

Brief Report

IMPROVEMENT IN CARDIAC FUNCTION IN THE CARDIAC VARIANT OF FABRY'S DISEASE WITH GALACTOSE-INFUSION THERAPY

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FABRY'S disease is an X-linked inborn error of glycosphingolipid catabolism caused by deficient activity of α -galactosidase A, a lysosomal exoglycosidase.^{1,2} In males with the classic form of the disease, there is little if any α -galactosidase A activity. As a result, undegraded glycosphingolipids accumulate, particularly in the vascular endothelium. These deposits cause the characteristic angiokeratomas, acroparesthesias, hypohidrosis, and corneal opacities of Fabry's disease. Death in early adulthood in affected persons may be due to vascular disease of the heart, kidney, or brain. These abnormalities are absent in males with the cardiac variant of the disease. Those with the cardiac variant typically present with a mild, late-onset disorder that is primarily limited to the heart, and there is no involvement of the vascular endothelium.¹⁻³ With advancing age, however, cardiac involvement progresses and leads to death.⁴ All of the previously described patients with the cardiac variant of Fabry's disease had mutations in the α -galactosidase A gene that encoded sufficient residual enzymatic activity to preclude the classic phenotype.^{1-3,5,6}

A recent clinical trial, reported elsewhere in this issue of the *Journal*, indicated that enzyme-replacement therapy may be safe and effective in patients with classic Fabry's disease.⁷ Enzyme infusions given every other week cleared the glycosphingolipid deposits in the vascular endothelium of the kidney, heart, and skin. In a finding of relevance to patients with the cardiac variant of the disease, *in vitro* studies indicated that residual α -galactosidase A activity can be increased by the addition of galactose to the medium of cultured fibro-

blasts from patients with specific mutations.^{5,8} Moreover, certain reversible competitive inhibitors of α -galactosidase A, such as 1-deoxygalactonojirimycin, can also increase the activity of the enzyme in cultured fibroblasts from patients with the cardiac variant of the disease.^{9,10} These findings suggest that treatment with galactose or a nontoxic, reversible competitive enzyme inhibitor may enhance the residual activity of α -galactosidase A, thereby improving the pathological and clinical manifestations of the cardiac variant of the disease.

We describe a 55-year-old man with the cardiac variant of Fabry's disease who had residual α -galactosidase A activity as the result of a missense mutation encoding a substitution of arginine for glycine at position 328 of the enzyme (G328R). He had severe myocardial disease and was a candidate for cardiac transplantation. Therapy with intravenous infusions of galactose, given every other day, was begun, and his condition has been monitored for more than two years.

CASE REPORT

A 55-year-old man presented with angina and dyspnea on mild effort and at rest (New York Heart Association [NYHA] functional class IV). Ten years before presentation, he had received a diagnosis of hypertrophic nonobstructive cardiomyopathy. During the preceding five years, he had had chronic atrial fibrillation and dyspnea with exertion and had been treated with digitalis, diuretics, and angiotensin-converting-enzyme inhibitors. There was no family history of Fabry's disease or hypertrophic cardiomyopathy, although his brother had died suddenly at the age of 50 years. The patient did not have angiokeratomas, acroparesthesias, hypohidrosis, or corneal opacities.

Cardiac auscultation revealed an irregular rhythm, a heart rate of 100 beats per minute, and no pathologic murmurs. The blood pressure was 130/80 mm Hg. Plain-film radiographs of the chest revealed slight enlargement of the heart. An electrocardiogram showed atrial fibrillation (mean ventricular rate, 90 to 100 beats per minute) with left ventricular hypertrophy as well as nonspecific ST-T-wave changes. Holter monitoring revealed an elevated heart rate (150 to 160 beats per minute) during mild exercise and frequent ectopic ventricular beats, with some couplets and triplets (Lown class IVa, where class I is characterized by occasional ventricular ectopic beats and class V by sustained ventricular tachycardia). Two-dimensional Doppler echocardiographic studies showed dilatation of the left atrium (diameter, 50 mm) and left ventricular end-diastolic and end-systolic diameters (68 and 53 mm, respectively), with a moderate reduction in left ventricular contractility (ejection fraction, 33 percent) (Table 1). Both the interventricular septum and the left ventricular posterior free wall were markedly thickened (interventricular septum, 20 mm; left ventricular posterior wall, 16 mm), particularly at the basal septum and apical levels. The right ventricular free wall was moderately hypertrophic. The valves were normal.

Spin-echo magnetic resonance imaging (MRI) (Signa Horizon, General Electric Medical Systems, Paris) was performed (repetition time, 25 msec; echo time, 25 msec; matrix, 256 by 160; number of excitations, 3), and cine gradient-echo sequences (repetition time, 30 msec; echo time, 12 msec; flip angle, 35 degrees; matrix, 256 by 128; number of excitations, 3), gated on the horizontal long and short axes, were obtained with a 0.5-T scanner (Vectra, General Electric Medical Systems). Diffuse and symmetric myocardial thickening was evident, particularly in the left midventricular and apical segments and in the right ventricular free wall, with heterogeneous signal intensity. The mean thicknesses of the left ventricular and right ventricular walls at end-diastole were 18 and 10 mm, respectively, and the left ventricular mass was 293 g. All of the mid-

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TABLE 1. RESULTS OF ECHOCARDIOGRAPHIC, HEMODYNAMIC, AND CARDIAC MRI STUDIES BEFORE AND DURING TREATMENT WITH INTRAVENOUS GALACTOSE.

