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SAFETY AND EFFICACY OF RECOMBINANT HUMAN α-GALACTOSIDASE A REPLACEMENT THERAPY IN FABRY'S DISEASE

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ABSTRACT

Background Fabry's disease, lysosomal α-galactosidase A deficiency, results from the progressive accumulation of globotriaosylceramide and related glycosphingolipids. Affected patients have microvascular disease of the kidneys, heart, and brain.

Methods We evaluated the safety and effectiveness of recombinant α-galactosidase A in a multicenter, randomized, placebo-controlled, double-blind study of 58 patients who were treated every 2 weeks for 20 weeks. Thereafter, all patients received recombinant α-galactosidase A in an open-label extension study. The primary efficacy end point was the percentage of patients in whom renal microvascular endothelial deposits of globotriaosylceramide were cleared (reduced to normal or near-normal levels). We also evaluated the histologic clearance of microvascular endothelial deposits of globotriaosylceramide in the endomyocardium and skin, as well as changes in the level of pain and the quality of life.

Results In the double-blind study, 20 of the 29 patients in the recombinant α-galactosidase A group (69 percent) had no microvascular endothelial deposits of globotriaosylceramide after 20 weeks, as compared with none of the 29 patients in the placebo group ($P<0.001$). Patients in the recombinant α-galactosidase A group also had decreased microvascular endothelial deposits of globotriaosylceramide in the skin ($P<0.001$) and heart ($P<0.001$). Plasma levels of globotriaosylceramide were directly correlated with clearance of the microvascular deposits. After six months of open-label therapy, all patients in the former placebo group and 98 percent of patients in the former recombinant α-galactosidase A group who had biopsies had clearance of microvascular endothelial deposits of globotriaosylceramide. Mild-to-moderate infusion reactions (i.e., rigors and fever) were more common in the recombinant α-galactosidase A group than in the placebo group.

Conclusions Recombinant α-galactosidase A replacement therapy cleared microvascular endothelial deposits of globotriaosylceramide from the kidneys, heart, and skin in patients with Fabry's disease, reversing the pathogenesis of the chief clinical manifestations of this disease. (N Engl J Med 2001;345:9-16.)

FABRY'S disease is an X-linked inborn error of glycosphingolipid catabolism due to deficient lysosomal α-galactosidase A activity.[1] In patients with the classic form of the disease, progressive accumulation of globotriaosylceramide and related glycosphingolipids in vascular endothelial lysosomes of the kidneys, heart, skin, and brain leads to the main disease manifestations. The clinical onset is in childhood and is characterized by severe acroparesthesias, angiokeratoma, corneal and lenticular opacities, and hypohidrosis. Over time, microvascular disease of the kidneys, heart, and brain progresses, leading to early death.[1] Treatment is limited to symptomatic management of pain and the end-stage complications of renal failure, cardiac disease, and strokes.

Early trials demonstrated the feasibility of enzyme replacement to correct the metabolic defect in Fabry's disease.[2-4] A phase 1 trial demonstrated reductions of globotriaosylceramide in the liver and in urinary sediment with a single dose of recombinant α-galactosidase A.[5] A phase 1 and 2 open-label dose-escalation study of replacement therapy with recombinant α-galactosidase A in 15 male patients with classic Fabry's disease demonstrated that repeated administration (a total of five infusions) was safe and effective in clearing plasma globotriaosylceramide and microvascular endothelial deposits of globotriaosylceramide from target tissues.[6] Plasma and tissue clearance of globotriaosylceramide was observed for all dose regimens, and the effect was most pronounced at higher doses. Therefore, we evaluated the safety and efficacy of recombinant α-galactosidase A replacement therapy in Fabry's disease in a multicenter, randomized, double-blind, placebo-controlled trial and subsequent open-label study.

METHODS

Patients

Eligible patients had an enzymatically confirmed diagnosis of classic Fabry's disease, had a level of activity of α-galactosidase A of less

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than 1.5 nmol per hour per milliliter in plasma or less than 4 nmol per hour per milligram in leukocytes,[7] and were at least 16 years old. Patients were excluded if their serum creatinine concentration exceeded 2.2 mg per deciliter (194.5 μmol per liter), if they were undergoing dialysis, or if they had undergone kidney transplantation.

