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Basic Research

Myocardial Alterations in the Murine Model of Fabry Disease Can Be Reversed by Enzyme Replacement Therapy

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ABSTRACT

Background: Fabry disease results from deficiency of alpha-galactosidase A (AGA), causing lysosomal storage of globotriaosylceramide in heart and other tissues. Since 2003, enzymatic replacement therapy with recombinant AGA agalsidase alfa (R-AGA) was approved for clinical use.

Methods: We evaluated whether, in mice knocked out for AGA (FM, n = 31), the myocardium was altered with respect to the wild-type mice (WT, n = 25) and whether alterations were reversed in FM treated with intravenous R-AGA, 0.5 mg/kg every other week during 2 months (FM-AGA, n = 12).

Results: Left ventricular (LV) contractility was depressed in FM, evaluated by LV $\Delta P/\Delta t$ (FM = 2832 ± 85 mm Hg/s, WT = 3179 ± 119 mm Hg/s; $P < 0.05$), papillary muscle contraction (FM = 39.8 ± 17.3 mg, WT = 67.5 ± 15.7 mg; $P < 0.05$), or shortening fraction measured by M-mode echocardiography (FM = 30% ± 6%, WT = 47% ± 2%; $P < 0.05$). LV stiffness (arrested hearts) decreased in FM (FM = 35.57 ± 3.5 mm Hg/20 μl; WT = 68.86 ± 6.12 mm Hg/20 μl; $P < 0.05$). FM myocytes showed augmented size, disorganized architecture, and

RÉSUMÉ

Introduction : La maladie de Fabry résulte d'un déficit d'alphagalactosidase A (AGA), causant une accumulation lysosomale de la globotriaosylcéramide dans le cœur et dans les autres tissus. Depuis 2003, l'enzymothérapie recombinante substitutive par l'agalsidase alfa (R-AGA) a été approuvée pour l'utilisation clinique.

Méthodes : Nous avons évalué si, chez les souris dont l'AGA a été inactivée (SF [souris ayant la maladie de Fabry], n = 31), le myocarde était modifié dans le cas des souris de phénotype sauvage (WT, n = 25), et si les altérations étaient réversibles chez les SF traitées par une R-AGA intraveineuse, à raison de 0,5 mg/kg toutes les 2 semaines durant 2 mois (SF-AGA, n = 12).

Résultats : La contractilité du ventricule gauche (VG) était abaissée chez les SF, évaluées par le VG $\Delta P/\Delta t$ (SF = 2832 ± 85 mmHg/s, WT = 3179 ± 119 mmHg/s; $P < 0,05$), la contraction du muscle papillaire (SF = 39,8 ± 17,3 mg, WT = 67,5 ± 15,7 mg; $P < 0,05$), ou la fraction de raccourcissement mesurée par l'échocardiographie en mode M (SF = 30 % ± 6 %, WT = 47 % ± 2 %; $P < 0,05$). La raideur du VG (cœur arrêté) a diminué chez les SF (SF = 35,57 ± 3,5 mmHg/20 μl; WT = 68,86 ± 6,12 mmHg/20 μl; $P < 0,05$). Les

Fabry disease results from the deficiency of the enzyme alpha-galactosidase A (AGA), which causes a progressive lysosomal storage of globotriaosylceramide (Gb3) and other substrates in different tissues including the heart. Although classic Fabry disease presents as a multisystemic affection, a "cardiac variant" is also observed, with manifestations circumscribed mainly to the heart.[1]

Around 1%-3% of male patients with left ventricular hypertrophy (LVH) have the cardiac variant of Fabry disease.[2,3] Cardiac involvement is due to structural and functional changes of the myocardium, conduction system, and valves. Gb3 was found in all cardiac tissues, with the greatest concentrations in the mitral valve and the LV myocardium. Another substrate, digalactosylceramide, was found to be increased in the right heart tissues.[4] Cardiac affection in patients with Fabry disease is frequent, resulting in hypertrophic changes of the myocardium, conduction delays, and thickening of the valves.[5] LVH is progressive, homogeneous, and concentric; occurs independently of blood pressure ;and could be associated in part with a proliferation factor present in plasma.[6] LVH is usually not associated with significant systolic or restrictive diastolic dys-

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intracytoplasmic vacuolization. Alterations reverted in FM-AGA: LV $\Delta P/\Delta t = 3281 \pm 456$ mm Hg/s and LV stiffness = 58.83 ± 2.15 mm Hg/20 μl, with normalization of myocyte architecture. No reversion was detected with AGA solvent.

Conclusions: The FM represent a mild, early stage of the disease, since myocardial alterations are not prominent and appear in nonhypertrophic hearts. Reversion of alterations in the FM-AGA suggests that enzymatic replacement therapy can be useful when administered in early stages of this disease.

myocytes des SF ont présenté une augmentation de leur taille, une désorganisation de l'architecture, et une formation de vacuoles intracytoplasmiques. Les altérations ont été rétablies dans l'AGA des SF : $LV\Delta P/\Delta t = 3281 \pm 456$ mm Hg/s et la raideur du VG = 58,83 ± 2,15 mmHg/20 μl, avec la normalisation de l'architecture des myocytes. Aucune réversion n'a été décelée avec le solvant AGA.