VARIABLE	BEFORE TREATMENT	AFTER 3 MONTHS OF TREATMENT	AFTER 2 YEARS OF TREATMENT
Echocardiographic data			
Left ventricular end-diastolic diameter (mm)	68	59	58
Left ventricular end-systolic diameter (mm)	53	45	44
Thickness of interventricular septum (mm)	20	16	16
Thickness of left ventricular posterior wall (mm)	16	14	14
Shortening fraction (%)	22	24	24
Left ventricular ejection fraction (%)	33	55	55
Hemodynamic data*			
Left ventricular end-diastolic pressure (mm Hg)	25	14	—
Cardiac index (liters/min/m ²)	1.7	2.9	—
Cardiac MRI data			
Mean thickness of left ventricular wall (mm)	18	15	14
Mean thickness of right ventricular wall (mm)	10	7	6
Left ventricular mass (g)	293	235	228
Left ventricular ejection fraction (%)	32	51	55

*Hemodynamic data were not obtained at two years, because cardiac catheterization was not performed at this time.

ventricular and apical segments were hypokinetic, and the midventricular inferior septum was dyskinetic; the left ventricular diameters at end-diastole were slightly increased, and global left ventricular function was depressed, with an ejection fraction of 31.5 percent (according to Simpson's biplane rule) (Table 1).

Because of his end-stage hypertrophic cardiomyopathy, the patient was considered for cardiac transplantation. Cardiac catheterization, coronary angiography, and left and right ventriculography were performed, and biventricular endomyocardial-biopsy specimens were obtained. The pulmonary and intracavity pressures were elevated. Ventricular angiography revealed moderate dilatation, with diffuse hypertrophy of both ventricles. The left ventricle had a spade-like shape, with moderately impaired contractility (left ventricular ejection fraction, 30 percent). The cardiac index was severely reduced (to 1.69 liters per minute per square meter of body-surface area). Coronary angiography showed that the epicardial coronary arteries were normal. Hematologic values, serum chemical values, and the results of urinalysis were normal; the blood urea nitrogen level was 17 mg per deciliter (6.1 mmol per liter), the serum creatinine level was 1.3 mg per deciliter (115 μ mol per liter), and the creatinine clearance was 75 ml per minute.

METHODS

Monitoring Procedures

Biopsy specimens were obtained after informed consent had been obtained from the patient and approval had been granted by the local ethics committee. Blood was collected from the coronary sinus for the isolation of lymphocytes and genomic DNA. Left ventricular and right ventricular endomyocardial biopsies were performed for morphologic and biochemical studies. A right ventricular endomyocardial-biopsy specimen (2.8 mg) was obtained through the femoral vein with a 7-French biptome (502-300L, Cordis, Miami) before and after the first infusions of galactose (3.1 mg). As a control, endomyocardial-biopsy specimens (2.6 to 3.8 mg) were obtained from the papillary muscles of patients with mitral stenosis who were undergoing mitral-valve replacement and who had given their informed consent.

During treatment, the serum galactose, glucose, and creatinine levels and hepatic function were assessed weekly. Electrocardiograms and two-dimensional echocardiograms were obtained monthly, and

cardiac MRI was performed three months and two years after the beginning of treatment.

Morphologic Studies

Endomyocardial-biopsy specimens were processed for routine histologic and histochemical analyses and for transmission electron microscopy. For light microscopy, specimens were fixed in 10 percent buffered formalin and embedded in paraffin, and 5- μ m sections were stained with hematoxylin and eosin, Miller's elastic Van Gieson stain,^{11,12} and Masson's trichrome. Frozen sections were stained with periodic acid-Schiff and Sudan black. For transmission electron microscopy, myocardial tissues were fixed in 2 percent glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) and embedded in Epon resin. Ultrathin sections were stained with uranyl acetate and lead hydroxide.

Biochemical and Molecular Studies

Lymphocytes were isolated by gradient centrifugation (Lymphoprep, Nycomed, Oslo, Norway), washed in phosphate-buffered saline, and homogenized in distilled water.^{13,14} The endomyocardial-biopsy specimens were homogenized in 1 mM citrate-phosphate buffer (pH 4.5) and then centrifuged. The resulting lymphocyte and endomyocardial supernatants were assayed with 4-methylumbelliferyl- α -D-galactoside¹⁵ for α -galactosidase A activity (measured as nanomoles per hour per milligram of protein). Genomic DNA was isolated from peripheral blood (Puregene DNA isolation kit, Gentra Systems, Minneapolis), and all of the α -galactosidase A coding regions and adjacent intronic regions were sequenced as previously described.¹⁶