Clinical and Biochemical Assessments

Evaluations including a medical history taking, routine chemical analyses, and hematologic indexes were obtained and a physical examination was performed at base line and before each infusion. Echocardiograms were obtained and plasma and 24-hour urinary sediments were collected at base line, after week 20 of the double-blind study, and after six months of open-label treatment. Glomerular filtration rates were measured in terms of inulin clearance at base line and after six months of the extension study. Concentrations of globotriaosylceramide in plasma, tissue, and urinary sediment[8] were determined by a quantitative enzyme-linked immunosorbent assay (ELISA).[9] Before each infusion, the presence or absence of antibody against recombinant α-galactosidase A was assessed by ELISA, and the results were confirmed by a radioimmunoprecipitation assay.[10]

Study Protocol

Enrollment in the double-blind study began on March 22, 1999, and ended on December 3, 1999. The open-label study began on October 26, 1999. Patients were randomly assigned to receive recombinant α-galactosidase A (agalsidase beta; Fabrazyme, Genzyme, Cambridge, Mass.) at a dose of 1 mg per kilogram of body weight or placebo (phosphate-buffered mannitol). Both agents were administered intravenously at a rate of 0.25 mg per minute every other week for 20 weeks (for a total of 11 infusions). Before each infusion patients were pretreated with 1000 mg of acetaminophen and 25 to 50 mg of hydroxyzine. Ibuprofen, prednisone, or both were also used in a few patients for infusion-related reactions. After the double-blind trial, all patients received recombinant α-galactosidase A in an open-label fashion at a dose of 1 mg per kilogram every other week, but the infusion rates were increased as tolerated, reducing the length of the infusion. The institutional review boards at all sites approved the double-blind and open-label protocols, and all patients gave written informed consent.

Tissue Assessments

Kidney specimens were obtained by ultrasound-guided biopsy, heart specimens were obtained through an endomyocardial catheter with the use of a bioptome, and 3-mm skin specimens were obtained by punch biopsy at base line, after infusion 11 (week 20), and after six months of the open-label study. Tissue sections (1 μm) were stained with methylene blue–azure II. Each of the three types of biopsy specimen was assessed for microvascular endothelial deposits of globotriaosylceramide by a different group of three pathologists. None of the nine pathologists were aware of the patients' treatment assignments or the times at which the specimens were obtained.

Specimens with no microvascular endothelial deposits of globotriaosylceramide 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 were given a score of 1; specimens that contained multiple vessels with multiple sites of single or multiple inclusions were given a score of 2; and specimens that had large accumulations of inclusions with some clusters at the juxtanuclear region and around cytoplasmic borders and bulging of the vessel lumens were given a score of 3. Renal-biopsy specimens that were initially given a score of 0 or 1 were reevaluated by the three renal pathologists with the use of a slightly modified scoring system. In this system, specimens with no inclusions were given a score of 0; those with one small granule (approximately 0.2 μm) were designated as having trace evidence; those with multiple discrete granules were given a score of 1; those with single or multiple aggregates of granules were given a score of 2; and those with aggregates of granules within the endothelium that caused the distortion of the luminal endothelial cell surface were given a score of 3.

Evaluation of Efficacy

An average of 233 capillaries in each renal-biopsy specimen were assessed by each renal pathologist. The primary efficacy end point of the double-blind study required more than 50 percent of the renal interstitial capillaries in each specimen to have a score of 0, less than 5 percent to have a score of 1 or greater, and the remainder to be designated as having trace evidence of microvascular endothelial deposits of globotriaosylceramide after week 20. For each biopsy specimen, a majority score was determined from the three pathologists' scores.

Secondary end points were also assessed at base line and after the week-20 infusion and consisted of the composite score for microvascular endothelial deposits of globotriaosylceramide in the heart, kidney, and skin specimens (scores were calculated per organ and summed for all organs) and the change from base line in the concentrations of globotriaosylceramide in urinary sediment and kidney specimens and the level of pain, as assessed by the short form of the McGill Pain Questionnaire.[11] Scores on this questionnaire can range from 0 to 45, with higher scores indicating severe pain intensity.

