

# Enzyme Replacement Therapy in Fabry Disease

## A Randomized Controlled Trial

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**F**ABRY DISEASE IS A RARE X-LINKED recessive glycosphingolipid storage disorder that is caused by a deficiency of the lysosomal enzyme  $\alpha$ -gal A ( $\alpha$ -galactosidase A).<sup>1</sup> Its incidence has been estimated to be 1:117 000 births.<sup>2</sup> Globotriaosylceramide (Gb<sub>3</sub>), the glycosphingolipid substrate of this enzyme, accumulates within vulnerable cells, tissues, and organs of affected patients. Affected cell types include endothelial cells, pericytes, smooth muscle cells of the vascular system, renal epithelial cells, myocardial cells, and dorsal root ganglia neuronal cells.<sup>3-5</sup>

Clinical onset of the disease typically occurs during childhood or adolescence with recurrent episodes of severe, debilitating neuropathic pain in the extremities. The neuropathic pain syndrome is thought to be secondary to a small-fiber peripheral neuropathy caused by destruction of dorsal root ganglion cells by progressive deposition of Gb<sub>3</sub>.<sup>3</sup> With increasing age, Gb<sub>3</sub> progressively accumulates throughout the body. Deposition of Gb<sub>3</sub> occurs within multiple sites throughout the nephrons and renal vasculature. Progressive glomerular injury

**Context** Fabry disease is a metabolic disorder without a specific treatment, caused by a deficiency of the lysosomal enzyme  $\alpha$ -galactosidase A ( $\alpha$ -gal A). Most patients experience debilitating neuropathic pain and premature mortality because of renal failure, cardiovascular disease, or cerebrovascular disease.

**Objective** To evaluate the safety and efficacy of intravenous  $\alpha$ -gal A for Fabry disease.

**Design and Setting** Double-blind placebo-controlled trial conducted from December 1998 to August 1999 at the Clinical Research Center of the National Institutes of Health.

**Patients** Twenty-six hemizygous male patients, aged 18 years or older, with Fabry disease that was confirmed by  $\alpha$ -gal A assay.

**Intervention** A dosage of 0.2 mg/kg of  $\alpha$ -gal A, administered intravenously every other week (12 doses total).

**Main Outcome Measure** Effect of therapy on neuropathic pain while without neuropathic pain medications measured by question 3 of the Brief Pain Inventory (BPI).

**Results** Mean (SE) BPI neuropathic pain severity score declined from 6.2 (0.46) to 4.3 (0.73) in patients treated with  $\alpha$ -gal A vs no significant change in the placebo group ( $P = .02$ ). Pain-related quality of life declined from 3.2 (0.55) to 2.1 (0.56) for patients receiving  $\alpha$ -gal A vs 4.8 (0.59) to 4.2 (0.74) for placebo ( $P = .05$ ). In the kidney, glomeruli with mesangial widening decreased by a mean of 12.5% for patients receiving  $\alpha$ -gal vs a 16.5% increase for placebo ( $P = .01$ ). Mean inulin clearance decreased by 6.2 mL/min for patients receiving  $\alpha$ -gal A vs 19.5 mL/min for placebo ( $P = .19$ ). Mean creatinine clearance increased by 2.1 mL/min (0.4 mL/s) for patients receiving  $\alpha$ -gal A vs a decrease of 16.1 mL/min (0.3 mL/s) for placebo ( $P = .02$ ). In patients treated with  $\alpha$ -gal A, there was an approximately 50% reduction in plasma glycosphingolipid levels, a significant improvement in cardiac conduction, and a significant increase in body weight.

**Conclusion** Intravenous infusions of  $\alpha$ -gal A are safe and have widespread therapeutic efficacy in Fabry disease.

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is associated with mesangial widening and ultimately with segmental and global glomerulosclerosis.<sup>6</sup> Patients often also develop hypertrophic cardiomyopathy,<sup>7</sup> coronary artery disease, valvular abnormalities,<sup>8,9</sup> dysrhythmias, and conduction disturbances.<sup>10</sup>

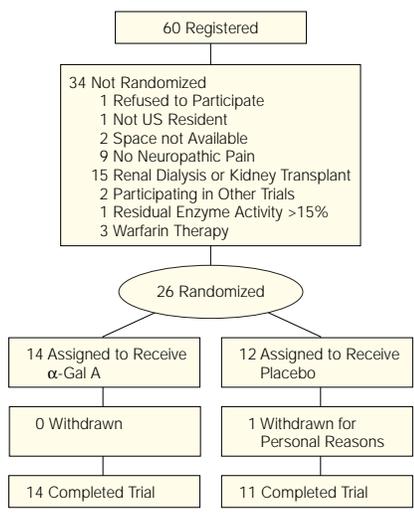
Death usually occurs during the fourth or fifth decade of life secondary to renal, cardiac, or cerebrovascular

complications.<sup>11</sup> To date, there has been no definitive therapy for Fabry disease.