RESULTS

Diagnostic Studies

The differential diagnosis of hypertrophic cardiomyopathy in this patient included Fabry's disease, and the typical morphologic changes in the endomyocardial-biopsy specimens (Fig. 1A, 2A, and 2B) suggested this diagnosis. Histologic examination revealed thickened endocardium in both ventricles, with prominent

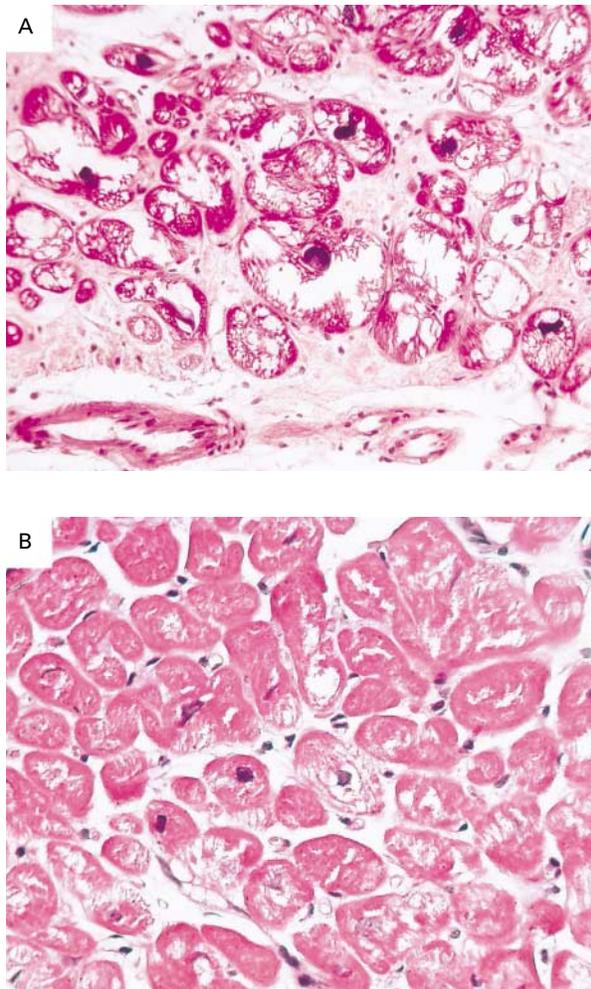


Figure 1. Left Ventricular Endomyocardial-Biopsy Specimens (Hematoxylin and Eosin, $\times 160$).

Before treatment, normal arterioles are surrounded by severely hypertrophic and extensively vacuolated myocardial fibers (Panel A). After three months of galactose infusions, the myocardial fibers appear smaller and are much less extensively vacuolated (Panel B).

smooth-muscle cells, a finding consistent with the presence of biventricular dilatation. The endomyocardial tissues contained regularly arranged and severely hypertrophic myocardial fibers (often larger than 40 μm in diameter), with large perinuclear vacuoles containing material that stained positive with periodic acid-Schiff and Sudan black. The interstitium was increased in width by areas of fibrosis. The arterioles appeared normal, and there was no evidence of endothelial or smooth-muscle involvement (Fig. 1A). Electron microscopy of the myocardial tissue revealed many concentric, lamellar figures in single membrane-bound vesicles (Fig. 2A); in cells where these vesicles

were infrequent, they were localized preferentially in the perinuclear area of the fibers (Fig. 2B). Remarkably, storage inclusions were not seen in the vascular endothelial cells, pericytes, interstitial histiocytes, or fibrocytes.

The morphologic diagnosis of Fabry's disease was substantiated by the finding that α -galactosidase A activity in the patient's lymphoblasts was deficient (141 nmol per hour per milligram of protein, or about 2.4 percent of the normal mean [\pm SD] activity, which is 5890 ± 2130 nmol per hour per milligram of protein). The patient's sister and the sister's three daughters had intermediate levels of lymphocyte α -galactosidase A activity (data not shown). Molecular analyses identified the α -galactosidase A G328R missense mutation in the patient, and these female family members were carriers of the mutation.

Effect of Galactose Infusions

After informed consent had been obtained from the patient, he was initially given a galactose infusion (1 g per kilogram of body weight, administered over a four-hour period) daily for three days. The α -galactosidase A activity in lymphocytes isolated from his coronary-sinus blood was then found to have increased from a mean of 156 ± 31.1 to 221 ± 40.4 nmol per hour per milligram of protein (Table 2). Similarly, the mean α -galactosidase A activity in endomyocardial-biopsy specimens increased from 50.4 ± 5.3 to 140 ± 1.5 nmol per hour per milligram of protein. On the basis of these results, treatment with galactose infusions (1 g per kilogram, given over a four-hour period every other day) was initiated. The effects of this regimen on cardiac function and endomyocardial glycosphingolipid deposition were evaluated after three months and then periodically during treatment.