Conclusions : Les SF représentent le stade précoce de la maladie, puisque les altérations du myocarde ne sont pas proéminentes et qu'elles apparaissent en l'absence d'hypertrophie cardiaque. La réversion des altérations de l'AGA chez les SF suggère que l'enzymothérapie substitutive puisse être utile lorsqu'administrée à un stade précoce de la maladie.

function.[5] With progression of the disease, the end-diastolic volume of the LV decreases and diastolic filling is impaired, resulting in a reduction of stroke volume and cardiac output. Recently, tissue Doppler imaging revealed reduced diastolic and systolic velocities even in the prehypertrophic stage of the disease.[7]

The mechanisms and pattern of LVH are different from those seen in other forms of infiltrative cardiomyopathies. Gb3 deposits in heart represent 1% of the increase in LV mass.[8] Cardiac tissue is characterized by hypertrophied cardiomyocytes, and the increase in cardiomyocyte size is attributable to intracytoplasmic glycosphingolipid vacuoles.[9] However, interstitial fibrosis is only slightly increased in Fabry disease.

Treatment of Fabry disease had been primarily symptomatic before the enzymatic replacement therapy (ERT), which was approved by the US Food and Drug Administration in April 2003. It is assumed that the missing enzyme, when intravenously infused, reaches the surface of the target cells and is internalized in the cytosol by binding to specific membrane receptors, and is finally incorporated in the lysosomes.

Abnormalities in cardiac contractile function can be produced by a decreased contractility of the myocardium, which can result in an inadequate cardiac output and/or a change in the passive properties of the myocardium, leading to an altered diastolic filling. In our laboratory, we have access to a colony of mice knocked out for the enzyme AGA (Fabry mice [FM]) and to their corresponding controls (wild-type mice [WT]), and we sought to study whether the myocardium of the FM did exhibit any of the defects just mentioned and whether these could be reversed by ERT. We were also interested in the possibility of myocardial structural alterations, and for that reason, histologic and morphometric studies were included in our protocol.

Methods

Animals

Mice knocked out for the enzyme AGA (AGA-null mice, FM) were bred from C57BL/6 hemizygous male mice (–/0) and homozygous female mice (–/–) kindly provided by Dr. R. Brady and Dr. A. Kulkarni (National Institutes of Health, Bethesda, MD, USA).[10] They were bred and maintained in the facility of the National University of La Plata. In addition, wild-type C57BL/6 littermates (WT) were used as controls. Both WT and FM were genotyped by polymerase chain reaction–based assay as previously described.[11] A total of 52 FM and 34 WT, 25 ± 3 weeks of age and weighing 30.2 ± 0.7 g, were used in the course of this study. All the animals used were male, and they were maintained in the central facility of the National University of La Plata School of Medicine, with air conditioning, a 12-hour light/dark cycle, and free access to food and water. The mice were housed in stainless steel cages with sterilized bedding (white pine wood shavings) that was changed every day. Tap water was provided in sterilized bottles with stainless steel nipples. Food was in the form of extruded chips (Purina nutrients) of the following composition (in %): humidity 10, proteins 24.6, lipids 7, ashes 6.4, raw fibre 4, hemicellulose 7.7, calcium 1.1, phosphorus 1.4, potassium 1.05, magnesium 0.2, sulfur 0.25, sodium 0.11, chloride 0.25, linoleic acid 2.25, linolenic acid 0.15, arginine 1.55, cysteine 0.42, glycine 1.4, histidine 0.59, isoleucine 0.97, leucine 2.25, lisine 1.2, methionine 0.4, phenylalanine 1.18, serine 0.09, treonine 0.9, valine 1.2, FDN 12, FDA 4.5, CNF 36, and TDN 82.

Myocardial performance

Experiments in intact hearts. The FM for the studies in intact hearts were divided into 3 groups: group 1 (n = 8) received no treatment; group 2 (n = 12) was treated with intravenous injections of the recombinant AGA enzyme agalsidase-alfa (Replagal; Shire Human Genetic Therapies, Cambridge, MA, USA) (0.5 mg/kg body weight) every other week during 2 months; and group 3 (n = 8) was injected according to the same protocol and time schedule but received only the solvent (placebo). ERT was initiated at 15 ± 2 weeks of age, and the studies were carried out at 25 ± 3 weeks of age. Each treated mice received 6 infusions (enzyme or placebo) every other week.

Active contraction was recorded in mice anesthetized with sodium pentobarbital 90 mg/kg IP and ventilated with a Harvard model 617 small rodent ventilator (Harvard Apparatus Co., Dover, Massachusettes, USA). After opening the chest, a short cannula connected to a P23Gb pressure transducer was introduced into the LV through the apex, and the LV pressure (LVP) and its first derivative ($\Delta P/\Delta t$) were measured and recorded for later analysis.