Previous studies have demonstrated that partially purified prepara-

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**Figure 1.** Study Flow



tions of  $\alpha$ -gal A are metabolically active.<sup>12,13</sup> Recently, 10 patients with Fabry disease were each treated with a single intravenous infusion of 5 escalating doses of highly purified  $\alpha$ -gal A.<sup>14</sup> This study showed that  $\alpha$ -gal A significantly reduced Gb<sub>3</sub> levels in the liver and in shed renal tubular epithelial cells in urine sediment. Immunohistochemical staining of liver tissue approximately 2 days after enzyme infusion identified  $\alpha$ -gal A in every cell type, suggesting diffuse uptake via the mannose-6-phosphate receptor. The tissue half-life in the liver was greater than 24 hours, consistent with that of other lysosomal enzymes.<sup>15-17</sup>

The goal of this study was to assess the safety and clinical efficacy of repeated intravenous administrations of  $\alpha$ -gal A for the treatment of patients with Fabry disease.

**METHODS**

**Patients**

Twenty-six hemizygous men 18 years of age or older, with Fabry disease confirmed by  $\alpha$ -gal A assay, participated in this study (FIGURE 1). All patients had neuropathic pain. The institutional review board of the National Institute of Neurological Disorders and Stroke approved the study. All the patients who participated in this study gave their

written informed consent prior to their inclusion in this trial.

**$\alpha$ -Gal A Production**

$\alpha$ -Gal A was produced in a genetically engineered continuous human cell line (Transkaryotic Therapies, Inc, Cambridge, Mass).  $\alpha$ -Gal A in the cell culture supernatant was harvested, and the enzyme was purified by a series of conventional chromatographic steps in facilities compliant with Good Manufacturing Practices. Purified  $\alpha$ -gal A was formulated and placed in vials containing sodium phosphate as a buffering agent (pH 5.8-6.2 at 4°C), polysorbate 20 as a stabilizing agent, and sodium chloride as an isotonic agent. The drug was diluted in 100 mL of normal saline for administration. The specific activity of the enzyme was  $3.4 \times 10^6$  nmol/h per milligram of protein and it was more than 99.5% pure.

**Treatment Regimen**

$\alpha$ -Gal A (0.2 mg/kg) was administered by intravenous infusion initially over a period of 20 minutes. Approximately midway into the trial, the infusion time was increased to 40 minutes to diminish the likelihood of mild infusion reactions (see “Safety,” below). Doses were administered every other week for 6 months (12 doses total). The placebo infusions, aside from the absence of  $\alpha$ -gal A, were identical to the enzyme infusions in composition, appearance, and method of administration.

**Treatment Assignment and Randomization**

A randomization schedule was prepared prior to the start of the study and was provided to an unblinded pharmacist in the research pharmacy at the National Institutes of Health. No other medical or sponsor personnel had access to the randomization code until the study was completed. Patients were randomized after the first evaluation was completed and the eligibility criteria were confirmed. Randomization was blocked to minimize imbalances between study groups.

**Clinical Outcome Measures**

**Neuropathic Pain.** The Brief Pain Inventory (BPI) short form contains 9 pain-related questions, each answered by circling a number on a 0 to 10 scale.<sup>18</sup> The BPI was completed by the patients at baseline, during each visit to the National Institutes of Health for enzyme infusion, and at the end of the study. At baseline and at weeks 8, 16, and 23, patients discontinued taking any neuropathic pain medications and completed the BPI within the following week, with the precise timing based on individual patient analgesic requirements. This procedure allowed the severity of the pain without pain medications to be assessed accurately while minimizing patients’ discomfort. Following pain medication withdrawal and BPI scoring, patients were able to remain without their chronic neuropathic pain medication regimens if they felt able to do so.

The primary efficacy end point was the effect of therapy on neuropathic pain while without pain medications, as measured by the “pain at its worst” item (question 3) from the BPI (“Please rate your pain by circling the one number that best describes your pain at its worst in the last week”). Other pain end points included the mean score of the BPI severity items (questions 3 through 6: “please rate your pain by circling the one number that best describes your pain at its least in the last week; please rate your pain by circling the one number that best describes your pain on the average; please rate your pain by circling the one number that tells how much pain you have right now”), and the BPI interference items, question 9 (“Circle the one number that describes how, during the past week, pain has interfered with your: A, general activity; B, mood; C, walking ability; D, normal work [includes both work outside the home and housework]; E, relations with other people; F, sleep; G, enjoyment of life”). Patients’ use of pain medication was recorded throughout the study. Neuropathic pain medications were defined to include carbamazepine, gabapentin, phenytoin, lamotrigine, nortriptyline, and amitriptyline.

**Renal Outcome Measures.** At baseline and at week 24, inulin clearance and creatinine clearance were used to estimate glomerular filtration rate, and renal biopsies were performed.

