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Enzyme replacement therapy in Japanese Fabry disease patients: The results of a phase 2 bridging study

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Summary: Fabry Disease (α-galactosidase A deficiency) is an X-linked hereditary disorder leading to the pathological accumulation of globotriaosylceramide (GL-3) in lysosomes, particularly in the vascular endothelium of the kidney, heart and brain. We report the results of an open-label phase 2 study that was undertaken to evaluate whether ethnic differences exist that would affect agalsidase beta (Fabrazyme) treatment of Fabry patients in the Japanese population, relative to safety and efficacy. The study design mirrored the design of the completed phase 3 clinical trial that led to approval of the product agalsidase beta. The 13 Japanese, male Fabry patients enrolled in the study received the enzyme replacement therapy over a period of 20 weeks as biweekly infusions. All selected efficacy end points showed improvements that were comparable with findings from the phase 3 study. These improvements included reductions of GL-3 accumulation in both kidney and skin capillary endothelial cells to (near) normal levels (92% of patients). Kidney and plasma GL-3 levels decreased by 51.9% and 100%, respectively, by ELISA. Renal function remained normal. Fabry-associated pain, and quality of life, showed improvement over baseline in multiple categories. Related adverse events were mild or moderate in intensity and mostly infusion-associated (fever and rigors). As expected, IgG antibody formation was observed in 85% of the patients, but had no effect on treatment response. These results suggest that treatment with agalsidase beta is safe and effective in Japanese patients with Fabry disease. With regard to safety and efficacy, no differences were observed as compared to the caucasian population.

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Fabry disease (McKusick 301500) is an X-linked inborn error of metabolism characterized by deficient activity of the lysosomal hydrolase α-galactosidase A (α-GalA; EC 3.2.1.22). The prevalence in males is estimated as 1:40 000 to 1:60 000 (Desnick et al 2001; Meikle et al 1999). In classically affected male individuals, residual activity of α-galactosidase A is (nearly) absent, which results in the pathological accumulation of α-galactosyl-terminated neutral glycosphingolipids, predominantly globotriaosylceramide (GL-3), in cellular lysosomes. Accumulation of GL-3 occurs in virtually all tissues of the body, but particularly in the endothelial, perithelial and smooth-muscle cells of blood vessels, ganglion cells of the autonomic nervous system, glomeruli and tubules of the kidney and the cardiomyocytes of the heart (Desnick et al 2001). In the classical phenotype, deterioration of renal function will ultimately progress to end-stage renal disease in the third to fifth decades of life (Desnick et al 2001). Cardiac manifestations causing significant morbidity may include cardiomyopathy, angina pectoris, congestive heart failure, myocardial ischaemia and arrythmias (Linhart et al 2002). Cerebrovascular involvement can lead to transient ischaemic attacks, stroke and other neurological disorders (Kolodny and Pastores 2002). The clinical spectrum of Fabry disease also includes a 'renal variant' and a 'cardiac variant' phenotype in patients without classic symptoms who predominantly develop end-stage renal disease (Nakao et al 2003) and cardiac manifestations (Elleder et al 1990; von Scheidt et al 1991), respectively. Heterozygotes are also prone to manifest disease symptoms owing to the phenomenon of random X-chromosome inactivation (lyonization) (Lyon 2002). The disease spectrum in female carriers may range from asymptomatic disease to the classic phenotype.

Treatment of Fabry disease used to be limited to supportive care such as management of pain and hypertension (Desnick and Wasserstein 2001). Clinical trials with two enzyme replacement therapies, agalsidase alfa (Replagal; Transkaryotic Therapies, Inc., Cambridge, MA, USA) (Schiffmann et al 2000, 2001) and agalsidase beta (Fabrazyme; Genzyme Corporation, Cambridge, MA, USA) (Eng et al 2001a,b) have led to the commercial availability of these two products: both agalsidase beta and agalsidase alfa in the European Union and only agalsidase beta in the United States.

A phase 2 open-label trial was undertaken in patients of Japanese descent to evaluate whether ethnic differences exist that would affect treatment of Fabry disease with agalsidase beta in this population, in relation to safety and efficacy. The study design, including the safety and efficacy end points, mirrored the design of the completed phase 3 double-blind study with agalsidase beta (Eng et al 2001b). This choice of design was based on the concern that the relatively small number of patients with Fabry disease in Japan would not adequately power a primary end point in an open-label, single treatment group study design. The results of this phase 2 bridging study are reported here.

PATIENTS AND METHODS

Study design, patients, treatment regimen: This multicentre, phase 2, open-label trial was designed to evaluate the efficacy and safety of agalsidase beta treatment

and enrolled 13 male patients with confirmed Fabry disease. The patients received 1 mg/kg of agalsidase beta administered as 11 biweekly infusions over 20 weeks. Participating study sites were The Jikei University School of Medicine, Chubu National Hospital, Osaka University School of Medicine, Nagoya University School of Medicine, and Kyusyu University School of Medicine. The protocol was approved by the relevant institutional review boards and the trial was conducted in accordance with the Good Clinical Practice guidelines.