The galactose infusions were well tolerated, and the results of liver-function tests performed every 90 days were normal during the first 2 years of treatment and have remained normal. After three months, electrocardiography and Holter-monitoring studies revealed a decrease in the heart rate at rest (mean, from 95 to 75 beats per minute) and during exercise (mean, from 160 to 120 beats per minute), with moderate improvement in the ST-T-wave changes. The frequency of ventricular ectopic beats was reduced from 12,720 per 24 hours to 562 per 24 hours, with no evidence of couplets or triplets. The QRS voltages remained unchanged. A two-dimensional Doppler echocardiographic study performed at three months showed improvement in cardiac contractility (left ventricular ejection fraction, 55 percent) and a reduction in myocardial-wall thicknesses (interventricular septum, 16 mm; left ventricular posterior wall, 14 mm) (Table 1).

MRI, performed with a 1.5-T scanner in the same planes and with the same sequences as in the pre-treatment studies, revealed moderate reductions in the mean left-ventricular-wall and right-ventricular-wall

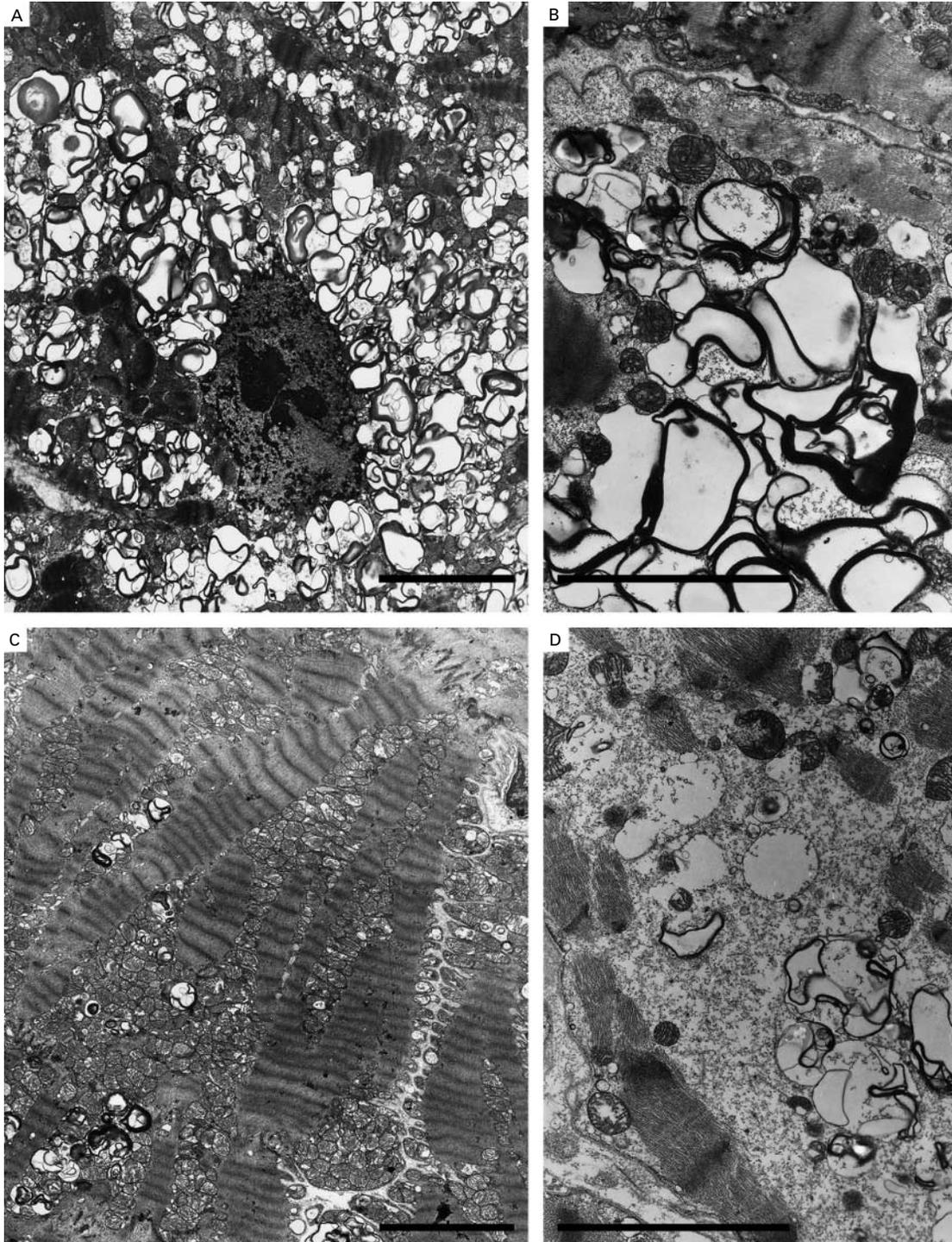


Figure 2. Transmission Electron Micrographs of Endomyocardial-Biopsy Specimens before Treatment with Intravenous Galactose (Panels A and B) and after Three Months (Panels C and D).