All biopsy specimens were coded so that the analysis would be blinded to treatment assignment, patient number, and order of biopsy. Two renal pathologists assessed renal biopsies and a consensus score was reached. Glomerular numbers were counted in paraffin and plastic sections and the total number of glomeruli was recorded. The mean glomerular number was 24, with a range of 2 to 52; only 1 biopsy specimen had fewer than 8 glomeruli. The morphology of each glomerulus was classified as normal (without mesangial changes); with mesangial widening (mesangial widening observed to an equal extent throughout the capillary tuft); with segmental glomerulosclerosis (a portion of the capillary tuft exhibited marked solidification or matrix expansion out of proportion to the remainder of the tuft, often accompanied by mesangial widening); or obsolescent (a globally sclerotic glomerulus with no patent capillary loops). The numerical fraction of glomeruli in these 4 categories was determined. The tubulointerstitial pathology score was determined as a sum of the following parameter scores, each rated on a scale of 0 to 3: tubular atrophy, interstitial inflammation, interstitial fibrosis, vascular hyalinosis, and vascular medial thickening. Glycolipid inclusions were assessed by examination of toluidine blue-stained semi-thin sections and a total score was calculated as a sum of scores for the following cellular compartments, each rated on a scale of 0 to 3: glomerular epithelial cells, glomerular endothelial/mesangial cells, proximal tubular epithelial cells, distal tubular epithelial cells, vascular endothelial cells, and vascular medial cells.

**Gb<sub>3</sub> Analysis.** Levels of Gb<sub>3</sub> were determined in plasma, 24-hour urine sediment, and in renal biopsy tissue. The analysis was performed essentially as previously described.<sup>14</sup> N-acetyl-ψ-lysine was added as an internal standard to calculate Gb<sub>3</sub> recovery.

**Table 1.** Demographic Characteristics\*

Characteristic	α-Gal A (n = 14)	Placebo (n = 12)
Age, mean (SE), y	34.0 (2.26)	34.4 (2.22)
Race, No. (%)		
White	13 (93)	11 (92)
Hispanic	1 (7)	1 (8)
Weight, mean (SE), kg	74.0 (3.1)	75.8 (5.0)
Duration of illness, mean (SE), y	12.8 (2.38)	12.1 (2.72)
Extent of involvement, No. (%)†		
1-3 Organ systems	3 (21)	3 (25)
4-6 Organ systems	11 (79)	7 (58)
>6 Organ systems	0	2 (17)
Residual α-gal A enzyme activity, mean (SE), % of normal	1.3 (0.37)	1.1 (0.66)

\*α-Gal A indicates α-galactosidase A.

†Organ system involvement was defined as neurologic (either central or peripheral), renal, cardiac, vascular (predominantly cerebrovascular), dermatologic, ocular/corneal, and gastrointestinal).

**Antibody Analyses.** Serum specimens were collected at baseline and weeks 9, 17, and 24 following the initial treatment. Anti-α-gal A antibodies were assayed using a plate enzyme-linked immunosorbent assay technique based on a goat anti-human IgG secondary antibody. For the immunoprecipitation assays, serum was diluted 1:2 and preincubated with purified α-gal A. Complexes were precipitated with protein sepharose A beads and analyzed by Western blot.

**Statistical Methods**

Data were summarized by treatment group with respect to demographics, baseline characteristics, and safety and efficacy variables. One patient randomized to the placebo group did not complete the study for personal reasons. All statistical tests were 2-sided and were performed at a significance level of <.05. All pain analyses were performed on an intent-to-treat basis. Missing data (4/104 BPI measurements) were imputed by the method of last observation carried forward. A second patient (randomized to α-gal A) had protracted bleeding as the result of the baseline kidney biopsy, requiring 2 vascular occlusive procedures. Because this complication likely affected renal function, it was prospectively determined that the patient be excluded from the renal analyses. A third patient (randomized to placebo) had entered end-stage renal disease at week 24 and had

creatinine clearance measured as 7 mL/min (0.12 mL/s), but due to low urine output did not undergo inulin clearance measurement or renal biopsy at week 24. Based on the ratio of creatinine clearance to inulin clearance at baseline, the week 24 inulin clearance was imputed as 4 mL/min for this patient. Renal pathologic analysis was conducted on all samples for which adequate tissue was available from the baseline and week 24 biopsies.

For the analysis of efficacy, a 1-way analysis of covariance (ANCOVA) model was used for the treatment effect of the primary efficacy variable with the baseline value for the variable of interest as the only covariate. In addition, a repeated measures analysis on raw data scores was used. Other continuous variables were analyzed similarly to the primary efficacy variable. The log-rank test was used in Kaplan-Meier analysis for the assessment of the time to permanent discontinuation of neuropathic pain medications. The total number of days with and without pain medications in each treatment group was compared using the t test. All results are given as mean (SE).

**RESULTS**

**Baseline Demographics**

The age distribution, race, weight, duration and severity of illness, and residual α-gal A activity were comparable in the 2 groups (TABLE 1). Mean pain score at baseline was higher in the placebo group compared with the α-gal A

group. Twenty-five of the patients completed the study and 1 (randomized to placebo) withdrew for personal reasons at week 22. Random differences between the groups for the various parameters at baseline were not systematic and were taken into account by ANCOVA.

