

Original Article

Long-term therapy with agalsidase alfa for Fabry disease: safety and effects on renal function in a home infusion setting

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Abstract

Background. Fabry disease is an X-linked disorder of glycosphingolipid catabolism that is the result of an intracellular deficiency in the lysosomal enzyme α -galactosidase A (α -Gal A). This enzymatic defect results in the accumulation of globotriaosylceramide (Gb₃) within cells and causes progressive neurological, cardiovascular and renal dysfunction. Our objective is to describe the safety and renal effects of long-term enzyme replacement therapy.

Methods. This was a single centre, prospective open-label treatment trial in 25 adult male Fabry patients who had completed a 6-month randomized placebo-controlled study and subsequently enrolled in an open-label extension study. Patients were treated every other week with agalsidase alfa (0.2 mg/kg) infused intravenously over 40 min. The main outcome measures were safety, antibody response and renal glomerular filtration rate (GFR).

Results. During the 4–4.5 years of enzyme replacement therapy, all eligible subjects were able to transition to home therapy. Eight patients developed persistent IgG antibodies to agalsidase alfa, but IgE antibodies were not detected in any patient. The development of IgG antibodies appeared not to affect any clinical end points. Estimated GFR remained stable in subgroups of patients with Stage I (GFR >90 ml/min) or Stage II (GFR 60–89 ml/min) chronic kidney disease at baseline. In contrast, in the subgroup of patients with Stage III chronic kidney disease (GFR 30–59 ml/min), the slope of the decline in GFR was reduced compared with comparable historical controls, suggesting that enzyme replacement therapy was slowing the decline of renal function in this susceptible population.

Conclusions. Long-term enzyme replacement therapy with agalsidase alfa is safe and may slow the

progressive decline in renal function that was commonly observed in adult males with Fabry disease.

Keywords: chronic renal disease; enzyme replacement therapy; Fabry disease; genetic disease; glomerular filtration rate; renal dysfunction

Introduction

Fabry disease is an X-linked disorder of glycosphingolipid catabolism that is the result of a deficiency in the lysosomal enzyme, α -galactosidase A (α -Gal A) [1]. This enzyme deficiency is caused by any of at least 356 mutations (Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff <http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>) and results in the cellular accumulation of glycolipids including globotriaosylceramide (Gb₃) in vascular endothelial and smooth muscle cells, cardiac myocytes, renal epithelial cells, dorsal root ganglion neurons, autonomic nervous system, brain and other organs and tissues [2–5]. Fabry disease occurs in people of all ethnicities and has a prevalence that has been estimated at 1 in 117 000 live births [6]. Female heterozygotes have been traditionally thought to be carriers who would not express the Fabry phenotype. However, it is becoming increasingly clear that the majority of females show, to varying degrees, the same signs and symptoms of Fabry disease as male hemizygotes [7–11].

The cellular accumulation of Gb₃ is likely responsible for all of the signs and symptoms of Fabry disease, although no evidence of its direct toxic effect has so far been shown. The initial manifestations of Fabry disease are usually angiokeratoma and recurrent episodes of neuropathic pain in the extremities occurring during childhood or adolescence [7,8]. Most affected patients also exhibit a decreased ability to sweat [7,12,13]. As the disease progresses, renal function deteriorates, usually beginning in the third or fourth decade of life, and in

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male patients often progresses to end-stage renal failure [14]. Left ventricular hypertrophy, conduction defects and valvular abnormalities are also commonly seen [3,7,11,15,16], as are structural and functional abnormalities of the cerebral circulation. Transient ischaemic attacks and large and small vessel strokes have been reported in both males and females [2,17–19]. All of these classic signs and symptoms of Fabry disease have been reported in heterozygous females, but their onset occurs on average about 10 years later than in males [10]. Renal, cardiac and cerebrovascular disease contribute to premature mortality at a median age of about 50–55 years in men and 70 years in women [10,14,20].

The incidence of chronic kidney dysfunction in male Fabry patients, characterized by a loss of renal filtering capacity, urinary excretion of protein and an increase in serum creatinine, increases rapidly after the age of 30 years [14]. In a study of the medical records of 105 male Fabry patients with long-term follow-up, Branton and colleagues reported that 50% had proteinuria by age 35 years with the onset of chronic renal insufficiency (CRI) (defined as serum creatinine ≥ 1.5 mg/dl) by age 42 years [14]. By the age of 60 years, all surviving patients had progressed to end-stage renal disease (ESRD) requiring dialysis and/or renal transplantation. Other authors have reported similar ages of onset and rates of progression of kidney dysfunction in Fabry disease [20–23].

Until recently, no specific treatment existed for Fabry disease. However, the results of clinical studies with two differently produced forms of α -Gal A suggest that enzyme replacement therapy may be both safe and effective. Schiffmann and coworkers reported clinical improvement in adult male Fabry patients treated for 24 weeks with biweekly infusions of agalsidase alfa compared with patients receiving placebo infusions. Enzyme-treated patients showed significant reductions in the severity of their neuropathic pain [24]. In addition, enzyme-treated patients in this study showed improvement in abnormal regional cerebral blood flow [17–19] and improvement in sweating as well as heat and cold sensory nerve function [12]. In a similar study, Eng and colleagues reported that biweekly infusions of agalsidase beta for 20 weeks decreased vascular endothelial deposits of Gb₃ in skin and the interstitial capillary endothelium in the kidney compared with patients receiving placebo [25].