Panel A shows an endomyocardial-biopsy specimen obtained before treatment. The cytoplasm of the myocardial cells is occupied mostly by single, membrane-lined vacuoles containing lamellar, electron-dense inclusions. The area of the myofibrils appears to be greatly reduced. The scale bar represents 10 μm . Panel B shows a pretreatment biopsy specimen at a higher magnification. The electron-dense lamellar inclusions occupy most of the vacuolar space. The scale bar represents 5 μm . The micrograph in Panel C shows the reduction in glycosphingolipid-containing vacuoles in the endomyocardium after three months of treatment. The area that previously was vacuolar appears to have been replaced, mostly by myofibrillar material. The scale bar represents 10 μm . A biopsy specimen viewed at a higher magnification (Panel D) shows that after three months of galactose treatment, some vacuoles were empty or had reduced amounts of the lamellar inclusion material. The scale bar represents 5 μm .

TABLE 2. α -GALACTOSIDASE A ACTIVITY IN LYMPHOCYTES AND ENDOMYOCARDIAL CELLS BEFORE AND AFTER THE INITIATION OF TREATMENT WITH INTRAVENOUS GALACTOSE.*

ASSESSMENT	α -GALACTOSIDASE A ACTIVITY	
	LYMPHOCYTES†	ENDOMYOCARDIAL TISSUE
	nmol/hr/mg of protein	
Before treatment	156±31.1	50.4±5.3
After three initial infusions (1 g/kg over 4 hr, daily)	221±40.4	140±1.5
Control‡	2270±182	429±35.9

*Values are the mean (\pm SD) results of three independent determinations.

†Lymphocytes were obtained from coronary-sinus blood before and after the three initial infusions.

‡Control values were determined with lymphocytes and endomyocardial tissue from a 50-year-old man with mitral stenosis.

thicknesses (15 mm and 7 mm, respectively), normal left ventricular diameters, and an improvement in left ventricular global function (left ventricular ejection fraction, 51 percent) (Table 1). The mass of the left ventricle decreased about 20 percent, to 235 g. No other left ventricular regional abnormalities were evident.

The patient's NYHA functional status improved from class IV to class I, and he was able to perform activities involving moderate effort without chest pain or dyspnea. After two years of continuous treatment with a galactose infusion every other day, the improvement in the patient's cardiac function was maintained, as indicated by the results of the two-dimensional echocardiographic and cardiac cine MRI studies (Table 1). MRI showed that the mean thicknesses of the left ventricular and right ventricular walls were 14.5 mm and 6.5 mm, respectively; the left ventricular ejection fraction was 55 percent.

The area of the endomyocardial storage vacuoles decreased with galactose treatment (Fig. 1B, 2C, and 2D and Table 3). Morphometric analysis of the left ventricular and right ventricular endomyocardial tissues after three months of treatment showed decreases in the mean vacuolar area, from 61±8.9 percent to 22±4.7 percent and from 35±7.1 percent to 26±5.7 percent of the total cell area, respectively. No significant variation of the long and short axes of the myocardial cells was seen after three months of treatment (Table 3). These microscopical observations were consistent with the macroscopical values measured by MRI and two-dimensional Doppler echocardiography.

DISCUSSION

Although the causes of many types of cardiomyopathy have recently been clarified (among them genetic, inflammatory, and autoimmune disorders¹⁷⁻¹⁹),

TABLE 3. MORPHOMETRIC ANALYSIS OF ENDOMYOCARDIAL-BIOPSY SPECIMENS BEFORE TREATMENT WITH INTRAVENOUS GALACTOSE AND AFTER 90 DAYS.*

ASSESSMENT	SHORT AXIS OF MYOCARDIAL CELLS	VACUOLAR AREA
	μ m	% of cell area
	Right ventricle	
Before treatment	29.5±6.7	35±7.1
After 90 days	28.1±8.1	26±5.7
Left ventricle		
Before treatment	35.2±7.5	61±8.9
After 90 days	32.0±8.2	22±4.7

*Values are the mean (\pm SD) measurements in at least 25 cells from 10 micrographs.

treatment is still a major challenge. Of the various types of cardiomyopathy, the cardiac variant of Fabry's disease may be more common than previously believed; in one study it was diagnosed in 3 percent of unselected men with left ventricular hypertrophy,²⁰ and in another it was diagnosed in 9 percent of male patients with hypertrophic nonobstructive cardiomyopathy.²¹ In the cardiac variant of Fabry's disease, residual α -galactosidase A activity prevents the characteristic manifestations of the classic disease, which are due to involvement of the vascular endothelium of the heart, kidney, or brain. Patients with the cardiac variant have little if any vascular involvement, and cardiac symptoms develop late in life.

The finding that galactose and other competitive inhibitors of α -galactosidase A can increase or stabilize the activity of the residual mutant α -galactosidase A in cultured cells from patients with the cardiac variant of Fabry's disease^{5,9,22} suggested that intravenous galactose may enter the cells and increase the stability and activity of the residual enzyme. This was the reasoning behind the trial of galactose therapy in our patient, whose lymphocytes retained α -galactosidase A enzymatic activity at about 7 percent of the level of activity in normal lymphocytes.