Statistical Analysis

We used chi-square tests to analyze the proportion of patients in the recombinant α-galactosidase A group and the placebo group with a renal-biopsy score of 0 after week 20 of the double-blind study and after six months of the open-label study. We used two-sample, two-tailed tests for all analyses. A P value of 0.05 or less was considered to indicate statistical significance. Changes in the concentrations of globotriaosylceramide in urinary sediment and kidney specimens on ELISA were ranked individually, and the rank-sum score for each patient was obtained. We used a two-sample Wilcoxon rank-sum test to assess the change from base line to the end of the double-blind study (after week 20). We used t-tests to compare the mean change in the level of pain from base line to the end of the double-blind study (after week 20) for each treatment group. The Genzyme Biostatistics group held the data and analyzed the data, with the help of consulting academic biostatisticians.

We used the 36-item Medical Outcomes Study Short-Form General Health Survey (SF-36)[12] to evaluate the patients' quality of life. This multi-item scale measures eight health-related aspects: physical function, social function, physical role, emotional role, mental health, energy, pain, and general health perception. Scores on each aspect can range from 0 (worst) to 100 (best). The results were evaluated according to established guidelines,[12] and we used a Wilcoxon signed-rank test to compare the mean change in scores from base line in each group. We used an analysis of variance to compare the differences between groups in the changes in the mean glomerular filtration rate from base line to six months of the open-label study.

RESULTS

Characteristics of the Patients

The base-line characteristics of the 58 patients assigned to the two treatment groups were similar (Table 1).

Double-Blind Study

Renal Capillary Endothelial Clearance of Globotriaosylceramide

The primary efficacy end point was the percentage of patients in each group who were free of microvascular endothelial deposits of globotriaosylceramide in renal-biopsy specimens (i.e., who had a score of 0) after 20 weeks of treatment (11 infusions) in the double-blind study. The end point was reached by 20 of the 29 patients in the recombinant α-galactosidase A

TABLE 1. BASE-LINE CHARACTERISTICS OF THE PATIENTS.*

CHARACTERISTIC	RECOMBINANT α-GALACTOSIDASE A GROUP (N=29)	PLACEBO GROUP (N=29)
Age (yr)		
Mean	32.0±9.4	28.4±11.4
Range	16–48	17–61
Weight (kg)	67.3±9.9	69.6±13.4
Height (cm)	175.7±8.3	175.6±8.3
Sex (no.)		
Male	27	29
Female	2	0
Race (no.)		
White	27	26
Nonwhite	2	3
Plasma globotriaosylceramide (ng/ml)	14.5±10.5	14.6±9.6
Glomerular filtration rate (ml/min)	83.0±22.0	96.6±35.3
Serum creatinine (mg/dl)†	0.8±0.2	0.8±0.2

*Plus–minus values are means ±SD.

†To convert the values for creatinine to micromoles per liter, multiply by 88.4.

group (69 percent), as compared with none of the 29 patients in the placebo group ($P<0.001$; odds ratio, 0.0). Eight of the remaining nine patients in the recombinant α-galactosidase A group had a score of 1 (the scores of six of these patients had improved, and the scores of two had not changed). The ninth patient had a missing biopsy specimen and so was assigned a score of 3. An analysis of sensitivity, in which a maximum of 1 percent of the capillaries could be given a score of 1 or greater, as opposed to the original requirement of less than or equal to 5 percent, did not change the outcome ($P<0.005$). These results for the three renal pathologists were uniform.

Secondary End Points

The individual scores for the kidney-, heart-, and skin-biopsy specimens as well as the composite scores for all three types of specimens were compared at base line and after the week-20 infusion (Table 2). Although both groups had similar base-line scores for each type of specimen ($P=0.53$), the patients in the recombinant α-galactosidase A group had significantly lower scores for each type of specimen after the week-20 infusion than did the patients in the placebo group ($P<0.001$ for all three comparisons). In addition, the median percent changes in the kidney and urinary concentrations of globotriaosylceramide differed between the patients in the recombinant α-galactosidase A group and the patients in the placebo group (23.3 percent decrease vs. 42.8 percent increase and 34.1 percent decrease vs. 6.2 percent decrease, respectively). The rank-sum scores for kidney and urinary-sediment concentrations of globotriaosylceramide had decreased significantly in the recombinant α-galactosidase A group, but not in the placebo group (median change, 32.5 percent decrease vs. 48.0 percent decrease; $P=0.003$).