Patient eligibility criteria, as well as the clinical, biochemical, biopsy tissue (kidney, heart, skin) and safety assessments, were identical to those applicable for the phase 3 double-blind study. Refer to the publication by Eng et al (2001b) for details. For the management of potential infusion-associated reactions, the patients were pretreated with acetaminophen ($\leqslant$500 mg) or ibuprofen ($\leqslant$200 mg) and hydroxyzine (up to 30 mg) administered 1 h prior to each infusion. The infusion rate was less than 0.25 mg/min.

Evaluation of efficacy: The renal efficacy end point was the proportion of patients with a zero score for GL-3 deposits at week 20 (11 infusions). Additional tissue efficacy end points included microvascular endothelial deposits of GL-3 in the heart, skin and other kidney cell types. Biopsy tissues were scored (0–1–2–3 scoring system) by three blinded, independent pathologists. Specimens with no microvascular endothelial deposits or only trace amounts (normal or nearly normal) were given a score of 0; specimens in which the majority of vessels had evidence of a single endothelial inclusion (mild GL-3 accumulation) were given a score of 1; those with multiple aggregates of granules in the majority of capillaries were given a score of 2 (moderate GL-3 accumulation); and those with numerous aggregates of granules within the endothelium (often bulging into the lumen) in the majority of vessels were given a score of 3 (severe GL-3 accumulation). Majority scores were calculated per organ as well as summed for all organs. Change from baseline to week 20 was also assessed for GL-3 concentrations (ELISA assay) in kidney, urine sediment and plasma. Quality of life measurements included the Short Form McGill Pain Questionnaire and SF-36 Health Status Survey.

Statistical analysis: An exact binomial matched pair procedure was used primarily for the analysis of GL-3 accumulation in the capillary endothelium of the kidney to analyse the proportions of patients with a score of 0 at baseline compared to week 20. This test was also used to analyse GL-3 accumulation in the other cell types in the kidney and skin. A one-sample Wilcoxon signed-rank test was used to determine whether there was a significant difference from zero in the median change score from baseline to week 20. Descriptive statistics (n, mean, standard deviation, minimum, median, maximum) were displayed at baseline, at week 20, and for changes from baseline to week 20 for multiple parameters, such as the kidney, urinary and plasma GL-3 (ELISA), GL-3 accumulation in additional cell types, and the quality of life results as measured by the Short Form McGill Pain Questionnaire and SF-36 Health Status Survey. Change in SF-36 parameters was analysed by the Wilcoxon signed-rank test.

RESULTS

Patients: Baseline characteristics and demographic data for the 13 patients enrolled in the study are presented in Table 1.

Kidney GL-3 clearance

Kidney capillary endothelial cells. Reduction in kidney capillary endothelial cell GL-3 accumulation from non-0 scores to a 0 score (clearance) by week 20 was achieved by 12/13 (92%) patients ($p < 0.001$). At baseline, GL-3 accumulation was mild (score = 1) for 10 of 13 (77%) patients and moderate (score = 2) for 3/13 (23%) patients. All patients (10/10) with mild accumulation showed clearance at week 20. The same observation was made for 2 of 3 (67%) patients who had moderate accumulation at baseline. The third patient exhibited a reduction from moderate to mild. The overall change in median histology score for all 13 patients was −1.0 ($p < 0.001$).

Other kidney cell types. All 11 patients with kidney glomerular endothelial cell GL-3 accumulation (non-0 score) at baseline achieved clearance (0 score) at week 20. Of these patients, 7/11 (64%) had severe GL-3 accumulation (score = 3) at baseline, and 4/11 (36%) had moderate accumulation (score = 2).

All 12 patients with kidney noncapillary (arteriolar) interstitial endothelial cell GL-3 accumulation (non-0 score) at baseline achieved a 0 score at week 20. Of these 12 patients, 10 (83%) had severe GL-3 accumulation and 2 (17%) had moderate GL-3 accumulation in noncapillary (arteriolar) interstitial endothelial cells at baseline.