In this article, we present long-term (a total of 4–4.5 years) safety and renal effects as well as the practicality of home infusions of enzyme replacement therapy with agalsidase alfa in the adult male Fabry patients who had completed the original double-blind, placebo-controlled study [24].

Methods

Patients

Fabry patients who completed the original 24-week, randomized, double-blind, placebo-controlled study of agalsidase alfa

were eligible to continue on open-label therapy [24]. Study patients were exclusively adult hemizygous males and all had to have neuropathic pain at the start of the original study as a requirement for enrollment. There was no specific requirement for renal dysfunction to be present at baseline in the original study.

Study design

This study was an open-label extension following an initial 6-month, randomized, placebo-controlled trial that was designed to evaluate the long-term safety and efficacy of enzyme replacement therapy with agalsidase alfa in adult male patients with Fabry disease. The institutional review board of the National Institute of Neurological Disorders and Stroke reviewed and approved the protocol, and all patients who participated in the study gave their informed written consent prior to enrollment in both the initial randomized placebo-controlled study and the open-label extension.

All patients received intravenous infusions of agalsidase alfa (0.2 mg/kg) administered every other week over a 40 min period during the entire duration of the open-label study. After the first year of open-label treatment in the hospital setting, their agalsidase alfa infusions could be administered at home. The investigators calculated the dose, and a visiting nurse, who was required by protocol to observe the patient for at least 1 h after completion of each infusion, administered the infusions. Safety parameters and renal function were evaluated at 6-month intervals.

Agalsidase alfa

Agalsidase alfa (ReplagalTM, Transkaryotic Therapies, Inc., Cambridge, MA), an α -Gal A enzyme with the same amino acid sequence and the same glycosylation pattern as the native human enzyme, was manufactured in a genetically engineered continuous human cell line by methods previously described [24]. Each dose (0.2 mg/kg) was diluted in 100 ml of normal saline and infused intravenously at a constant rate over 40 min. Pre-medications were not routinely used during this study unless the patient had experienced an infusion-related reaction during a previous infusion. In these few cases, patients were pre-medicated orally with a steroid and/or H₁ and H₂ histamine receptor antagonists or NSAIDs. These pre-medications were subsequently withdrawn in the majority of patients without the return of infusion-related symptoms.

Safety

Serious adverse events were monitored throughout the study. Other safety assessments were made every 3 months during the first year and every 6 months thereafter. These assessments included electrocardiograms (ECGs), clinical laboratory tests, and serum assays for IgG and IgE anti-agalsidase alfa antibodies. Anti-agalsidase alfa antibodies were assayed using an Enzyme-linked Immunosorbent Assay (ELISA) as previously described [24]. A positive antibody response was defined as a time-point to baseline absorbance ratio of 2.0 or greater, provided the absorbance of the sample was ≥ 0.04 absorbance units. A persistently positive antibody response was defined by serial positive antibody titres including the last assessment made. A transiently positive antibody response was defined by the development of positive antibody titres

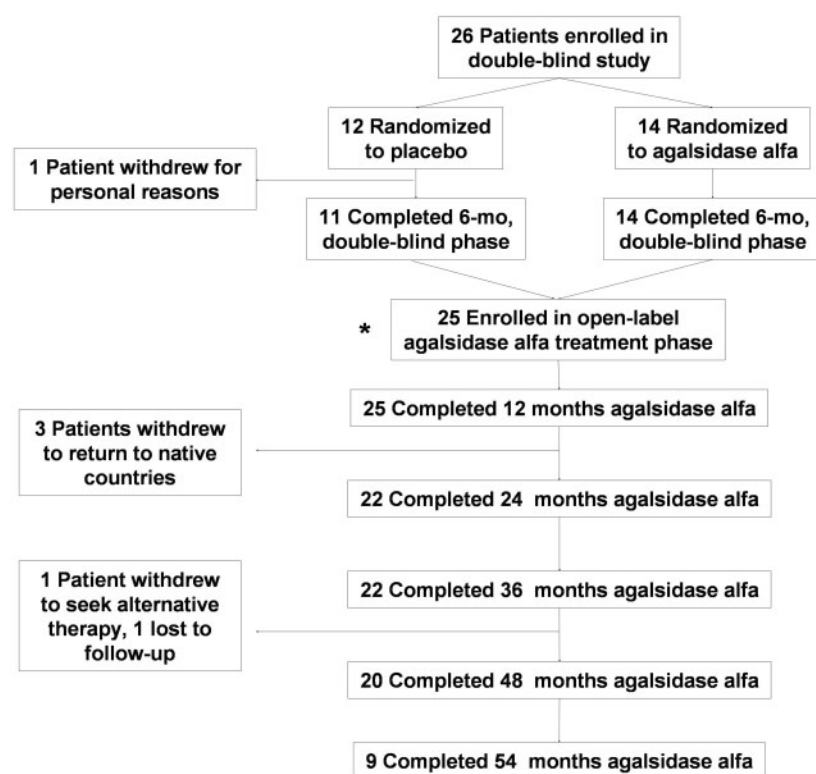


Fig. 1. Flow chart of patient disposition. Only patients in the active treatment arm of the double-blind phase of the study had the opportunity to be treated for 54 months. *One patient who had been in the original placebo group, began peritoneal dialysis before entering the open-label phase and received a kidney transplant within the first 12 months of active therapy.

that subsequently became negative, including the last assessment made.