**Neuropathic Pain and Pain-Related Quality of Life**

FIGURE 2 presents the mean BPI short-form results (question 3, “pain at its worst”) for the measurements without pain medication for the 2 treatment groups of the intent-to-treat population. There was a consistent and progressive decline in the pain scores in the  $\alpha$ -gal A treatment group and essentially no change in the placebo group. There was a significant difference for the

change from baseline in pain scores between the 2 treatment groups favoring the  $\alpha$ -gal A treatment group ( $P = .02$ ). A subgroup analysis showed no significant difference in pain response between patients with and without infusion reactions (data not shown). There was also a significant decline in overall pain severity in the  $\alpha$ -gal A treatment group (TABLE 2,  $P = .02$ ). Treatment with  $\alpha$ -gal A also improved pain-related quality of life (Table 2,  $P = .05$ ).

In the  $\alpha$ -gal A treatment group, 11 of 14 were taking neuropathic pain medication(s) at the time of the first infusion of study drug, as were 11 of 12 in the placebo group. Four patients in the  $\alpha$ -gal A treatment group of the 11 who were taking neuropathic pain medication(s) at the start of the study were able to discontinue these pain medications for the duration of the trial. Discontinuation of pain medication occurred between weeks 1 and 8 of the study with a mean time to discontinuation for these responders of 30.5 days. In contrast, no patient in the placebo group of 11 taking neuropathic pain medication(s) was able to discontinue these pain medications ( $P = .03$ ).

For those patients who were taking neuropathic pain medications, the mean (SE) number of days that patients in the  $\alpha$ -gal A treatment group were able to remain without pain medications during the study was 74.5 (22.5) days, compared with 12.9 (6.11) days for the placebo group ( $P = .02$ ). The days that

patients in the placebo group were able to remain without pain medications were largely accounted for by the 3 periods of pain medication withdrawal required by the protocol.

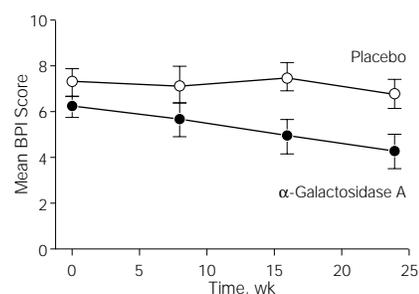
**Renal Pathology**

Therapy with  $\alpha$ -gal A was associated with improvement in glomerular histology (TABLE 3). There was a 21% increase in the fraction of normal glomeruli (glomeruli without mesangial widening or sclerosis) in patients treated with  $\alpha$ -gal A and a 27% decrease in the fraction of normal glomeruli in patients randomized to placebo ( $P = .01$ ). Furthermore, in the  $\alpha$ -gal A treatment group, the fraction of glomeruli with mesangial widening exhibited a significant decrease compared with an increase in the placebo population ( $P = .01$ ). Although there was a significant increase in the fraction of glomeruli with segmental sclerosis in the  $\alpha$ -gal A treatment group, the relative increases were small compared with the changes in normal glomeruli and glomeruli with mesangial widening. There was no significant difference in the fraction of obsolescent glomeruli between the 2 groups. No significant change in total score for tubulointerstitial pathology or for the total Fabry inclusion score was seen in this 6-month trial. When the individual inclusion scores were examined, there was a decrease in glycolipid inclusions within the vascular endothelium in the enzyme group and an increase in the placebo group ( $P = .002$ ).

**Renal Function**

Glomerular filtration rate was assessed in 2 ways. First, analysis of inulin clearance showed a trend in favor of enzyme treatment, with the placebo group experiencing a 3-fold greater decline than the  $\alpha$ -gal A treatment group ( $P = .19$ , TABLE 4). The range of changes was broader in the placebo group ( $-70$  to  $8$  mL/min/1.73 m<sup>2</sup>) than in the  $\alpha$ -gal A treatment group (range  $-28$  to  $15$  mL/min/1.73 m<sup>2</sup>). In general, patients in the placebo group with a normal inulin clearance at baseline ex-

**Figure 2.** BPI “Pain at Its Worst” Scores for Patients While Not Receiving Neuropathic Pain Medications



BPI indicates Brief Pain Inventory. Points represent the mean of each treatment group flanked by SE bars at each time point ( $P = .02$ , analysis of covariance).