Skin GL-3 clearance

Skin capillary endothelial cells. Twelve of 13 (92%) patients achieved a reduction of skin capillary endothelial cell GL-3 from non-0 scores to a 0 score (clearance) at week 20 ($p < 0.001$). For these 12 patients, at baseline GL-3 accumulation was severe

Table 1 Patient details

Age (years)	
Mean ± SD	26.6 ± 5.5
Range	16–34
Weight (kg) (mean ± SD)	59.1 ± 8
Sex (*n*)	
Male	13
Female	0
Plasma α-GAL activity	BDL[a]
Leukocyte α-GAL activity	BDL[a]
Serum creatinine (mg/dl) (mean ± SD)	1.1 ± 0.28
Completed study *n* (%)	13 (100)

[a] BDL, below detectable level (<0.78 nmol/h per ml for plasma α-GAL and <0.78 nmol/h per mg for leukocyte α-GAL)

(score = 3) for 5 patients, moderate (score = 2) for 6, and mild (score = 1) for 1 patient. One patient with moderate GL-3 accumulation at baseline achieved a reduction to mild at week 20. The overall change in median histology score for all patients was −2.0 ($p < 0.001$).

Other skin cell types. Of patients with deep-vessel endothelial cell GL-3 accumulation in the skin (non-0 score) at baseline, 10/12 (83%) patients achieved reduction to a 0 score at week 20, and 2/12 (17%) patients achieved a decrease in GL-3 accumulation from moderate (score = 2) to mild (score = 1) at week 20.

Heart GL-3 clearance—heart capillary endothelial cells: Only one patient met the criteria for baseline cardiac biopsy as defined in the protocol. The cardiac capillary endothelial cells from this patient showed reduction of GL-3 from 'mild' (score = 1) GL-3 accumulation in cardiac capillary endothelial cells at baseline, to complete clearance at week 20.

GL-3 clearance—kidney, urine, plasma (by ELISA): The results of GL-3 clearance in the kidney, urinary sediment and plasma are shown in Table 2. In the 13 patients, the median kidney, urinary, and plasma GL-3 levels decreased by 51.9% ($p = 0.003$), 0.003), 55.4% ($p = 0.244$), and 100% ($p < 0.001$), respectively, between baseline and week 20.

Creatinine clearance: Creatinine clearance was used as a measure of the change in renal filtration function from baseline to week 20. The median creatinine clearance was 125.9 ml/min (mean ± SD = 126.6 ± 41.8) at baseline and 120.2 ml/min (mean ± SD = 115.3 ± 30.4) at week 20. This difference was not statistically significant ($p = 0.216$; Wilcoxon signed rank test). Median serum creatinine levels also

Q1 Table 2 Mean change from baseline to week 20 (11 infusions) for GL-3 levels in kidney, urine, and plasma (ELISA)

Organ		*Baseline*	*Week 20*	*% Change from baseline (week 20)*	*p-Value*[a]
Kidney (ng/mg)	Mean ± SD	2972 ± 1529	1667 ± 1760	−46.2	0.003
	Median	3149	1182	−51.9	
	Range	341–5098	171–6122		
Urine (nmol/filter)	Mean ± SD	4085 ± 2077	2687 ± 2514	65.1[b]	0.244
	Median	3680	1278	−55.4[b]	
	Range	62–7340	313–8080		
Plasma (ng/µl)	Mean ± SD	3.9 ± 2.7	0.2 ± 0.8	−89.4	<0.001
	Median	3.6	0	−100	
	Range	0–9	0–3		

[a] *p*-Value derived from a Wilcoxon signed rank test on change from baseline to week 20
[b] Values are correct. The upper end of the range (min–max) consisted of only a few patients; therefore, the median fell on a negative value

remained relatively stable at week 20 (not shown), suggesting maintenance of renal function.

Clinical assessments: Results from laboratory tests indicate that treatment with r-hαGAL appears to have no toxic effect. Ophthalmic, ECG, and echocardiogram findings further support this observation.

Quality of life assessments: Overall, median pain scores were at the low end at baseline and showed slight improvement at week 20 in all parameters. The median change score for present pain intensity (PPI) approached statistical significance ($p = 0.063$). Likewise, there was improvement in all categories for the SF-36 Health Status Survey. Statistically significant improvement was observed in median values for the General Health ($p = 0.023$) and the Mental Component Scale (MCS) scores ($p = 0.048$). The median values from baseline to week 20 for the category Role – Emotional approached statistical significance ($p = 0.063$). *p*-Values presented in this section refer to the Wilcoxon signed rank test.

Safety: All patients completed the study and each received all 11 infusions of agalsidase beta. All patients reported at least one adverse event (AE). Relation of AEs to the study drug were defined as possible, probable, definite or unknown. The most frequently reported related AEs were rigors (chills) and fever. These were infusion-associated reactions (related events that occurred on the same day as the infusion) and were mild or moderate in intensity (Table 3). These events were often managed with antihistamines and antipyretics or with a reduction in the infusion rate.

One patient experienced a serious adverse event considered to be related to the infusion. The patient was hospitalized overnight for observation owing to persistent malaise (related to fever) and limb pain after the infusion (probably related). The patient recovered without sequelae.