Measurements of renal function

Glomerular filtration rate was estimated (eGFR) using the four-variable Modification of Diet in Renal Disease (MDRD) method using serial measurements of serum creatinine throughout the 4–4.5 years of the study [26]. GFR was also measured directly by the inulin clearance method, but only for the first 30 months of the study. Due to the occurrence of a worldwide shortage of inulin, this direct method of measuring GFR could not be used for the entire duration of the study. For purposes of classification, subjects were also classified by their initial stage of renal function as defined by the National Kidney Foundation Guidelines where stage I chronic kidney disease (CKD) represented a GFR of ≥ 90 ml/min/1.73 m², stage II, 60–89 ml/min/1.73 m², and stage III, 30–59 ml/min/1.73 m² [27]. Total protein excretion was measured from 24 h urine collections. All assays were performed in one hospital laboratory using its standard clinical chemistry methods.

Metabolic effects

Gb₃ in plasma samples was quantified by HPLC as nanomoles per millilitre as previously described [2]. Twenty-four hour urine specimens were collected from each patient and the total volumes recorded. Duplicate aliquots of 50 ml were frozen in polypropylene centrifuge tubes and stored

at -70°C . Additional aliquots were taken for creatinine determination. Urine specimens in 50 ml conical tubes were centrifuged at $23\,700\times g$ for 1 h at 4°C and the sediments resuspended in 1 ml of distilled water. Gb₃ in the urine sediment samples was then quantified by HPLC and expressed as nanomoles of Gb₃ per gram creatinine.

Statistical analysis

Pre-treatment baseline values were based on measurements made just before beginning active treatment with agalsidase alfa for all patients. Patients who completed at least 36 months of treatment were considered to have completed the study for the purpose of this report. Comparison of changes in eGFR, plasma Gb₃ concentration and urinary Gb₃ excretion from baseline to the last time point were performed with two-sided, paired *t*-tests.

Results

All 25 male Fabry patients who completed the original 6-month double-blind, placebo-controlled study were included in this open-label extension study. At the beginning of the enzyme replacement therapy, their average age was 36.8 years and the mean time interval since diagnosis of their Fabry disease was 13.6 years. Other demographic characteristics of this study population have been previously reported [24]. Figure 1 shows the patient disposition during the study.

One patient, who received placebo in the double-blind phase of the study, was started on dialysis for ESRD prior to the start of his open-label agalsidase alfa therapy and received a renal transplant within the next 12 months. For that reason, data from this patient were excluded from the analysis of effects on renal function and included only in the safety and plasma Gb₃ analyses. Of the 25 patients entering this extension study, 14 had received agalsidase alfa and 11 had received placebo during the original 6-month double-blind phase. Three patients withdrew from this open-label study after being treated with agalsidase alfa for 12 or 18 months to return to their native countries, where they continued to receive agalsidase alfa therapy on either a compassionate use or commercial basis, and continued to have periodic blood samples drawn for detection of anti-agalsidase alfa antibodies. One patient withdrew from the study after a total of 36 months of treatment with agalsidase alfa to enroll in a clinical study of agalsidase beta.

Treatment compliance

Over 98% of planned infusions of agalsidase alfa were performed. Eleven patients missed one or more infusions during the entire duration of the study. One patient (Patient 25) accounted for about one-third of the total missed infusions; he missed 12 infusions during his first year of treatment, including 5 consecutive treatments. However, this period of noncompliance was not associated with any serious adverse events.

Safety

Long-term treatment with agalsidase alfa was well tolerated and the majority of adverse events reported was consistent with the natural history of untreated Fabry disease and were not attributed to agalsidase alfa therapy. During the 4–4.5 year study, four patients suffered a cerebrovascular accident or a transient ischaemic attack, one patient suffered a myocardial infarction and required a coronary bypass, and another patient underwent a coronary angioplasty. With the exception of infusion-related reactions, none of the serious or non-serious adverse events were considered by the investigator to be related to agalsidase alfa therapy.

Infusion-related reactions

Thirteen of 25 patients (52%) in this study experienced an infusion reaction during or shortly after one or more infusions. These reactions typically consisted of facial flushing and rigors. In response to the frequency and severity of infusion reactions reported during the initial double-blind phase of the study, when the 0.2 mg/kg dose of agalsidase alfa was administered over 20 min, the duration of the infusions was lengthened from 20 min to 40 min, and all patients who had experienced infusion reactions were premedicated orally with

H₁ and H₂ receptor antagonists and/or a steroid prior to subsequent infusions. Agalsidase alfa infusions were not permanently discontinued in any patient due to infusion reactions.