Passive myocardial properties were studied in hearts arrested by exposure to KCl 15%. A needle with a small balloon at its tip was introduced through a small opening at the LV apex. The needle was connected to an infusion pump delivering at a rate of 10 μl/min, and the resulting pressure was measured through a side tubing connected to a P23Gb pressure transducer (Gould Inc, Cleveland, OH, USA). Passive pressure-volume curves were generated up to a pressure of ≈60 mm Hg.

Ultrasound studies. Mice were administered one-third of the usual dose of pentobarbital sodium IP to produce light sedation for 10 minutes with minimal cardiovascular depression. Echocardiographic examination was performed in 11 animals (6 FM and 5 WT) using a Mindray Digiprince DP 6900 cardiac ultrasound machine with an 8.5-MHz imaging transducer at high frame rate. After good-quality 2-dimensional short-axis images of the LV were obtained, M-mode tracings of >10 consecutive beats were recorded and digitized to be measured using a computerized review station with Image Pro-Plus software. Measurements were made from 3 consecutive cardiac cycles, and the operator was kept blind with respect to the type of animals under examination.

Experiments in isolated papillary muscles. After anaesthesia with sodium pentobarbital (FM = 6, WT = 4), the thorax was widely opened and the heart was rapidly excised and placed in cold physiologic salt solution (PSS) of the following composition (in mM): NaCl 130, KCl 4.7, Na_2HPO_4 1.17, $MgSO_4$ 1.16, HCO_3Na 24.0, $CaCl_2$ 1.6, and glucose 11.0, bubbled with a mixture of 5% CO_2 and 95% O_2. The LV wall was opened along its longitudinal axis, and a suitable papillary muscle was selected and isolated. One end was traversed with a small stainless steel pin, and the opposite was tied with atraumatic 6-0 silk to form a ring. The muscle was then excised and placed into a thermostatized chamber suffused with PSS at 37°C (1 ml/min), and both ends were fixed to a stationary stainless steel hook and a miniature force transducer (Sensor One, Sausalito, CA, USA). Muscles were stimulated with depolarizing pulses at 0.5 Hz through field electrodes; by progressive stretching, the optimal length for force development was identified. Preparations were then stabilized during 30 minutes at the optimal length. After that period, the frequency of stimulation was suddenly raised to 1 Hz and sustained for 5 minutes; then it was returned to 0.5 Hz until stabilization. In each experiment, the developed force (F) was recorded at the end of each of the experimental interventions.

Morphometric studies

Anatomical measurements. After weighing and anesthetizing the mice (FM = 9, WT = 9) with sodium pentobarbital, the thorax was widely opened and the heart was rapidly excised and placed in cold PSS. The right and left ventricle were dissected with microscissors and weighed individually in an analytical balance (Mettler, Switzerland). The tibia was removed and its length was measured with a digital caliper. The weight of both ventricles was divided by body weight and by tibial length and was compared between FM and WT.

Electron microscopy. LV sections were fixed in 2.5% buffered glutaraldehyde, postfixed in a 1% solution of osmium tetroxide, dehydrated in ethanol, and embedded in epoxy resin according to standard protocols for electron microscopy. Sections (1- to 2-mm thick) were stained with alkaline 1% solution of toluidine blue and examined for proper orientation. Thin sections were double stained with uranyl acetate and lead citrate and examined with a JEM 1200EX II transmission electron microscope (JEOL, Tokyo, Japan). These microscopic studies were performed by the Histology Department, Faculty of Veterinary Sciences, National University of La Plata.

Light microscopy. LV slices were fixed in a neutral buffered formaldehyde solution and paraffin sections were obtained following standard techniques. Two types of histochemical studies were performed on these sections: (1) Sudan staining, which is used for demonstrating fats, triglycerides, and lipoproteins present in tissues as biological stains, and (2) picrosirius red method, one of the best understood techniques of collagen histochemistry.

Data acquisition

The signals from the pressure and force sensors were amplified and then digitized with an analog-digital board (DT9802; Data Translation, Marlboro, MA, USA) mounted in a desktop computer. Online display for controlling the procedure and files for later processing were obtained with the appropriate software (DT Measure Foundry; Data Translation).

Statistics

The results were expressed as mean ± 1 SEM, and the differences between means were evaluated using a statistical package (Sigmastat 2.0; Jandel Scientific Software, San Rafael, CA, USA). When only 2 groups were involved, the Student's *t* test (paired or unpaired) was used, while for multiple group comparisons, the 1-way ANOVA was used. The particular modifications of these tests as dictated by the circumstances are indicated in "Results" for each specific protocol. The correlation between variables was studied with linear regression. A value of $P < 0.05$ was considered significant.

Results

Table 1 shows the anatomic parameters measured in FM and WT. All of the parameters measured were significantly greater in WT than in FM, with the exception of right ventricle weight and tibial length, whose difference did not reach significance.

Figure 1 shows the experimental setups for assessing myocardial performance in the intact heart and in isolated papillary muscles. The instrumentation is similar to that used in rat hearts, since we did not find any reference about this type of study in Fabry mice.