Although both groups had low scores on all five scales of the short form of the McGill Pain Questionnaire at base line, statistically significant decreases in the scores were observed at week 20 in both treatment groups (Fig. 1). There was no significant difference between groups after week 20 in any pain assessment ($P>0.05$ for all comparisons), possibly because of a placebo effect.

Clearance of Globotriaosylceramide in Plasma

Figure 2 shows clearance of globotriaosylceramide from plasma by week 14 of treatment with recombinant α-galactosidase A; in contrast, the plasma concentrations in the placebo group did not change significantly during the double-blind study ($P<0.001$ for the comparison between the groups). Plasma concentrations of globotriaosylceramide were undetectable (<1.2 ng per microliter) after week 20 in all 20 patients who had no microvascular endothelial deposits of globotriaosylceramide in renal-biopsy specimens after week 20 of treatment. Five of eight patients in the recombinant α-galactosidase A group who had a renal score of 1 after week 20 had undetectable plasma concentrations of globotriaosylceramide after week 20, and three had concentrations ranging from 12 to 94 percent (mean, 35.3 percent) of their base line values. The patient who had been assigned a score of 3 because of a missing biopsy specimen at week 20 had a plasma globotriaosylceramide concentration of 3.9 ng per microliter.

Quality of Life

Patients in the recombinant α-galactosidase A group had significant improvements in two components of the SF-36 (physical role and emotional role), whereas patients in the placebo group had significant improvements in the physical role and body-pain components of the SF-36.

Open-Label Extension Study

All 58 patients enrolled in the open-label study. After six months of treatment with recombinant α-galactosidase A, 98 percent of patients in whom a biopsy was performed at this time (42 of 43) had a score of 0 on histologic analysis of microvascular endothelial deposits of globotriaosylceramide in kidney specimens, 96 percent (45 of 47) had such results for skin specimens, and 75 percent (24 of 32) had such results for heart specimens (Table 3). The results were similar when the analysis included only the patients who crossed over from placebo to recombinant α-galactosidase A: 100 percent, 96 percent, and 67 percent, respectively. In 95 percent of the patients who had had a biopsy during the open-label study and who received

TABLE 2. MEAN CHANGES IN INDIVIDUAL AND COMPOSITE SCORES FOR MICROVASCULAR ENDOTHELIAL DEPOSITS OF GLOBOTRIAOSYLCERAMIDE IN KIDNEY-, HEART-, AND SKIN-BIOPSY SPECIMENS FROM BASE LINE TO AFTER THE WEEK-20 INFUSION.*

SPECIMEN	NO. OF PATIENTS	BASE LINE	WEEK 20	MEAN CHANGE	P VALUE†
			score		
Kidney					<0.001
Recombinant α-galactosidase A	29	1.9±0.8	0.4±0.7	−1.6±1.2	
Placebo	29	2.2±0.7	2.1±0.8	−0.1±1.1	
Heart					<0.001
Recombinant α-galactosidase A	29	0.9±0.4	0.3±0.5	−0.6±0.7	
Placebo	29	0.9±0.5	1.2±0.6	0.2±0.8	
Skin					<0.001
Recombinant α-galactosidase A	29	2.1±0.7	0.0±0.0	−2.1±0.7	
Placebo	29	2.3±0.8	2.2±0.7	−0.1±1.0	
Composite					<0.001
Recombinant α-galactosidase A	29	4.9±1.5	0.7±0.8	−4.2±1.8	
Placebo	29	5.4±1.4	5.5±1.6	0.1±2.0	

*Plus–minus values are means ±SD.

†P values are for the mean changes and were calculated with use of a t-test.