Home infusion

The safety and feasibility of home infusion of agalsidase alfa was demonstrated during this long-term open-label maintenance study. Twenty-two patients had infusions in the home setting. A total of 1528 home infusions were administered (range of 42–73 infusions per patient). No additional safety concerns were raised by administration of agalsidase alfa in the home setting by a visiting nurse. Specifically, no patient experienced the new onset of infusion reactions after switching to home therapy.

Renal function

One patient received a kidney transplant prior to starting enzyme replacement therapy, therefore, effects on renal function were evaluated in only 24 patients. Of these 24 patients, 12 had stage I renal disease (eGFR: ≥ 90 ml/min/1.73 m²) at baseline, 8 had stage II (eGFR: 60–89 ml/min/1.73 m²), and 4 had stage III (GFR: 30–59 ml/min/1.73 m²) renal disease. In Table 1, individual eGFR data at baseline and during the study for each patient according to baseline eGFR subgroup are shown. Eleven patients were treated with angiotensin converting enzyme (ACE) inhibitors during this study, including all four patients with Stage III CKD and two other patients who progressed to CRI. Four other patients were treated with angiotensin receptor antagonists.

Mean eGFR remained relatively stable for up to 36–54 months of enzyme replacement therapy with agalsidase alfa for the entire patient population. Mean baseline eGFR was 88.4 ± 26.0 ml/min/1.73 m² (mean $\pm$ SD, $n = 24$) and after 48 months of treatment, mean eGFR declined only slightly to 75.1 ± 32.7 ml/min/1.73 m² (Table 1, Figure 2; $P = 0.039$). This small decrease in mean eGFR appeared to be driven primarily by the marked declines in GFR noted in the four patients with stage III renal disease at baseline (Table 1, Figure 1). In these latter patients, mean eGFR fell from 47.1 ± 9.4 ml/min/1.73 m² to 24.8 ± 14.5 ml/min/1.73 m² ($P = 0.098$) after 48 months of therapy (representing an average rate of decline of ~ 5.2 ml/min/1.73 m² per year). None of these patients with stage III renal disease progressed to end-stage renal failure while receiving long-term enzyme replacement therapy with agalsidase alfa. Mean serum creatinine increased from 1.17 ± 0.08 mg/dl (mean $\pm$ SEM) at baseline to 1.58 ± 0.29 mg/dl ($P = 0.065$) after 48 months of agalsidase alfa therapy. Again, this increase in the mean serum creatinine was primarily driven by the changes observed in the subgroup of four patients with Stage III renal disease at baseline (Figure 3).

Table 1. Estimated GFR (ml/min/1.73 m²) in male Fabry disease patients treated with agalsidase alfa for up to 54 months

Patient	Months										Final
	0	6	12	18	24	30	36	42	48	54	
Stage I CKD											
4	115.8	124.2	107.6	123.2	122.3	121.5	141.7	120.6	120.6		120.6
11	99.8	99.3	113.8	132.1	131.4	131.4	97.9	97.9	97.4		97.4
12	90.0	80.6	80.5	89.6							
13	103.3	90.9	102.7	102.1	102.1	101.5	101.5	89.4	100.9		100.9
14	103.6	96.6	96.1	110.1	95.7	84.8	109.2	94.9	94.9		94.9
15	95.7	114.3	100.3	113.2	112.2	111.1	111.1	97.6	97.6	109.3	109.3
16	142.6	115.5	101.3	131.0	113.2	100.3	128.5	99.3	88.2	88.2	88.2
17	125.2	144.9	143.8	123.2	122.3	122.3	121.5	121.5	120.6		120.6
21	109.7	117.6	116.9	116.9	116.3	116.3	134.9	100.9	100.4	100.4	100.4
22	108.4	95.3	107.6								
23	110.3	102.7	102.7	116.9	116.9	116.3	116.3	100.9	89.4	100.4	100.4
25	100.3	84.1	93.9	105.3	93.3	92.6	83.0	82.5			82.5
Mean	108.7	105.5	105.6	114.9	112.6	109.8	114.6	100.6	101.1	99.6	101.5
SD	14.1	18.5	15.3	12.7	12.2	14.6	17.9	12.2	11.9	8.7	12.4
Stage II CKD											
1	68.7	71.6	71.3	55.1	54.8	54.8	44.2	35.1	41.3		41.3
2	79.6	65.7	60.0	55.4	51.1	44.6	44.4				44.4
7	87.1	77.3	76.9	85.9	96.6	69.3	76.3	69.0	75.9		75.9
8	74.9	78.4	78.4	78.0	87.1	86.7	77.7	63.7	69.9	69.6	69.6
9	87.1	86.7	97.4	97.4	97.0	85.9	85.5	76.6	76.3		76.3
18	83.5	93.3	92.6	104.6	92.0	103.9	91.5	81.9	81.9	81.4	81.4
19	82.5	103.9	81.9	103.3	102.7	102.7	102.1	73.2	72.8	80.5	80.5
27	65.3	71.3	65.0	70.9							
Mean	78.6	81.0	77.9	81.3	83.0	78.3	74.5	66.6	69.7	77.2	67.1
SD	8.2	12.8	12.8	19.9	21.1	22.9	22.4	16.6	14.5	6.6	17.0
Stage III CKD											
3	33.7	32.0	29.1	28.9	25.6	22.1	19.4	15.1	13.1		13.1
6	48.2	40.9	43.4	40.8	40.6	32.6	30.9	25.8	24.7		24.7
10	55.4	55.4	33.6	30.6	33.5	31.9	24.5	21.2	16.1	14.2	14.2
26	51.1	57.5	52.9	57.2	62.1	62.1	56.8	39.9	45.2	48.5	48.5
Mean	47.1	46.5	39.8	39.4	40.5	37.2	32.9	25.5	24.8	31.4	25.1
SD	9.4	12.1	10.6	13.0	15.7	17.3	16.6	10.6	14.5		16.4