All the passive experiments performed in the 4 experimental groups are averaged in Figure 2, showing that a significant difference in distensibility exists between the WT and FM. In the ERT group of FM, a significant increment of the distensibility was observed compared to FM and placebo FM groups;

Table 1. Anatomic parameters in Fabry mice (FM) and wild-type (WT) (n = 9 in each group)

Parameter	Unit	FM	WT
Body weight (BW)	g	27 ± 1	30 ± 1*
Left ventricle weight (LVW)	mg	100 ± 4.6	128 ± 6*
Right ventricle weight (RVW)	mg	24.3 ± 3.7	32.3 ± 1.7†
Tibial length (TL)	mm	18.9 ± 0.5	19.9 ± 0.3†
LV/BW	mg/g	3.7 ± 0.09	4.24 ± 0.2*
LV/TL	mg/mm	5.32 ± 0.18	6.43 ± 0.3*
RV/BW	mg/g	0.88 ± 0.11	1.07 ± 0.05*
RV/TL	mg/mm	1.26 ± 0.16	1.63 ± 0.09*

* $P < 0.05$ between FM and WT (unpaired Student's *t* test).
† P = NS.

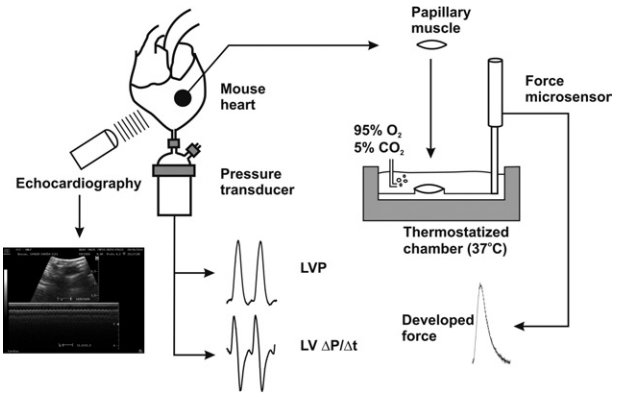

Figure 1. Schematic representation of the experimental setup used for the active and passive myocardial studies. LVP, Left ventricular pressure; LV $\Delta P/\Delta t$, first derivative of LVP.

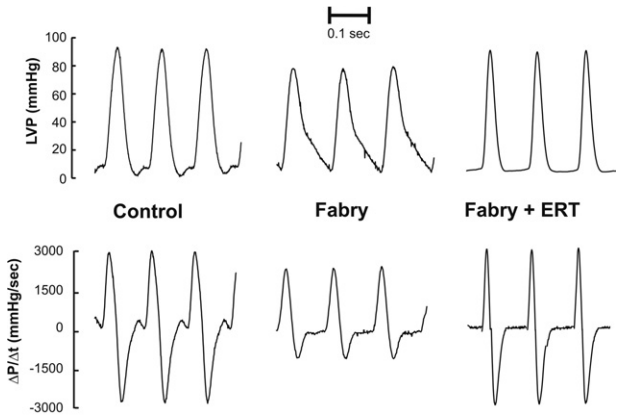

Figure 3. Three individual measurements of myocardial contractility, evaluated by the left ventricular pressure (LVP) and its first derivative ($\Delta P/\Delta t$). The Fabry mouse (**middle**) showed a depressed response with respect to the control (**left**), and this was reversed after enzymatic replacement therapy (**right**).

however, no difference was revealed in the passive behaviour in comparison to the WT group (1-way ANOVA, $P < 0.05$).

Active contractility was decreased in the FM with respect to the WT. Representative tracings of LVP and $\Delta P/\Delta t$ can be seen in Figure 3, showing a depression of both variables that was corrected after ERT. The data from all the experimental groups are represented in Figure 4, showing modest but yet significant depressions of LVP and $\Delta P/\Delta t$ (max) that were corrected by enzyme treatment (1-way ANOVA, $P < 0.05$). To be sure of the decrease in contractility observed in FM, the ratio of the maximum rate of rise of LVP divided by the pressure at the moment such maximum occurs was also calculated[12] as a more sensitive index of contractility. This ratio was (in s^{-1}): WT = 67.6 ± 4.6, FM = 52.8 ± 3.4, FM-ERT = 70.6 ± 9.8, FM-PLAC = 53.8 ± 6.7—the statistical significance was the same as in the case of $\Delta P/\Delta t$ (max).

Figure 5 shows the force developed by papillary muscles of FM and WT isolated and electrically stimulated. It can be seen that the preparations from FM developed significantly less force than the WT specimens, and within each group the increment in the frequency of stimulation from 0.5 Hz to 1.0 Hz produced a significant depression of the contractile response (negative staircase phenomenon) that was percentually similar in both FM and WT.