**Table 2.** BPI Severity and Interference Scores\*

Assessment	$\alpha$ -Gal A (n = 14)	Placebo (n = 12)	P Value
BPI severity, mean (SE)			
Baseline	3.8 (0.44)	5.4 (0.45)	.02
Week 8/9	3.1 (0.54)	5.2 (0.67)	
16/17	3.3 (0.67)	5.2 (0.59)	
23/24	2.7 (0.54)	4.7 (0.65)	
BPI pain-related quality of life, mean (SE)†			
Baseline	3.2 (0.55)	4.8 (0.59)	.05
Week 8/9	3.2 (0.61)	4.1 (0.71)	
16/17	2.8 (0.67)	4.6 (0.75)	
23/24	2.1 (0.56)	4.2 (0.74)	

\* $\alpha$ -Gal A indicates  $\alpha$ -galactosidase A; BPI, Brief Pain Inventory.  
†P values by repeated measures analysis of variance.

perienced a greater decrease in renal function than patients with depressed renal function at baseline.

Second, analysis of creatinine clearance showed an improvement in renal function with  $\alpha$ -gal A therapy compared with a decline with placebo treatment ( $P=.02$ , Table 4). Although there is no standard definition of undercollection or overcollection of a 24-hour urine sample, to confirm the robustness of the data we performed a subset analysis in which we prospectively determined that 24-hour urine collections must have less than 35% deviation from the mean creatinine appearance for each patient. This resulted in the elimination of 3 of 97 urine collections. Even with these exclusions, the  $\alpha$ -gal A group gained 1.9 mL/min/1.73m<sup>2</sup> and the placebo group lost 10.5 (19.9) mL/min/1.73m<sup>2</sup> ( $P=.06$ ).

Five patients in the enzyme group and 3 receiving placebo had a urinary protein excretion greater than 1 g/24 h, while the other patients had protein excretion below that level. The degree of proteinuria was evenly distributed between the 2 treatment groups. There was no consistent change in proteinuria seen in either group, but there was a large individual variability in the amount of protein excreted over 24 hours. One patient in the placebo group progressed to end-stage renal disease during the course of the study and began peritoneal dialysis.

**Cardiac Conduction System Effects**

There was a significant decrease in QRS-complex duration as measured by electrocardiography, with a decrease of 2.4 (3.90) milliseconds (94.1 [4.85] to 91.7 [2.14] milliseconds) in the treatment group vs an increase of 3.6 (1.17) milliseconds (94.0 [3.39] to 97.6 [3.37] milliseconds) in the placebo group ( $P=.047$ ). Furthermore, 1 patient in the  $\alpha$ -gal A treatment group began the study with a right bundle-branch block pattern that completely resolved during  $\alpha$ -gal A therapy.

**Metabolic Effects**

Patients treated with  $\alpha$ -gal A had a greater than 50% decrease in their plasma Gb<sub>3</sub>

levels, whereas patients receiving placebo had a small mean decrease in their plasma Gb<sub>3</sub> levels (TABLE 5,  $P=.005$ ). Similarly, treatment with  $\alpha$ -gal A resulted in a decrease of renal tubular glycosphingolipid levels as detected in 24-

hour urine sediments (Table 5).  $\alpha$ -Gal A recipients showed a mean decrease in urine sediment Gb<sub>3</sub> levels of 30%, while patients in the placebo group had a mean increase of 15% ( $P=.05$ ). The patients treated with  $\alpha$ -gal A had a 21% de-

**Table 3. Renal Pathology\***

Assessment	Mean (SE) %		P Value
	$\alpha$ -Gal A (n = 12)	Placebo (n = 9)	
Normal glomeruli			
Baseline	39.9 (6.6)	59.6 (6.8)	.01
Week 24	48.0 (8.9)	43.6 (10.1)	
Change	8.1 (4.4)	-16 (7.6)	
Glomeruli with mesangial widening			
Baseline	38.2 (4.3)	23.9 (3.8)	.01
Week 24	25.7 (6.0)	40.4 (9.5)	
Change	-12.5 (5.0)	16.5 (7.7)	
Glomeruli with segmental sclerosis			
Baseline	2.8 (1.4)	6.0 (1.8)	.048
Week 24	6.8 (2.5)	3.0 (1.9)	
Change	4.0 (2.1)	-3.0 (1.6)	
Obsolescent glomeruli			
Baseline	19.1 (7.1)	10.5 (5.1)	.87
Week 24	19.5 (6.0)	13.0 (5.1)	
Change	0.4 (5.0)	2.5 (3.4)	

\* $\alpha$ -Gal A indicates  $\alpha$ -galactosidase A.

**Table 4. Renal Function Tests**

Assessment	$\alpha$ -Gal A (n = 13)	Placebo (n = 11)
Creatinine clearance, mean (SE), mL/min/1.73 m <sup>2</sup> *		
Baseline	92.7 (6.2)	100.6 (12.2)
Week 24	94.8 (7.7)	84.5 (10.6)
Change	2.1 (3.4)	-16.1 (6.2)
Inulin clearance, mean (SE), mL/min/1.73 m <sup>2</sup> †		
Baseline	77.2 (5.57)	90.9 (12.07)
Week 24	71.0 (4.47)	71.5 (9.66)
Change	-6.2 (3.10)	-19.5 (7.12)

\* $P = .02$  using analysis of covariance (ANCOVA) with the baseline value as covariate. Normal range for creatinine clearance is 90-130 mL/min/1.73 m<sup>2</sup>. To convert mL/min to mL/s, multiply creatinine clearance values by 0.01667.