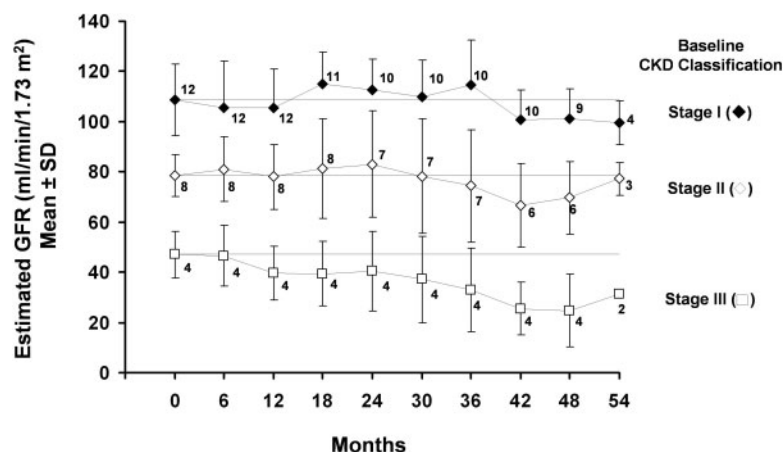


Fig. 2. The effect of treatment with agalsidase alfa on eGFR in male patients with Fabry disease. Patients with stage I chronic kidney disease (CKD) (GFR < 90 ml/min) are represented by filled diamonds, patients with stage II CKD (GFR 60–89 ml/min) are represented by open diamonds, and patients with stage III renal disease (GFR 30 ml/min) are represented by open squares. The horizontal lines show the mean baseline GFR for each of these subgroups. The number of patients represented by each point is shown next to the symbols. The low *n* at 54 months reflects the fact that only patients who were treated with agalsidase alfa during the initial 6-month double-blind portion of the study were treated for 54 months.

The degree of proteinuria at baseline in this patient population varied widely, ranging from 100 mg/24 h to 7.5 g/24 h, with a median of 353 mg/24 h. Thus, at baseline, all 24 patients had either proteinuria or

microalbuminuria. Fourteen of the 24 patients had proteinuria in excess of 300 mg/24 h. In the 20 patients completing at least 36 months of agalsidase alfa treatment and who had a valid final measurement of

protein excretion, the post-treatment median protein level was 543 mg/24 h, with a median increase of approximately 7 mg/24 h (range, -3.00 g/24 h to 2.72 g/24 h). Three patients with proteinuria at baseline improved to microalbuminuria (below 300 mg/24 h) at the end of the study, whereas three baseline microalbuminuric patients developed proteinuria during the 36 months of the study.

Metabolic effects

The mean plasma Gb₃ level at baseline was 11.4 ± 0.8 nmol/ml (mean $\pm$ SEM, Figure 4). Plasma Gb₃ decreased to a mean of 5.8 ± 0.4 nmol/ml over the first 6 months of treatment with agalsidase alfa and remained decreased for the duration of the study ($P < 0.001$ for all time points compared with baseline).

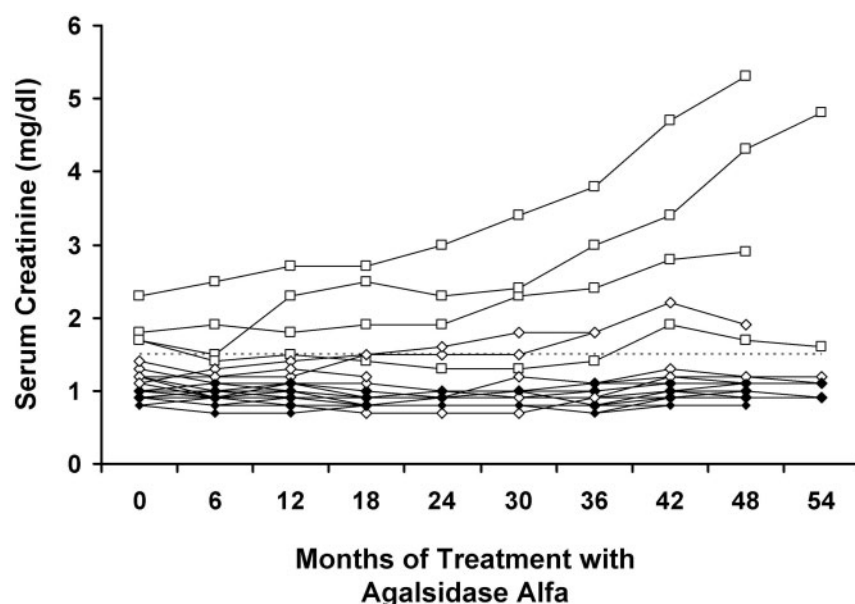


Fig. 3. The effect of treatment with agalsidase alfa on serum creatinine in male patients with Fabry disease. Symbols are the same as in Figure 1. Symbols connected by lines represent individual patients. The dotted line represents a serum creatinine of 1.5 mg/dl.