Figure 1. Change in Levels of Pain from Base Line to Week 20 of the Double-Blind Study in the Recombinant α-Galactosidase A Group (Panel A) and the Placebo Group (Panel B).

The short form of the McGill Pain Questionnaire was used to assess the level of sensory pain, affective pain, pain as measured on a visual analogue scale (VAS), and the present pain intensity (PPI). On this scale, higher scores indicate greater pain. The total pain score is the sum of the sensory and affective pain scores. There were significant reductions in all the mean pain measures within each treatment group, but no significant differences between the two groups.

recombinant α-galactosidase A during the double-blind study, the renal scores were maintained or further decreased after six months of open-label treatment. In addition, renal function, as measured by the glomerular filtration rate, did not change substantially from base line in either group after week 20 of the double-blind study (P=0.19) or after six months of open-label treatment (P=0.81).

Safety

No significant changes from base line in the echocardiograms, electrocardiograms, or other safety as-

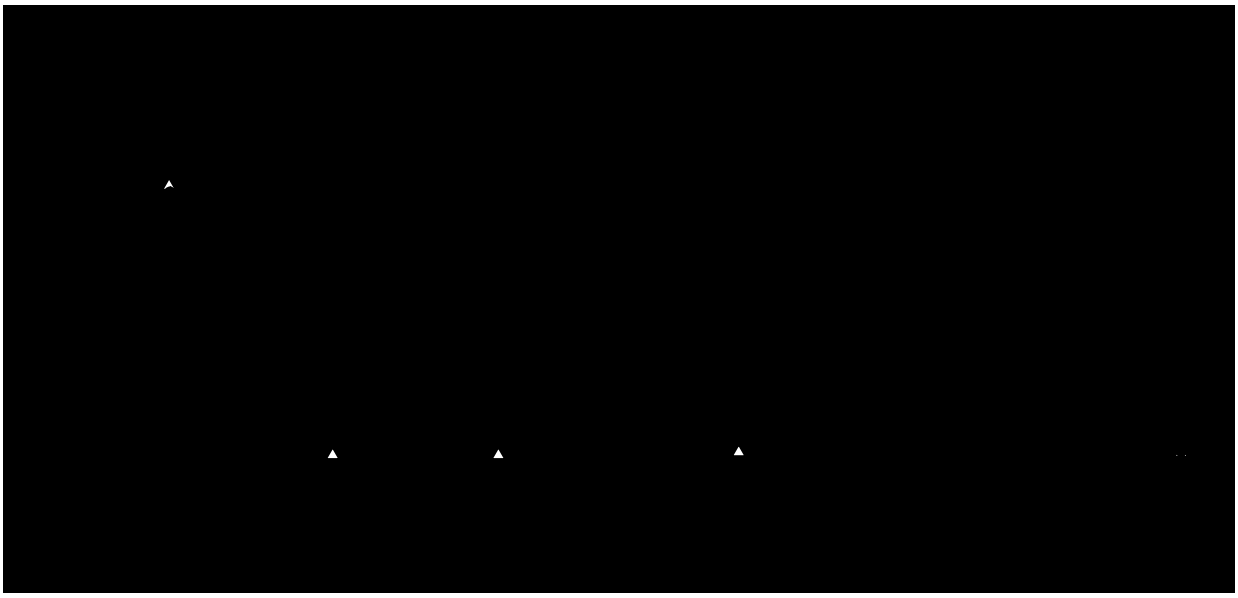

Figure 2. Median Plasma Concentrations of Globotriaosylceramide in the Recombinant α-Galactosidase A Group and the Placebo Group in the Double-Blind Study and the Open-Label Study.