After we observed that three daily galactose infusions increased α -galactosidase A activity in his circulating lymphocytes and endomyocardial cells, the patient received galactose infusions every other day for two years, and he continues to receive them. Not only were the infusions well tolerated, but marked improvement in cardiac contractility (seen as an increase in the left ventricular ejection fraction from 33 percent to 55 percent), a moderate reduction in ventricular-wall thickness, and a 20 percent reduction in cardiac mass were documented after three months of treatment. These improvements, which have persisted for more

than two years, were confirmed by the findings of independent observers, by two-dimensional echocardiography, and by cardiac MRI studies. Cardiac transplantation was no longer required in this patient, because of the clinical improvement (from NYHA functional class IV to class I) during galactose-infusion therapy. The patient has returned to full-time work as a bus driver.

The mechanism by which galactose and other re-

versible competitive inhibitors enhance the activity and stability of mutant α -galactosidase A has been the subject of recent studies.^{9,10} The substrates of wild-type α -galactosidase A are neutral glycosphingolipids with terminal α -galactosyl residues. Galactose and other reversible competitive inhibitors of the enzyme act as “chemical chaperones”^{23,24} that enhance the stability of the mutant enzyme by binding to its active sites (Fig. 3). In this way, the chaperones promote the prop-

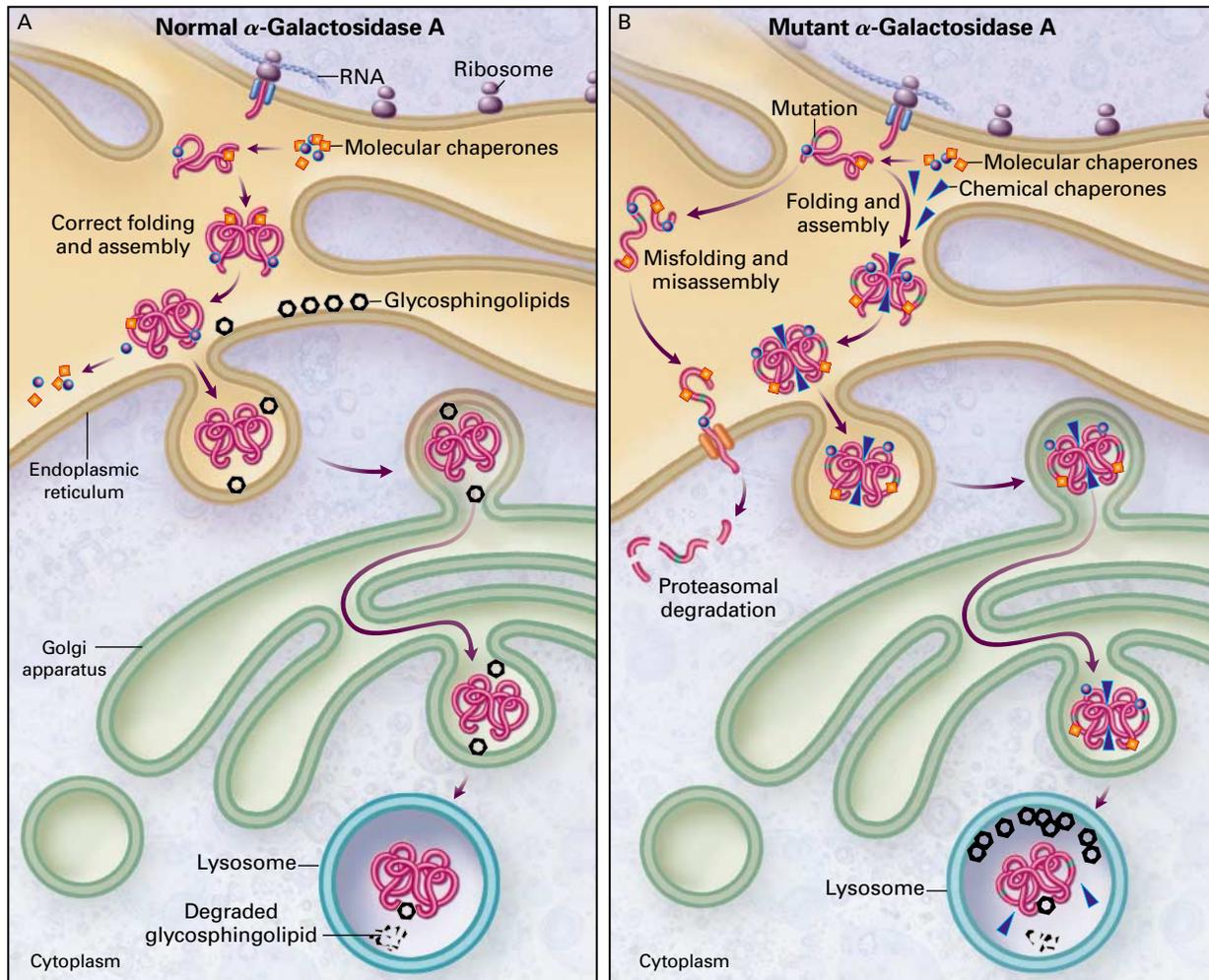


Figure 3. Proposed Mechanism of Enzyme Stabilization by Chemical Chaperones.