IgG seroconversion occurred in 11/13 (85%) patients. The mean time to seroconversion was 63.3 days. Seroconversion did not affect the patient's response to treatment. No IgE antibody formation was detected in any of the patients.

Table 3 Related adverse experiences occurring in >10% of patients

	Severity of adverse experience (% of patients)	
WHOART preferred term	*Mild*	*Moderate*
Rigors	3 (23)	2 (15)
Fever	2 (15)	2 (15)
Malaise	2 (15)	0
Dyspnoea	2 (15)	0
Rhinitis	2 (15)	0
Hypertension	1 (8)	1 (8)

AE counted once (most severe occurrence) if reported more frequently. There were no adverse experiences of severe intensity

DISCUSSION

This phase 2 open-label trial in patients of Japanese descent was conducted to evaluate whether ethnic differences exist in relation to safety and efficacy that could affect treatment of Fabry disease with agalsidase beta in this population. The study design, including the safety and efficacy end points, paralleled the design of the completed phase 3 double-blind study with agalsidase beta (Eng et al 2001b). The renal efficacy end point was the proportion of patients with clearance of GL-3 deposits from capillary endothelial cells at week 20 (11 infusions). Additional tissue efficacy end points included microvascular endothelial deposits of GL-3 in the heart, skin and other kidney cell types.

In the current study, the percentages of patients who achieved GL-3 clearance (zero score) from capillary endothelial cells after 20 weeks of agalsidase beta treatment were 92% ($p < 0.001$) for the kidney and 92% ($p < 0.001$) for skin. For those patients who received active drug during the double-blind portion of the completed phase 3 study, these percentages were 69% and 100%, respectively. After the 6-month open label extension portion of the phase 3 study, zero scores were reached in 98% and 96% of the patients, respectively. Clearance of GL-3 deposits (0 score) was observed for other cell types of the kidney as well, i.e. for all patients with glomerular endothelial cell GL-3 accumulation or noncapillary interstitial cell GL-3 accumulation. Although not assessed during the current study, variable responses to enzyme therapy may be observed for cell types such as podocytes and interstitial smooth-muscle cells. This may be due to either greater total GL-3 accumulation or/and relatively lower accessibility of enzyme therapy. Prolonged treatment may be necessary to remove a lifetime of accumulated GL-3. Clearance of GL-3 in heart tissue was not directly comparable to patients from the phase 3 study because a biopsy specimen was only obtained from one of the patients in the current study. This patient achieved clearance at 20 weeks.

Other efficacy end points, i.e. kidney, urine and plasma GL-3 measurements, creatinine clearance and quality of life measurements, also showed improvement and were comparable with findings from the phase 3 double-blind study. Median kidney, urinary and plasma GL-3 levels decreased by 51.9% ($p = 0.003$), 55.4% (not statistically significant) and 100% ($p < 0.001$), respectively, between baseline and week 20. For urinary GL-3, some outlier patients in our study may have shifted mean values for this category. Creatinine clearance and median serum creatinine levels also remained normal, suggesting maintenance of renal function. However, a longer duration of follow-up is needed to assess the change in renal function over time.

Patients had low baseline values for measurement of pain as determined by the McGill Pain Questionnaire but did show an overall improvement in all categories evaluated by the questionnaires. However, this study was not designed as a pain study and patients were not chosen on the basis of the presence of pain. In addition, there was no restriction on the use of pain medications. The statistically significant improvement in the General Health and Mental Component Scale scores of the SF-36 Health Status Survey are particularly encouraging since Fabry disease is a chronic disease and can have a significant impact on patients' quality of life. Long-term treatment is

needed to establish this particular effect more conclusively, as patients were allowed to continue on prophylactic pain medications while participating in the study.

The profile of adverse events considered in relation to treatment in the current phase 2 study is consistent when compared to the agalsidase beta treatment group in the phase 3 study and its extension. Infusion-associated events such as rigors, fever, dyspnoea and rhinitis coincided with the development of IgG antibodies, an expected response with the infusion of recombinant protein therapy. The proportion of patients who developed IgG antibodies was almost identical compared with patients in the phase 3 study, i.e. 85% and 83%, respectively. Seroconversion did not affect the patient's response to treatment. No patients developed IgE antibodies.

We conclude that the results of this phase 2 trial demonstrate that agalsidase beta is effective in the treatment of Fabry disease in Japanese patients and is well tolerated, as there were no safety issues. Because classical Fabry disease is progressive in nature, it often culminates in renal failure, cardiac failure and/or stroke resulting in death in the third to fifth decades of life. The long-term administration of agalsidase beta may significantly reduce the physical effects of Fabry disease and improve quality of life and possibly life expectancy. Results from the current study and the completed phase 3 study suggest that there are no ethnic differences between the Japanese and caucasian Fabry patient populations with regard to safety and efficacy of agalsidase beta treatment.

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