Electron micrographs of the LV of mice are shown in Figure 6 (top). Myocardial cells from FM are expanded with vacuolar spaces. Concentric lamellar bodies were not observed. The regular myocardial structure is destroyed by vacuolization and with more disarray in the myocardium from FM compared to that from WT. Micrographs from FM after ERT show a tendency toward a reduction and resolving of vacuoles content and a reorganization of the tissue architecture.

Figure 6 (bottom) shows optical microscopic images of myocardium from WT (*1* and *3*) and FM (*2* and *4*). Sudan

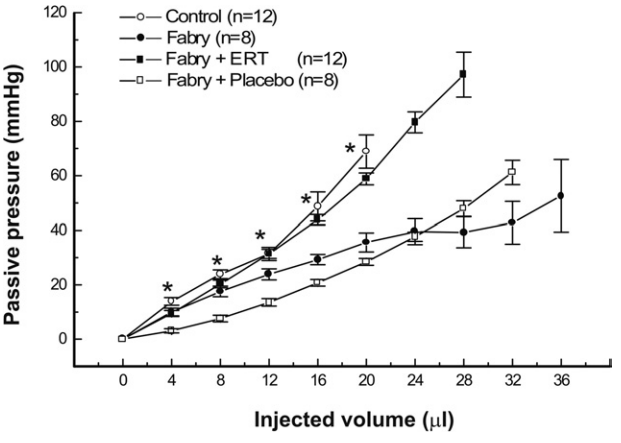

Figure 2. Passive pressure-volume curves in arrested hearts of the four experimental groups. The hearts of the untreated Fabry mice and those treated with placebo are significantly more compliant than the control hearts. After enzymatic replacement therapy, the Fabry hearts behave similarly to the normal control animals. $*P < 0.05$ with respect to Fabry hearts.

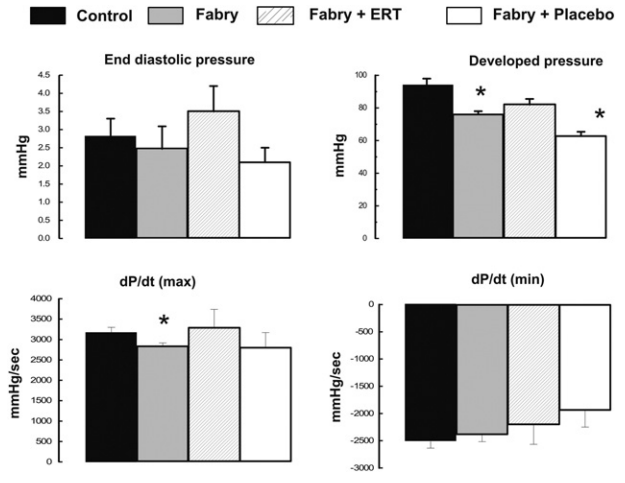

Figure 4. Average results of all the experiments similar to those detailed in Figure 3. The developed pressure and the positive $\Delta P/\Delta t$ were significantly depressed in the Fabry mice, and this was reversed by enzymatic replacement therapy. The changes in negative $\Delta P/\Delta t$ and end-diastolic pressure did not reach statistical significance. $*P < 0.05$ with respect to control hearts.

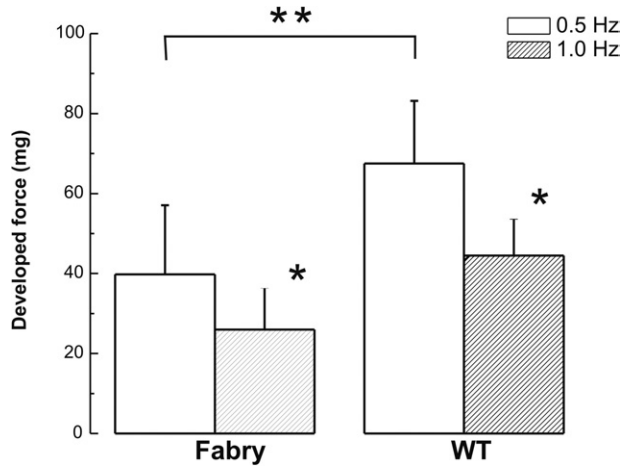

Figure 5. Force developed by papillary muscles, showing a depressed contractility in Fabry mice with respect to their controls. In both cases, increasing the pacing frequency elicited a similar depression of the contraction (negative staircase phenomenon). $*P < 0.05$ with respect to pacing at 0.5 Hz. $**P < 0.05$ between Fabry and WT hearts.

staining (Fig. 6, top) shows increased accumulation of lipids in the FM (*2*) compared to the WT (*1*). Picrosirius red staining for collagen demonstration is shown at the bottom, in WT (*3*) and FM (*4*). Digital analysis of the images revealed that the percentage occupied by collagen was 0.65% ± 0.32% in 4 WT and 0.71% ± 0.21% in 4 FM (NS, Student's *t*-test).

Figure 7 (left) shows one of the echocardiographic studies performed in the mice (5 FM and 6 WT). Figure 7 (right) shows that the fractional shortening of the LV was significantly reduced in FM with respect to the WT controls. In addition, end-diastolic volume was greater in the FM than in the WT (0.36 ± 0.05 ml vs 0.24 ± 0.01 ml, $P < 0.05$). Thicknesses of the interventricular septum and LV posterior wall did not show significant differences.