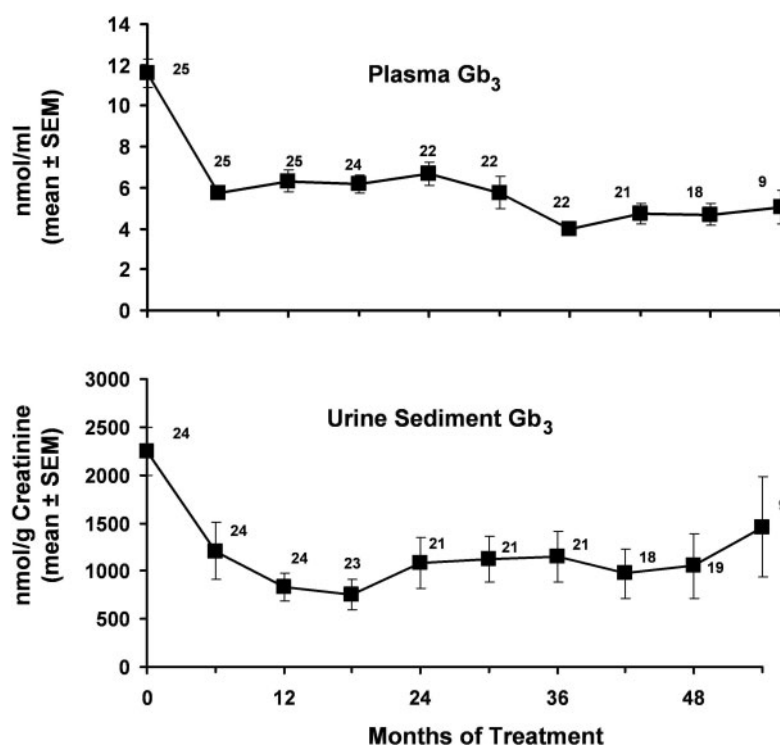


Fig. 4. The effect of agalsidase alfa on plasma Gb₃ levels and urine sediment Gb₃ in male Fabry patients. The numbers next to each point represent the number of patients. $P < 0.001$ for all time points compared with baseline. Normal urinary Gb₃ excretion is below 50 nmol/g creatinine/24 h.

After 48 months of enzyme replacement therapy with agalsidase alfa, mean plasma Gb₃ was 5.0 ± 0.6 mmol/ml, which represents a 65% mean reduction from baseline ($P < 0.001$). Urine sediment Gb₃ at baseline was 2566 ± 299 nmol/g creatinine (mean $\pm$ SEM, Figure 4). Mean urine sediment Gb₃ also decreased over the first 6 months of treatment and remained reduced for the remainder of the 4–4.5 year period of enzyme replacement therapy ($P < 0.001$ for all time points). A mean decrease of 59.7% was observed in the 21 patients with urine

sediment Gb₃ data available after 36 months of agalsidase alfa treatment.

IgG antibody formation to agalsidase alfa

Fourteen of 25 patients (56%) evaluated in this study showed an IgG antibody response to agalsidase alfa at one or more time points. Six of these patients reverted to IgG-negative by the end of the study, and therefore eight patients were persistently positive for IgG. No patient tested positive for IgE antibodies at any time point.

As suggested by Figure 5, the antibody status did not seem to influence change in renal function over time. In order to investigate whether these IgG antibodies might affect the metabolic actions of enzyme replacement, the time courses of changes in plasma Gb₃ and urine sediment Gb₃ were compared between subgroups of patients who tested persistently positive for IgG antibodies, and those who tested negative or had only a transient IgG response. As shown in Figure 6, all subgroups of patients demonstrated an initial decrease in both plasma and urine sediment Gb₃. However, in the subgroup of eight patients who generated a persistent IgG antibody response, mean urine sediment Gb₃ levels were observed to rise, nearly reaching mean baseline levels after 12 months of enzyme replacement. In contrast, in those 15 patients who had only a transiently positive antibody response or who remained antibody negative

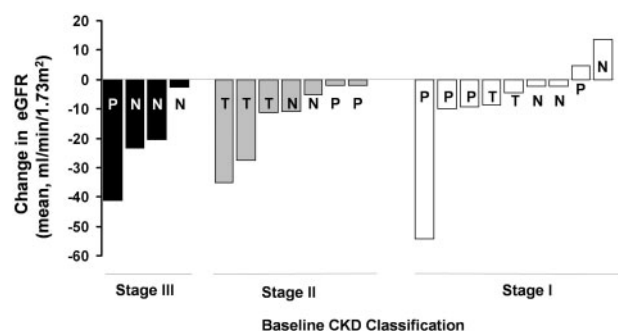


Fig. 5. The effect of treatment with agalsidase alfa on eGFR in patients with Fabry disease and their anti-agalsidase alfa antibody response. Only patients who completed at least 36 months of treatment are presented. The letters associated with each bar indicate the IgG antibody response observed in that patient. P = persistently positive, T = transiently positive and N = no antibody response during the study.