† $P = .19$  using ANCOVA with the baseline value as covariate. Normal value is >90 mL/min/1.73 m<sup>2</sup>.

**Table 5. Gb<sub>3</sub> Analyses\***

Assessment	$\alpha$ -Gal A	Placebo	P Value
Plasma Gb <sub>3</sub> , mean (SE), nmol/mL	(n = 14)	(n = 11)	
Baseline	12.14 (0.907)	10.96 (1.087)	.005
Week 24	5.58 (0.536)	10.192 (1.271)	
Change	-6.56 (0.751)	-0.77 (0.479)	
Urine sediment Gb <sub>3</sub> , mean (SE), nmol/g of creatinine	(n = 14)	(n = 11)	
Baseline	2369 (308)	2162 (383)	.05
Week 24	1683 (443)	2495 (333)	
Change	-686 (298)	333 (400)	
Kidney Gb <sub>3</sub> , mean (SE), nmol/mg of tissue	(n = 11)	(n = 9)	
Baseline	19.5 (1.68)	19.0 (3.59)	.27
Week 24	15.6 (1.60)	18.1 (3.18)	
Change	-4.0 (2.2)	-0.9 (1.8)	

\*Gb<sub>3</sub> indicates globotriaosylceramide;  $\alpha$ -gal A,  $\alpha$ -galactosidase A.

crease from baseline in their kidney Gb<sub>3</sub> levels, while patients in the placebo group had a 4% decrease (Table 5,  $P = .27$ ).

### Body Weight

Patients treated with  $\alpha$ -gal A gained an average (SD) of 1.5 (0.6) kg (73.4 [3.3] to 75.0 [3.5] kg), compared with an average loss of 1.4 (1.3) kg in the placebo group (73.8 [4.8] to 72.4 [0.8 kg]) ( $P = .02$ ).

### Safety

$\alpha$ -Gal A was well tolerated. The vast majority of adverse events (eg, constipation, abdominal pain crisis, and hearing loss) were symptoms that are typically observed in patients with Fabry disease and were not thought to be related to the study drug. The 1 patient in the placebo group who developed renal failure requiring peritoneal dialysis continued in the study and was receiving peritoneal dialysis at the time of his final visit.

Eight of 14 patients receiving  $\alpha$ -gal A experienced mild infusion reactions, generally consisting of rigors within 45 minutes following the infusion. These reactions were readily controlled with regimens of antihistamines and low-dose corticosteroids, which were subsequently tapered. All patients who experienced these reactions were able to continue with  $\alpha$ -gal A infusions at a reduced infusion rate, and subsequent reactions were generally milder than the initial reaction.

No patient developed an IgE, IgA, or IgM antibody to  $\alpha$ -gal A. Three of the 14 patients who received  $\alpha$ -gal A developed a low-titer (approximately 1:10) IgG antibody. Nine patients were positive by the immunoprecipitation assay with titers of approximately 1:2. Patients who developed an immune response to  $\alpha$ -gal A subsequently became desensitized with reductions in antibody levels over time. Subset analyses demonstrated that the low-titer antibodies appeared to have no clinically significant effect on the safety or efficacy of the  $\alpha$ -gal A. In addition, the presence of antibodies did not correlate with the incidence of infusion reactions.

### COMMENT

This study has demonstrated widespread effects of  $\alpha$ -gal A enzyme replacement therapy on a number of clinically significant aspects of Fabry disease. Compared with placebo,  $\alpha$ -gal A reduced the level of severe incapacitating neuropathic pain; improved pain-related quality of life, renal pathology, and cardiac function; may have improved renal function; and partially corrected the underlying metabolic defect as reflected by significant Gb<sub>3</sub> reductions and weight gain.

The level of neuropathic pain decreased approximately 2 units on the BPI, where a 1-unit decrease is considered clinically significant (Charles Cleland, MD, M. D. Anderson Cancer Center, Houston, Tex, oral communication, November 1998). In addition, the decrease in pain passed through level 5 on the BPI.<sup>18-25</sup> This level is most clinically sensitive to the effects of changes in patients' levels of pain.<sup>23</sup> Consistent with the marked decrease in pain, most patients receiving  $\alpha$ -gal A were either able to discontinue their chronic neuropathic pain medication regimens for the duration of the trial or to markedly decrease their use of pain medications. The placebo effect may have been mitigated in the placebo group by the exacerbations of neuropathic pain that they incurred during periodic withdrawals of pain medications. The lack of a difference between patients with and without infusion reactions argues against the possibility that study blinding was compromised by adverse effects from the active drug.