Discussion

Abnormalities of diastolic and systolic function are characteristics of Fabry cardiomyopathy and can usually be detected before the onset of LVH. In our work, we aimed to study myocardial function in a Fabry disease murine model through the use of a direct measurement of morphometric and hemodynamic parameters, to evaluate and compare the abnormalities observed in human patients with the those in the mild Fabry disease murine model. The main findings of our study are the functional and morphologic alterations in the myocardium of FM and the possibility of their reversion by ERT.

Physical size of the WT was ~10% greater than that of the FM, and this was reflected in higher values for the RV, the LV, and tibial length. Even when the RVW and LVW were normalized by body weight or tibial length, the difference between WT and FM persisted, ruling out the presence of cardiac hypertrophy in the FM, as was described by others.[13]

In our murine model, the passive studies in which we infused PSS in arrested hearts at a constant rate revealed that the hearts of FM were more compliant than those of WT (ie, they accommodated more volume for any given pressure). This alteration is similar to the usual findings in myocardial infarction and ventricular dilation,[14-16] and the opposite of findings in hypertrophy due to chronic pressure overload, where the compliance is decreased.[17] It seems more probable that the augmented compliance is associated with the myocardial infiltration detected in the FM hearts, since its regression paralleled the structural changes induced by the ERT.

LV performance as evaluated by LVP and $\Delta P/\Delta t$ (max) demonstrated that myocardial contractility was slightly but significantly depressed in FM, although there were no differences in end-diastolic pressure and $\Delta P/\Delta t$ (min). In comparison with the only reference that we located in the literature about similar measurements performed in FM,[18] we found that our figures for LVP were similar (although they did not find significant differences in LVP between FM and WT), but our values for $\Delta P/\Delta t$ (max) were lower than those in the mentioned report. This difference could be explained by the different instrumentation used (ie, direct measurement with the associated high impedance of the fluid-filled catheter in our study, and a miniaturized pressure-volume transducer in Yoshimitsu et al, which implies practically a null impedance). In our study, we detected a decreased contractility in the FM, which resulted in diminished LV pressure and $\Delta P/\Delta t$ in the intact hearts. Moreover, we confirmed the decrease in contractility by using the ratio dP/dt (max)/LVP, which is a much more sensitive index of contractility.[12] The decreased myocardial contractility that we found could not be attributed to systemic influences

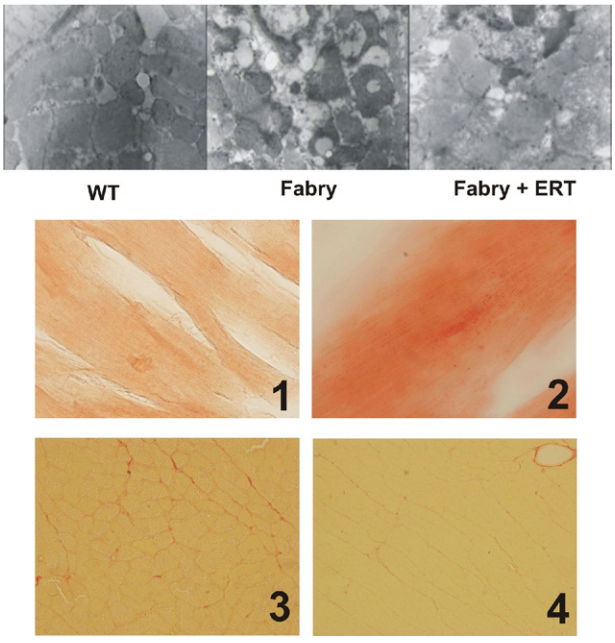

Figure 6. (**Top**) Three electron microscope images of left ventricular myocardium showing (left to right) the wild-type mice (WT), the changes induced by the disease in the Fabry mice (FM), and the tendency toward reversion of these changes by enzymatic replacement therapy. (**Bottom**) Optical microscope images of left ventricular myocardium. (**1**) Sudan red staining in WT; (**2**) same staining in FM showing intracellular lipid accumulation; (**3** and **4**) picrosirius staining in WT and FM, respectively, showing no changes in the amount of collagen tissue.