Plasma levels of globotriaosylceramide were determined by a quantitative enzyme-linked immunosorbent assay at base line and week 20 of the double-blind study and after six months of open-label treatment. Plasma globotriaosylceramide values that were below the limit of detection (<1.2 ng per microliter) were recorded as 0. All patients who had been in the placebo group during the double-blind study received recombinant α-galactosidase A during the open-label study.

sessments in either group were observed after week 20 of the double-blind study or after six months of the open-label study. The infusions were generally well tolerated. Rigors and fever were the only treatment-related adverse events that occurred significantly more frequently in the recombinant α-galactosidase A group than in the placebo group during the double-blind study (P=0.004) (Table 4). Although not considered to be related to recombinant α-galactosidase A therapy, skeletal pain was the only other adverse event that occurred more frequently among enzyme-treated patients during the double-blind study (P=0.02). Transient mild-to-moderate infusion-associated reactions occurred in 59 percent of patients (34 of 58) during double-blind or open-label treatment. Reducing the infusion rate, administering preventive medications, or both measures controlled these reactions. A single patient had a positive skin test to recombinant α-galactosidase A after his eighth infusion during the open-label study, and treatment was discontinued.

IgG seroconversion occurred in 51 of the 58 patients who received recombinant α-galactosidase A (88 percent) during the study. Seroconversion did not affect the primary or secondary efficacy end points. For example, the distribution of the scores for renal specimens (0 vs. not 0) did not differ significantly between patients who did seroconvert and those who did not. In addition, 8 of 29 patients in the original recombinant α-galactosidase A group who had renal scores of

TABLE 3. NUMBER OF PATIENTS WITH A SCORE OF 0 ON HISTOLOGIC ANALYSIS OF MICROVASCULAR CAPILLARY ENDOTHELIAL DEPOSITS OF GLOBOTRIAOSYLCERAMIDE IN BIOPSY SPECIMENS AFTER 20 WEEKS OF DOUBLE-BLIND TREATMENT AND 6 MONTHS OF OPEN-LABEL TREATMENT WITH RECOMBINANT α-GALACTOSIDASE A.

SPECIMEN AND PREVIOUS DOUBLE-BLIND TREATMENT	NO. EVALUATED AT 12 MO	NO. WITH SCORES OF 0 (%)	MONTHS OF RECOMBINANT α-GALACTOSIDASE A TREATMENT
Kidney			
Placebo	22	22 (100)	6
Recombinant α-galactosidase A	21	20 (95)	12
Total	43	42 (98)	—
Heart			
Placebo	15	10 (67)	6
Recombinant α-galactosidase A	17	14 (82)	12
Total	32	24 (75)	—
Skin			
Placebo	23	22 (96)	6
Recombinant α-galactosidase A	24	23 (96)	12
Total	47	45 (96)	—

TABLE 4. ADVERSE EVENTS THAT OCCURRED IN AT LEAST 10 PERCENT OF PATIENTS IN THE RECOMBINANT α-GALACTOSIDASE A GROUP DURING THE DOUBLE-BLIND STUDY.

ADVERSE EVENT	RECOMBINANT α-GALACTOSIDASE A GROUP (N=29)	PLACEBO GROUP (N=29)
	no. (%)	
Rigors	14 (48)*	0
Fever	7 (24)†	1 (3)
Headache	5 (17)	2 (7)
Chills	4 (14)	0
Pain related to Fabry's disease	3 (10)	1 (3)
Hypertension	3 (10)	0

*P=0.004 by Fisher's exact test.

†P=0.024 by Fisher's exact test.

1 after week 20 had a reduction in their scores to 0 during the open-label study. IgG titers had decreased in 15 of 26 patients in the recombinant α-galactosidase A group (58 percent) who seroconverted during the double-blind study when the titers were assessed after 12 months of treatment. In addition, one IgG-positive patient with a low titer became seronegative during this period. These observations serve to reduce concern about potential reactions associated with seroconversion.

DISCUSSION

During the past decade the safety and effectiveness of enzyme-replacement therapy have been demonstrated in patients with type 1 Gaucher's disease.[13,14] In patients with this lysosomal storage disease, the infusion of human placental or recombinant acid β-glucosidase metabolized the accumulated substrate, reversed the disease-related abnormalities, and markedly improved the quality of life.[13,14] We report the results of a randomized, double-blind, placebo-controlled trial and the first six months of an open-label extension study that demonstrate the safety and effectiveness of enzyme replacement in a second lysosomal disorder, Fabry's disease.