These changes were corroborated by changes in all of the other questions on the BPI. The severity items revealed a significant decrease in the level of pain in the  $\alpha$ -gal A group and the analysis of the interference items that measure pain-related quality of life also revealed a significant decrease in the level of pain in the  $\alpha$ -gal A group. Taken together, these data suggest that there is a time-dependent and sustained effect of  $\alpha$ -gal A therapy on pain in Fabry disease. The improvement in neuropathic pain seen in this study may reflect the initial mo-

bilization of Gb<sub>3</sub> from damaged dorsal root ganglion cells by  $\alpha$ -gal A.<sup>26</sup>

The measurements of glomerular filtration rate showed differences between placebo and  $\alpha$ -gal A groups that favored the treatment group, although there was insufficient statistical power to prove a treatment effect in this relatively short-duration study. All the patients who completed this study were subsequently enrolled into an open-label maintenance study in which they received  $\alpha$ -gal A for 1 year. We found that the decline in renal function associated with the placebo group was halted, and at 1 year a statistically significant improvement in renal function in the placebo patients was observed. An additional year of therapy in the patients originally treated with  $\alpha$ -gal A demonstrated that renal function remained stable after 18 months of  $\alpha$ -gal A therapy (R. S. et al, unpublished data, 2001). These results strongly suggest that the findings of the current study are representative of the effect of  $\alpha$ -gal A on renal function.

The pathologic hallmark of Fabry renal disease is accumulation of glycosphingolipid within the glomerular epithelial, mesangial, distal tubular epithelial, vascular endothelial, and vascular smooth muscle cells. Progression of disease is associated with mesangial expansion and ultimately glomerulosclerosis.<sup>27-29</sup> Therapy with  $\alpha$ -gal A was associated with improved glomerular histology and with reduced mesangial widening. Lipid deposition in the kidney affects predominantly glomerular epithelial cells but also endothelial, tubular, mesangial, and interstitial cells. It has been suggested that in Fabry renal disease, degenerative glomerular changes are not related to glomerular lipid deposition and instead may be due to ischemic damage.<sup>27</sup> Importantly, the overall glomerular architecture was improved by therapy with  $\alpha$ -gal A, and, in addition, treatment with  $\alpha$ -gal A also reduced the extent of glycolipid storage deposits within renal vascular endothelial cells.

Glomerular diseases associated with metabolic disorders have rarely been

found to reverse with therapy. In the case of diabetic nephropathy in patients who undergo pancreatic transplantation, reversal of basement membrane thickening and mesangial expansion occurs; this effect is seen only after 5 to 10 years following normalization of blood glucose levels.<sup>30</sup> Thus,  $\alpha$ -gal A administration in Fabry disease may represent the first metabolic disease affecting the glomerulus that improves with medical therapy, even with a relatively short duration of treatment.

The beneficial clinical effects of  $\alpha$ -gal A were associated with a significant reduction in the total glycosphingolipid burden in the treated patients. The reduction of neuropathic pain and improvement in renal pathology occurred despite incomplete clearance of accumulated Gb<sub>3</sub>. It is possible that with further clearance of stored material there will be further clinical improvements including pain reduction and further preservation of renal function. The many organ systems and cell types affected by these improvements suggest that the enzyme is taken up diffusely throughout the body by mannose-6-phosphate receptors.<sup>14</sup>

Repeated administration of this  $\alpha$ -gal A preparation was demonstrated to be safe and well tolerated. Drug reactions were mild, easily treated, and could be prevented with anti-inflammatory premedication. Lengthening of the infusion time in subsequent studies from 20 minutes to 40 minutes has markedly reduced the incidence of these reactions. Currently, with the lengthening of the infusion time, less than 10% of patients who have received  $\alpha$ -gal A for the first time have experienced infusion reactions. The development of antibodies is not surprising given the fact that the majority of patients with Fabry disease do not synthesize a full-length enzyme.<sup>26</sup> Even these low-titer antibodies decreased over time, indicating the induction of tolerance. Patients with and without the low-titer antibodies responded to  $\alpha$ -gal A similarly, and accordingly it appears that the antibodies were of no clinical significance.

The clinical efficacy data from this study suggest that this fully human  $\alpha$ -gal A preparation is delivered to multiple different tissues throughout the body including those of nerves, kidneys, heart, blood vessels, and liver. Based on the improvement of multiple functional, metabolic, and pathologic parameters, repeated administration of  $\alpha$ -gal A is expected to improve the overall prognosis of patients with Fabry disease.

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#### REFERENCES

- Brady RO, Gal AE, Bradley RM, Martensson E, Warshaw AL, Laster L. Enzymatic defect in Fabry's disease. *N Engl J Med.* 1967;276:1163-1167.
- Meikle PJ, Hopwood JJ, Clague AE, Carey WF. Prevalence of lysosomal storage disorders. *JAMA.* 1999;281:249-254.
- Kahn P. Anderson-Fabry disease: a histopathological study of three cases with observations on the mechanism of production of pain. *J Neurol Neurosurg Psychiatry.* 1973;36:1053-1062.
- Kaye EM, Kolodny EH, Logigian EL, Ullman MD. Nervous system involvement in Fabry's disease. *Ann Neurol.* 1988;23:505-509.
- DeVeber GA, Schwarting GA, Kolodny EH, Kowall NW. Fabry disease: immunocytochemical characterization of neuronal involvement. *Ann Neurol.* 1992;31:409-415.
- Seth KJ, Roth DA, Adams MB. Early renal failure in Fabry's disease. *Am J Kidney Dis.* 1983;2:651-654.
- Becker AE, Schooli R, Balk AG, van der Heide RM. Cardiac manifestations of Fabry's disease. *Am J Cardiol.* 1975;36:829-835.
- Sakuraba H, Yanagawa Y, Igarashi T, et al. Car-

diovascular manifestations in Fabry's disease. *Clin Genet.* 1986;29:276-283.