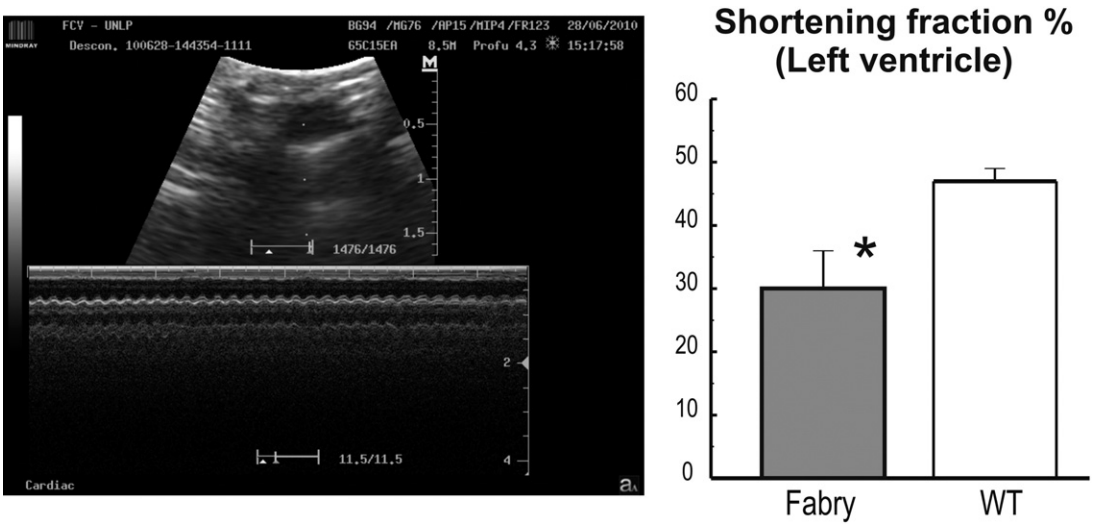

Figure 7. (**Left**) One of the echocardiographic studies performed in the mice (5 Fabry mice [FM] and 6 wild-type mice [WT]). (**Right**) Bar graph showing that the fractional shortening was significantly reduced in FM with respect to the WT controls. $*P < 0.05$ with respect to WT hearts.

that could have been present in the intact preparations, since it was also present in isolated papillary muscles. In addition, the negative staircase phenomenon detected after doubling the pacing of these isolated preparations was similar in FM and WT. Since this phenomenon is related to the calcium handling by the sarcoplasmic reticulum.[19] we can assume that this part of the excitation-contraction coupling was unaffected by the disease.

This dissociation between functional damage and fibrosis is not novel in cardiac physiology and has been demonstrated previously in other pathologic settings. For example, it has been demonstrated that cardiac remodelling in spontaneously hypertensive rats can be ameliorated by inhibition of the Na-H exchanger despite the persistence of an elevated blood pressure.[20,21]

Regarding the possible mechanism responsible for this altered myocardial functionality, a recent report indicates that matrix metalloproteinase (MMP) activity is augmented in patients with Fabry disease, leading eventually to a diminished contractility and heart failure.[22] ACE inhibitors, which constitute a frontline treatment for heart failure, prevent remodelling by inhibiting MMPs.[23] It would be interesting to explore this relationship in the FM model and to see if it is linked to the myocardial alterations detected.

Tissue Doppler imaging can provide early detection of cardiac involvement in Fabry disease, before onset of LVH and even in Fabry females, and represents a noninvasive tool for the diagnosis of myocardial dysfunction and for the assessment of cardiac improvement during ERT.[7] Fabry cardiomyopathy is characterized by reduced myocardial contraction and relaxation velocities, paralleled by an increase in LV filling pressure, detectable even before development of LVH.[7] Tissue Doppler imaging examination in patients with Fabry disease has shown a reduction of LV filling pressure after ERT, documenting an improvement of diastolic function during ERT.[24]

Fractional shortening studies demonstrate that LV systolic function progressively deteriorates in untreated patients with Fabry disease.[25] Weidemann and colleagues have shown that tissue Doppler–derived strain rate improves during treatment with ERT, suggesting that contractile function is reversibly impaired in Fabry disease cardiomyopathy.[26] Coincident with these reports, the echocardiographic studies that we performed in FM and WT demonstrated a significant reduction of the ejection fraction in the FM, an index that is widely used as an indicative of LV contractility.[27-30]

Recently, hearts from old patients with Fabry disease on ERT were analyzed by histologic examination. The myocardium of the right and left ventricles showed prominent myocyte hypertrophy with severe vacuolization and myocyte disarray with replacement fibrosis around the myocytes.[31] Lamellar bodies, the hallmark of Fabry disease in skin or kidney tissues, were not observed in myocardium from human patients.[32] In our study, hearts from Fabry mice showed similar myocardial changes, with vacuolization and myocyte disarray and absence of lamellar bodies. Fibrosis was not observed in hearts from Fabry mice, probably because of the mild nature of this murine model.[33] Myocyte disarray may contribute to systolic and diastolic dysfunction.

Alterations observed in human patients on ERT suggest that this procedure had been either ineffective or barely capable of slowing down the progression of the disease, probably because ERT was initiated after heart involvement was already well advanced.[31,34] However, in the murine model we could observe a regression of alterations after exposure to ERT. It could imply a benefit of early treatment, before the onset of irreversible damage.

The main conclusions of this study are that (1) the phenotype observed in hearts from FM resembles the cardiac phenotype of patients with Fabry disease at an early stage of the disease. This model could be of help in assessing pathophysiologic mechanisms leading to heart disease and analyzing the effect of therapeutic interventions like ERT. (2) Passive and active myocardial alterations are dissociated from fibrosis (ie, they are present before a significant remodelling can be detected).