In patients with classic Fabry's disease, the chief debilitating manifestations result primarily from the progressive accumulation of microvascular endothelial deposits of globotriaosylceramide, leading to ischemia and infarction, particularly in the kidneys, heart, and brain.[1] In contrast, patients with the cardiac variant of the disease have residual α-galactosidase A activity (<10 percent of normal levels) and do not have vascular endothelial accumulation of glycosphingolipid.[1,15,16] In these patients, left ventricular hypertrophy and mild proteinuria typically develop late in life, the life span is normal, and the classic manifestations of the disease, including angiokeratoma, acroparesthesias, hypohidrosis, and renal failure, are absent.[1] Thus, the reversal of the underlying vascular endothelial abnormalities in patients with classic Fabry's disease should be therapeutic.

We found that 11 infusions of recombinant α-galactosidase A at a dose of 1 mg per kilogram over a 20-week period safely and effectively cleared the abnormalities in the capillary endothelium of the kidneys, heart, and skin of patients with classic Fabry's disease. The primary efficacy end point of our study directly addressed a fundamental cause of the most common and devastating feature of classic Fabry's disease: renal failure. After 20 weeks of treatment, complete or almost complete clearance of the accumulated renal microvascular endothelial deposits of globotriaosylceramide was achieved in 69 percent of the patients in the recombinant α-galactosidase A group, as compared with none of the patients in the placebo group ($P<0.001$). In addition, the concentration of globotriaosylceramide was significantly reduced in the urinary sediment of patients in the recombinant α-galactosidase A group, providing indirect evidence of the clearance of glycosphingolipids in renal tubules. Similar results were achieved with respect to the clearance of microvascular endothelial deposits of globotriaosylceramide from the heart ($P<0.001$) and skin ($P<0.001$).

The open-label extension study confirmed the results of the double-blind study and demonstrated that clearance was maintained or that microvascular endothelial deposits of globotriaosylceramide were further reduced in all three types of specimens assessed in patients who were treated with recombinant α-galactosidase A for about one year. Notably, the percentage of patients with clearance of the microvascular endothelial deposits of globotriaosylceramide in the endomyocardium increased from 67 percent after 20 weeks to 82 percent after 6 months of open-label treatment, indicating that the clearance of globotriaosylceramide may be tissue specific, depending on the dose and duration of treatment, the level of enzyme uptake, and the degree of substrate accumulation. Taken together, the results of the double-blind and open-label studies confirm that recombinant α-galactosidase A replacement therapy cleared the accumulated microvascular endothelial deposits of globotriaosylceramide and reversed the chief underlying abnormality in Fabry's disease. On the basis of the results of the preclinical,[17] phase 1 and 2 dose-escalation,[6] and double-blind studies, the plasma globotriaosylceramide level may be correlated with the accumulation of this glycosphingolipid in tissue and may provide a noninvasive indicator of systemic substrate clearance, analogous to serum glucose levels in patients with diabetes.

Most patients with the classic form of the disease have episodic acroparesthesias that are debilitating and markedly impair their quality of life. Patients in a phase

1 and 2 open-label study reported decreased severity of pain related to Fabry's disease.[6] In our double-blind study, the severity of pain and the quality of life, as assessed by standardized instruments, were significantly improved in both groups, making it impossible to differentiate treatment-related effects from a placebo effect. For ethical reasons, patients who had been dependent on prophylactic drugs, analgesics, or both for years continued to take such medications during the study, a factor that may have minimized baseline scores and subsequent differences between groups. Studies are needed to determine the long-term effects of treatment with recombinant α-galactosidase A, perhaps with the use of instruments specifically designed to assess pain related to Fabry's disease and quality-of-life issues.

In general, the infusions were well tolerated, and all 58 patients completed the double-blind trial and entered the open-label study. The possibility of infusion-related reactions was anticipated, since patients with classic Fabry's disease have no detectable α-galactosidase A activity, protein, or both.[1] Therefore, we purposely kept the infusion rates slow to maintain blinding, and we administered prophylactic medications to all patients to minimize any infusion-related reactions. During the open-label study, we increased the infusion rates, and in the case of many patients, the infusion lasted two hours. In 88 percent of patients, IgG antibodies against recombinant α-galactosidase A developed; however, seroconversion did not affect primary or secondary efficacy results, nor did the antibodies have a neutralizing effect, as occurs in patients with hemophilia A in whom inhibitors develop.[18,19] After approximately one year of treatment with recombinant α-galactosidase A, IgG titers had decreased in 58 percent of patients with seroconversion and became undetectable in one patient. On the basis of previous experience with long-term enzyme-replacement therapy,[10,20] such findings suggest that immunologic tolerance may develop in these patients.