Panel A shows the processing of normal α -galactosidase A. Newly synthesized α -galactosidase A is translocated into the endoplasmic reticulum, where molecular chaperones facilitate its proper folding and dimerization by specialized processing enzymes. The molecular chaperones then dissociate from the folded, dimerized enzyme, which moves to the Golgi apparatus and then to lysosomes, where the enzyme is stable and active in the acidic environment of these organelles. Panel B shows the processing of mutant α -galactosidase A. Most mutations in the α -galactosidase A gene encode α -galactosidase A molecules that are misfolded, misassembled, or aggregated in the endoplasmic reticulum, where they are degraded, presumably by the ubiquitin–proteasome pathway. However, certain missense mutations decrease the stability of the enzyme, but the conformation of the active site is retained. Most of this type of mutant enzyme is degraded in the endoplasmic reticulum. However, these mutant forms of α -galactosidase A may be stabilized by chemical chaperones, such as galactose, that bind to the active site of the enzyme, promote folding, and stabilize the mutant enzyme. Some of the enzyme then reaches the lysosomes, where it retains low levels of activity. In the lysosomes, the accumulated glycosphingolipid substrates displace the chemical chaperones and are hydrolyzed by the enzyme. Modified from Kuznetsov and Nigam.²³

er folding, dimerization, and processing of the enzyme, thereby preventing the proteasomal degradation of misfolded, mutant enzymes. The stabilized enzyme is transported through the Golgi apparatus to lysosomes, where it may be even more stable, because of the acidic environment of those organelles (Fig. 3). In the lysosome, accumulated glycosphingolipid (the usual substrate of α -galactosidase A) has a much higher affinity for the active site of the enzyme than galactose. As a result, it displaces galactose from the active site and becomes susceptible to degradation by enzymatic hydrolysis.

Studies of different mutant α -galactosidase A enzymes have shown that the residual enzymatic activity encoded by two other mutations in the cardiac variant of Fabry's disease — one encoding the substitution of glutamic acid for glutamine at position 279 (Q279E) and one encoding the substitution of glutamine for arginine at position 301 (R301Q) — is markedly increased by galactose²² and 1-deoxygalactonojirimycin (another reversible competitive inhibitor of the enzyme),^{9,10} whereas the activity of the G328R mutant enzyme is minimally enhanced. Such in vitro experiments may be used to predict which patients with the cardiac variant of Fabry's disease (or even the classic disease) will benefit from galactose therapy.

Of the more than 160 mutations described in unrelated patients with Fabry's disease,² a mutation encoding the substitution of histidine for arginine at position 112 (R112H), R301Q, and G328R have been identified in patients with either the classic phenotype or the cardiac-variant phenotype.²⁵⁻²⁸ These missense mutations presumably allow extremely low levels of residual activity that can be stabilized by certain substrates.

For patients with classic Fabry's disease, enzyme replacement appears efficacious.^{29,30} For patients with the cardiac variant whose residual α -galactosidase A activity can be enhanced in vitro, chaperone-mediated therapy with galactose or other inhibitors may prove safe and therapeutically effective, as in our patient. Patients with unexplained left ventricular hypertrophy or hypertrophic nonobstructive cardiomyopathy should be evaluated for Fabry's disease by means of assays of their plasma α -galactosidase A activity, and those with residual α -galactosidase A activity that increases in response to an initial trial of galactose supplementation in vitro should be considered for treatment with a non-toxic, reversible competitive inhibitor such as galactose, at least until enzyme-replacement therapy or gene therapy³¹ proves efficacious. Our study supports the use of chaperone-mediated therapy for late-onset lysosomal storage diseases and possibly other genetic disorders.^{2,9}