9. Desnick RJ, Blieden LC, Sharp HL, Hofschire PJ, Moller JH. Cardiac valvular abnormalities in Fabry disease. *Circulation.* 1976;54:818-825.

10. Mehta J, Tuna N, Moller JH, Desnick RJ. Electrocardiographic and vectorcardiographic abnormalities in Fabry's disease. *Am Heart J.* 1977;93:699-705.

11. Crutchfield KE, Patronas NJ, Dambrosia JM, et al. Quantitative analysis of cerebral vasculopathy in Fabry disease patients. *Neurology.* 1998;50:1746-1749.

12. Brady RO, Tallman JF, Johnson WG, et al. Replacement therapy for inherited enzyme deficiency: use of purified ceramidetrihexosidase in Fabry's disease. *N Engl J Med.* 1973;289:9-14.

13. Desnick RJ, Dean KJ, Grabowski GA, Bishop DF, Sweeley CC. Enzyme therapy XII: enzyme therapy in Fabry's disease. *Proc Natl Acad Sci U S A.* 1979;76:5326-5330.

14. Schiffmann R, Murray GJ, Treco D, et al. Infusion of  $\alpha$ -galactosidase A reduces tissue globotriaosylceramide storage in patients with Fabry disease. *Proc Natl Acad Sci U S A.* 2000;97:365-370.

15. Crawley AC, Brooks DA, Muller VJ, et al. Enzyme replacement therapy in a feline model of Maroteaux-Lamy syndrome. *J Clin Invest.* 1996;97:1864-1873.

16. Kakkis ED, Matynia A, Jonas AJ, Neufeld EF. Overexpression of the human lysosomal enzyme  $\alpha$ -L-iduronidase in Chinese hamster ovary cells. *Protein Expr Purif.* 1994;5:225-232.

17. Mistry PK, Wraight EP, Cox TM. Therapeutic delivery of proteins to macrophages: implications for treatment of Gaucher's disease. *Lancet.* 1996;348:1555-1559.

18. Cleeland CS, Gonin R, Hatfield AK, et al. Pain and its treatment in outpatients with metastatic cancer. *N Engl J Med.* 1994;330:592-596.

19. Larue F, Colleau SM, Brasseur L, Cleeland CS. Multicentre study of cancer pain and its treatment in France. *BMJ.* 1995;310:1034-1037.

20. Breitbart W, McDonald MV, Rosenfeld B, et al. Pain in ambulatory AIDS patients. I: pain characteristics and medical correlates. *Pain.* 1996;68:315-321.

21. Lydick E, Epstein RS, Himmelberger D, White CJ. Area under the curve: a metric for patient subjective responses in episodic diseases. *Qual Life Res.* 1995;4:41-45.

22. Shacham S, Dar R, Cleeland CS. The relationship of mood state to the severity of clinical pain. *Pain.* 1984;18:187-197.

23. Serlin RC, Mendoza TR, Nakamura Y, Edwards KR, Cleeland CS. When is cancer pain mild, moderate, or severe? grading pain severity by its interference with function. *Pain.* 1995;61:277-284.

24. Daut RL, Cleeland CS, Flanery RC. Development of the Wisconsin Brief Pain Questionnaire to assess pain in cancer and other diseases. *Pain.* 1983;17:197-210.

25. Cleeland CS, Ryan KM. Pain assessment: global use of the Brief Pain Inventory. *Ann Acad Med Singapore.* 1994;23:129-138.

26. Desnick RJ, Ioannou YA, Eng CM.  $\alpha$ -Galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease.* 8th ed. New York, NY: McGraw Hill; 2001:3733-3774.

27. Gubler M-C, Lenoir G, Grünfeld J-P, Ullmann A, Droz D, Habib R. Early renal changes in hemizygous patients with Fabry's disease. *Kidney Int.* 1978;13:223-235.

28. Pabico RC, Atanacio BC, McKenna BA, Pamukoglu T, Yodaiken R. Renal pathologic lesions and functional alterations in a man with Fabry's disease. *Am J Med.* 1973;55:415-425.

29. Sirvent AE, Enriquez R, Antolin A, et al. Fabry's disease presenting with oligoanuric end-stage renal failure. *Nephrol Dial Transplant.* 1997;12:1503-1505.

30. Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med.* 1998;339:69-75.