In conclusion, we found that a dose of 1 mg of recombinant α-galactosidase A per kilogram every other week for about six months to one year safely and effectively reversed the accumulation of microvascular endothelial deposits of globotriaosylceramide in the kidneys, heart, and skin. Continued treatment may be required to reduce the deposition of glycosphingolipids in other types of cells, to which less enzyme is delivered,[17] particularly renal tubular epithelial cells, podocytes, and cardiomyocytes. Further experience will determine effective regimens for initial reversal and subsequent control of the accumulated glycosphingolipids in the capillary endothelium and other types of cells.

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APPENDIX

In addition to the authors, the following members of the International Collaborative Fabry Disease Study Group participated in the study: **Investigators** — M. Banikazemi, J. Ibraham, and A.P. Cheng (New York); L.J. Raffel (Los Angeles); P. Cochat (Lyons, France); M. Azizi and X. Jeunemaitre (Paris); A. Vellodi (London); J.E. Wraith (Manchester, United Kingdom); C.J. Chaves, K.B. Kanis, I. Linfante, and R. Llinas (Boston); D.K. Bosman, H.S.A. Heymans, C.E.M. Hollak, and F.A. Wijburg (Amsterdam); **Expert pathologists** — *Kidney:* R.B. Colvin (Boston); S. Dikman (New York), and H. Rennke (Boston); *Heart:* H.T. Aretz (Boston), J. Fallon (New York), and R. Mitchell (Boston); *Skin:* H.R. Beyers and S. Granler (Boston) and R. Phelps (New York); and *General Pathology:* R.E. Gordon (New York); **Specialty consultants** — S. Brodie, S.A. Gass, M. Goldman, D. Mehta, and J. Winston (New York); R. Bouvier, B.P. Denis, L. Dubourg, A. Fouilhoux, A. Hadj-Aïssa, M. Laville, I. Maire, B. Ranchin, and M.T. Vanier (Lyons, France); A. Hickey, J. Jordan, S. Jordan, S.S. Khan, and E. Maguen (Los Angeles); C. Amrein, B. Diebold, J.N. Fiessinger, M. Froissart, J.P. Grunfeld, J. Julien, L.H. Noel, C. Orssaud, and L. Poenaru (Paris); M.H. Griffiths, D. Holdright, N. Phelps-Brown, S. Sporton, R. Woolfson, V.C. Worthington, and E.P. Young (London); M. Bhushan, A. Cooper, E. O'Riordan, R. Radford, S.G. Ray, and R.S. Reeve (Manchester, United Kingdom); F.G. Berson, M.S. Kruskall, and W.J. Manning (Boston); W.J.W. Bos, D.K. Bosman, F.J.W. ten Kate, R.T. Krediet, K.I. Lie, J.J. Piek, L.J.J.M. Prick, and J.H.S. Smitt (Amsterdam); **Study coordinators and nurses** — M. Nunn, A. Nieto, R.A. Denchy, and A. Kowalski (New York); J. Exantus, M.T. Dupret, S. Garnier, and S. Walbilic (Lyons, France); A.G. Verne and B. Williams (Los Angeles); M.C. Bernard and V. Remones (Paris); J. Morrison, D.G. Burke, L.G. Fulford, M. Jackson, R. Lobo, S. Sporton, and V.C. Worthington (London); B.M. Kenny (Manchester, United Kingdom); L. Baron (Boston); A. Vyth (Amsterdam); **Genzyme personnel** — R. Moscicki, T. Braakman, M. Goldberg, M. O'Callaghan, R. Cintron, S. Richards, P.K. Tandon, M.A. Fitzpatrick, M. Yelmene, and M. Nichols.

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