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REFERENCES

- Desnick RJ, Ioannou YA, Eng CM. α -Galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular bases of inherited disease. 7th ed. Vol. 2. New York: McGraw-Hill, 1995:2741-84.
- Idem. α -Galactosidase A deficiency: Fabry disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic & molecular bases of inherited disease. 8th ed. Vol. 3. New York: McGraw-Hill, 2001:3733-74.
- von Scheidt W, Eng CM, Fitzmaurice TF, et al. An atypical variant of Fabry's disease with manifestations confined to the myocardium. *N Engl J Med* 1991;324:395-9.
- Elleder M, Dorazilova V, Bradova V, et al. Fabry's disease with isolated disease of the cardiac muscle, manifesting as hypertrophic cardiomyopathy. *Cas Lek Cesk* 1990;129:369-72. (In Czech.)
- Ishii S, Kase R, Sakuraba H, Suzuki Y. Characterization of a mutant α -galactosidase gene product for the late-onset cardiac form of Fabry disease. *Biochem Biophys Res Commun* 1993;197:1585-9.
- Nagao Y, Nakashima H, Fukuhara Y, et al. Hypertrophic cardiomyopathy in late-onset variant of Fabry disease with high residual activity of α -galactosidase A. *Clin Genet* 1991;39:233-7.
- Eng CM, Guffon N, Wilcox WR, et al. Safety and efficacy of recombinant human α -galactosidase A replacement therapy in Fabry's disease. *N Engl J Med* 2001;345:9-16.
- Okumiya T, Ishii S, Takenaka T, et al. Galactose stabilizes various missense mutants of α -galactosidase in Fabry disease. *Biochem Biophys Res Commun* 1995;214:1219-24.
- Fan JQ, Ishii S, Asano N, Suzuki Y. Accelerated transport and maturation of lysosomal α -galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. *Nat Med* 1999;5:112-5.
- Asano N, Ishii S, Kizu H, et al. In vitro inhibition and intracellular enhancement of lysosomal α -galactosidase A activity in Fabry lymphoblasts by 1-deoxygalactonojirimycin and its derivatives. *Eur J Biochem* 2000;267:4179-86.
- Elleder M, Bradova V, Smid F, et al. Cardiocyte storage and hypertrophy as a sole manifestation of Fabry's disease: report on a case simulating hypertrophic non-obstructive cardiomyopathy. *Virchows Arch A Pathol Anat Histopathol* 1990;417:449-55.
- Miller PJ. An elastin stain. *Med Lab Technol* 1971;28:148-9.
- Boyum A. Isolation of leucocytes from human blood: a two-phase system for removal of red cells with methylcellulose as erythrocyte-aggregating agent. *Scand J Clin Lab Invest Suppl* 1968;97:9-29.
- Hall CW, Liebaers I, Di Natale P, Neufeld EF. Enzymatic diagnosis of the genetic mucopolysaccharide storage disorders. *Methods Enzymol* 1978;50:439-56.
- Desnick RJ, Allen KY, Desnick SJ, Raman MK, Bernlohr RW, Krivit W. Fabry's disease: enzymatic diagnosis of hemizygotes and heterozygotes: α -galactosidase activities in plasma, serum, urine, and leukocytes. *J Lab Clin Med* 1973;81:157-71.
- Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH, Desnick RJ. Nature and frequency of mutations in the α -galactosidase A gene that cause Fabry disease. *Am J Hum Genet* 1993;53:1186-97.
- Bonne G, Carrier L, Richard P, Hainque B, Schwartz K. Familial hypertrophic cardiomyopathy: from mutations to functional defects. *Circ Res* 1998;83:580-93.
- Kamisago M, Sharma SD, DePalma SR, et al. Mutations in sarcomere protein genes as a cause of dilated cardiomyopathy. *N Engl J Med* 2000;343:1688-96.
- Towbin JA, Bowles NE. Genetic abnormalities responsible for dilated cardiomyopathy. *Curr Cardiol Rep* 2000;2:475-80.
- Nakao S, Takenaka T, Maeda M, et al. An atypical variant of Fabry's disease in men with left ventricular hypertrophy. *N Engl J Med* 1995;333:288-93.
- Kuhn H, Köhler E, Hort W, Frenzel H. Concealed myocardial storage disease (Fabry's disease): pitfalls in the diagnosis of hypertrophic non obstructive cardiomyopathy. *Circulation* 1982;66:Suppl II:II-117. abstract.
- Okumiya T, Ishii S, Kase R, Kamei S, Sakuraba H, Suzuki Y. α -Galactosidase gene mutations in Fabry disease: heterogeneous expressions of mutant enzyme proteins. *Hum Genet* 1995;95:557-61.

23. Kuznetsov G, Nigam SK. Folding of secretory and membrane proteins. *N Engl J Med* 1998;339:1688-95.
24. McClellan AJ, Frydman J. Molecular chaperones and the art of recognizing a lost cause. *Nat Cell Biol* 2001;3:E51-E53.
25. Ishii S, Kase R, Sakuraba H, et al. Human α -galactosidase gene expression: significance of two peptide regions encoded by exons 1-2 and 6. *Biochim Biophys Acta* 1994;1204:265-70.
26. Ashton-Prolla P, Tong B, Shabbeer J, Astrin KH, Eng CM, Desnick RJ. Fabry disease: twenty-two novel mutations in the α -galactosidase A gene and genotype/phenotype correlations in severely and mildly affected hemizygotes and heterozygotes. *J Investig Med* 2000;48:227-35.
27. Eng CM, Niehaus DJ, Enriquez AL, Burgert TS, Ludman MD, Desnick RJ. Fabry disease: twenty-three mutations including sense and antisense CpG alterations and identification of a deletion hot-spot in the α -galactosidase A gene. *Hum Mol Genet* 1994;3:1795-9.
28. Sakuraba H, Oshima A, Fukuhara Y, et al. Identification of point mutations in the α -galactosidase A gene in classical and atypical hemizygotes with Fabry disease. *Am J Hum Genet* 1990;47:784-9.
29. Eng CM, Banikazemi M, Gordon RE, et al. A phase 1/2 clinical trial of enzyme replacement in Fabry disease: pharmacokinetic, substrate clearance, and safety studies. *Am J Hum Genet* 2001;68:711-22.
30. Schiffmann R, Kopp JB, Austin H, et al. Efficacy and safety of enzyme replacement therapy for Fabry disease demonstrated by a double-blind placebo-controlled trial. *Am J Hum Genet* 2000;67:Suppl 2:38. abstract.
31. Zeigler RJ, Yew NS, Li C, et al. Correction of enzymatic and lysosomal storage defects in Fabry mice by adenovirus-mediated gene transfer. *Hum Gene Ther* 1999;10:1667-82.

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