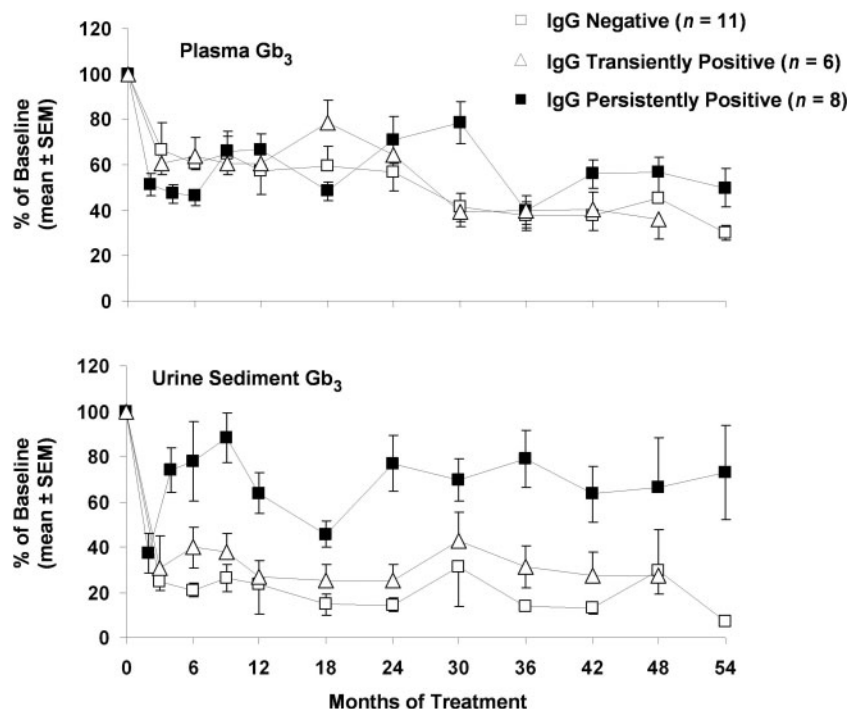


Fig. 6. Effect of IgG antibodies on the biological activity of agalsidase alfa. The filled symbols represent the patients who had demonstrated a persistently positive IgG antibody response while being treated and the open symbols represent patients who had no IgG antibody response (squares) or only a transiently positive response (triangles). The ns indicate the number of patients in each category at baseline. For the IgG-negative group in the urine Gb₃ plot, baseline n is 10, reflecting the fact the patient who underwent a kidney transplant was not included in any renal analyses.

throughout, mean urine sediment Gb₃ levels remained below 20% of the mean baseline level. The effect of a persistently positive IgG antibody response on plasma Gb₃ levels was much less dramatic. More importantly, the presence or absence of persistently positive IgG antibodies against agalsidase alfa did not appear to correlate with the magnitude or direction of changes in eGFR in individual patients (Figure 5).

Discussion

The results of this long-term, open-label study demonstrate that agalsidase alfa administered at a dose of 0.2 mg/kg by intravenous infusion over 40 min on an every-other-week basis was safe and well tolerated for up to 4.5 years. Because the treatment was well tolerated, all eligible patients were able to transition to administration of their infusions in the home setting. The incidence of serious adverse events was consistent with the natural course of Fabry disease, and none were attributed by the investigators to the treatment with agalsidase alfa. No deaths occurred and no patients withdrew from the study because of adverse events. One important safety consideration was the incidence and time course of the IgG antibody response to repeated infusions of agalsidase alfa. The generation of an IgG antibody response with repeated dosing was anticipated because the exogenous α -Gal A would be viewed as 'a foreign protein' by the subgroup of male Fabry patients with undetectable circulating α -Gal A levels. In fact, the incidence of anti-agalsidase alfa IgG antibody formation remained relatively low over the 4–4.5 year course of the study, as did antibody titres. Among those patients who generated any IgG antibody response, there was evidence of development of immunological tolerance (defined by the disappearance of a positive antibody test) in 43% of patients. The development of immunological tolerance to chronic administration of agalsidase alfa is consistent with clinical experience with repeated administration of other therapeutic proteins, for example alglucerase for Gaucher disease [28]. Most importantly, the development of anti-agalsidase alfa IgG antibody formation did not appear to affect clinical efficacy parameters including changes in renal function. However, since the number of patients studied was small, definitive conclusions regarding the clinical significance of IgG antibodies cannot be made at this time. The absence of an IgE antibody response, however, is critical for the long-term safety of long-term administration of enzyme replacement therapy. It is noteworthy, therefore, that no patient receiving agalsidase alfa developed IgE antibodies at any time during this study. In addition to the present study, 280 additional male Fabry patients have been treated with agalsidase alfa in published clinical trials without a single instance of IgE antibody formation being reported [29].

