guía práctica para el diagnóstico, tratamiento y seguimiento de la enfermedad de Fabry. Rev. Nefrol. Diál. Traspl., volumen 27 – Número – 2007.

[12] Amartino H, Politei J, Raskovsky V. On behalf of Grupo Argentino de Diagnóstico y Tratamiento de la Enfermedad de Fabry (GADYTEF). Guía práctica para el diagnóstico, tratamiento y seguimiento de la enfermedad de Fabry. Nefrol. Argentina, volumen IV – Número 3 – Año 2006.

[13] R.M. Lang, M. Bierig, R.B. Devereux, F.A. Flachskampf, E. Foster, P.A. Pellikka, et al., Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology, J. Am. Soc. Echocardiogr. 18 (2005) 1440–1463.

[14] D.P. Germain, J. Charrow, R.J. Desnick, N. Guffon, J. Kempf, R.H. Lachmann, et al., Ten-year outcome of enzyme replacement therapy with agalsidase beta in patients with Fabry disease, J. Med. Genet. 52 (2015) 353–358.

[15] M.R. Patel, F. Cecchi, M. Cizmarik, I. Kantola, A. Linhart, K. Nicholls, et al., Cardiovascular events in patients with Fabry disease natural history data from the Fabry registry, J. Am. Coll. Cardiol. 57 (2011) 1093–1099.

[16] J. Politei, B.L. Thurberg, E. Wallace, D. Warnock, G. Serebrinsky, C. Durand, et al., Gastrointestinal involvement in Fabry disease. So important, yet often neglected, Clin. Genet. 89 (2016) 5–9.