The relatively low incidence (12%) of infusion reactions without routine pre-medication allowed the

transition of patients from receiving their infusions in the hospital outpatient clinic to the home setting for all the eligible patients in this study [29]. Demonstration of the safety of treatment at home is important because it allows patients to be treated with less disruption of their activities of daily living, and furthermore, offers the potential of greater acceptance of, and compliance with, life-long enzyme replacement therapy. In addition, the convenience of 40 min infusions with few side effects may also lead to initiation of enzyme replacement therapy in asymptomatic adults or even children before signs and symptoms of major organ damage become evident. Early treatment may be more successful in altering the natural course of Fabry disease than some current treatment guidelines that recommend initiating therapy only after significant organ damage has become clinically evident.

In addition to the evaluation of safety, serial measurements of renal function were also made to evaluate the potential beneficial effects of long-term agalsidase alfa treatment in adult male Fabry patients. Not surprisingly, kidney function appeared to remain stable in the subgroups of patients with stage I or II renal disease. In contrast, the patient subgroup with stage III renal disease at baseline showed a mean rate of decline in eGFR of ~ 5.2 ml/min/1.73 m² per year (Table 1 and Figure 1). None of the four patients in this subgroup deteriorated to ESRD while receiving agalsidase alfa treatment in this study. However, in the subsequent 2 years following the end of this 4–4.5 year extension study, despite continuing agalsidase alfa infusions at the same dose and frequency, two of these stage III patients were started on dialysis.

In a study by Branton *et al.* [14] that followed 105 male Fabry patients before the advent of enzyme replacement therapy, the incidence of CRI, as defined by serum creatinine ≥ 1.5 mg/dl, increased in male patients above the age of 35 years, and once CRI developed, the decline in renal function to ESRD was rapid, averaging 4 ± 3 years. Others have reported a similar age of onset of CRI and the subsequent development of ESRD in untreated adult male Fabry patients [20–23]. Branton and co-workers identified 14 patients with CRI and documented a mean rate of decline of GFR of 12.2 ml/min per year over the next 4–4.5 years in this group [14]. This group was similar in age and baseline GFR as the four patients with stage III kidney disease in the present study, and thus, taken together, these results suggest that agalsidase alfa enzyme replacement therapy slowed the progression of decline in kidney function in this renal function subgroup. However, it is important to recognize that without a concurrent control group, this conclusion remains tentative.

Evidence for a beneficial metabolic effect of agalsidase alfa on the Fabry kidney is provided by the decrease in plasma and urinary sediment Gb₃ levels observed during the study. The decrease in urinary sediment Gb₃ is taken as evidence of a decrease in the Gb₃ content of shed renal tubular epithelial cells. Larger, long-term studies will be required to confirm

the suggestion that agalsidase alfa helps to preserve kidney function in Fabry patients.

Treatment with agalsidase alfa has also been reported to have other beneficial effects. Pain in the extremities (acroparesthesia) due to Fabry involvement in the peripheral nervous system is a common finding in Fabry disease and is often the initial symptom in childhood [7,8]. The anatomical origin of this neuropathic pain is not well understood, but has been attributed to Gb₃ accumulation in dorsal root ganglion cells [30]. Agalsidase alfa was shown to reduce the severity of neuropathic pain in the double-blind phase of the present study [24], a response that persisted for up to 2 years of chronic therapy [12]. Beneficial effects on sweating and other indices of sensory nerve function (warm and cold sensation) have also been reported [12]. Preliminary results from studies (mostly open-label) of agalsidase alfa demonstrated regression of left ventricular hypertrophy in both male and female Fabry patients [31]. Despite evidence of a beneficial central nervous system effect as evidenced by normalization of abnormally elevated regional cerebral blood flow with long-term enzyme replacement with agalsidase alfa, as reported by Moore *et al.*, four of these patients did suffer strokes while receiving enzyme infusions [17–19].

Study limitations

The relatively small patient numbers and the lack of a parallel placebo-treated group beyond the initial 6 months of the randomized double-blind phase limit the strength of the conclusions of this study. Although these results suggest that long-term agalsidase alfa therapy might have slowed the decline in kidney function in patients with severely compromised kidney function, it is important to recognize that there were only four patients in this patient subgroup and that no concurrent control group was studied. It is entirely possible that more aggressive use of concomitant medical therapy with ACE inhibitors and/or angiotensin II receptor blockers might have contributed to the slower decline in GFR observed in these severely affected patients compared with that reported in Fabry patients not receiving enzyme replacement therapy. Finally, longer-term outcome studies in larger populations of treated and untreated patients will be necessary to show that enzyme replacement therapy will reduce the morbidity associated with Fabry disease and extend the lifespan of these patients. As with any rare genetic disease, it is difficult to enroll large numbers of patients into clinical studies. This difficulty is even more pronounced when studying Fabry disease, because the heterogeneous progression of the disease makes it difficult to obtain a cohort of patients who are at a similar stage of the disease. Nonetheless, this study suggests that adult male patients with Fabry disease safely tolerate long-term enzyme replacement therapy with agalsidase alfa and this therapy potentially slows the progression of renal disease in those patients with advanced kidney involvement.

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