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Disclosures

The authors have no conflicts of interest to disclose.

References

1. 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.
2. 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.
3. Monserrat L, Gimeno-Blanes JR, Marin F, et al. Prevalence of Fabry disease in a cohort of 508 unrelated patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 2007;50:2399-403.
4. Desnick RJ, Blieden LC, Sharp HL, et al. Cardiac valvular anomalies in Fabry disease. Clinical, morphologic, and biochemical studies. Circulation 1976;54:818-25.
5. Linhart A, Palecek T, Bultas J, et al. New insights in cardiac structural changes in patients with Fabry's disease. Am Heart J 2000;139:1101-8.
6. Barbey F, Brakch N, Linhart A, et al. Cardiac and vascular hypertrophy in Fabry disease. Evidence for a new mechanism independent of blood pressure and glycosphingolipid deposition. Arterioscler Thromb Vasc Biol 2006;26:839-44.
7. Pieroni M, Chimenti C, Russo A, et al. Tissue Doppler imaging in Fabry disease. Curr Opin Cardiol 2004;19:452-7.
8. 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. Virch Arch A Pathol Anat Histopathol 1990;417:449-55.
9. Chimenti C, Hamdani N, Boontje N, et al. Myofilament degradation and dysfunction of human cardiomyocytes in Fabry disease. Am J Pathol 2008;172:1482-90.
10. Ohshima T, Murray GJ, Swaim WD, et al. Alpha-galactosidase A deficient mice: a model of Fabry disease. Proc Natl Acad Sci USA 1997;94: 2540-4.
11. Gelderman MP, Oliver KL, Yazdani AT, et al. Preclinical studies with plant-produced α-galactosidase A in Fabry mice show potential for replacement therapy. Preclinica 2004;2:67-74.
12. Perez NG, Piaggio MR, Ennis IL, et al. Phosphodiesterase 5A inhibition induces Na^+/H^+ exchanger blockade and protection against myocardial infarction. Hypertension 2007;49:1095-103.
13. Takahashi H, Hirai Y, Migita M, et al. Long-term systemic therapy of Fabry disease in a knockout mouse by adeno-associated virus-mediated muscle-directed gene transfer. Proc Natl Acad Sci USA 2002;99:13777-82.
14. Nahrendorf M, Hiller K-H, Hu K, et al. Cardiac magnetic resonance in small animal models of human heart failure. Med Image Analysis 2003;7: 369-75.
15. Fletcher PJ, Pfeffer JM, Pfeffer MA, et al. Left ventricular diastolic pressure-volume relations in rats with healed myocardial infarction: effects on systolic function. Circ Res 1981;49:618-26.
16. Patten R, Aronovitz MJ, Deras-Mejia L, et al. Ventricular remodeling in a mouse model of myocardial infarction. Am J Physiol 1998;274:H1812-20.
17. Braunwald E. Heart Disease: A Textbook of Cardiovascular Medicine. Philadelphia/London/Toronto: WB Saunders, 1981:1446.
18. Yoshimitsu M, Higuchi K, Daywood F, et al. Correction of cardiac abnormalities in Fabry mice by direct intraventricular injection of a recombinant lentiviral vector that engineers expression of α-galactosidase A. Circ J 2006;70:1503-8.
19. Bers DM. Excitation-Contraction Coupling and Cardiac Contractile Force. 2nd ed. Dordrecht: Kluwer Academic, 2001:Ch 9.
20. Camilion de Hurtado MC, Portiansky EL, Perez NG, et al. Regression of cardiomyocyte hypertrophy in SHR following chronic inhibition of the Na /H exchanger. Cardiovasc Res 2002;53:862-8.
21. Cingolani HE, Rebolledo OR, Portiansky EL, et al. Regression of hypertensive myocardial fibrosis by Na^+/H^+ exchange inhibition. Hypertension 2003;41:373-77.
22. Shah JS, Hughes DA, Tayebjee MH, et al. Extracellular matrix turnover and disease severity in Anderson-Fabry disease. J Inherit Metab Dis 2007; 30:88-95.
23. Brower GL, Levick SP, Janicki JS. Inhibition of matrix metalloproteinase activity by ACE inhibitors prevents left ventricular remodeling in a rat model of heart failure. Am J Physiol 2007;H3057-64.
24. Kovacevic-Preradovic T, Zuber M, Attenhofer Jost CH, et al. Anderson-Fabry disease: long-term echocardiographic follow-up under enzyme replacement therapy. Eur J Echocardiogr 2008;9:729-35.
25. Shah JS, Lee P, Hughes D, et al. The natural history of left ventricular systolic function in Anderson-Fabry disease. Heart 2005;91:533-4.
26. Weidemann F, Breunig F, Beer M, et al. Improvement of cardiac function during enzyme replacement therapy in patients with Fabry disease: a prospective strain rate imaging study. Circulation 2003;108:1299-301.
27. Rottman JN, Gemin NI, Brown M. Echocardiographic evaluation of ventricular function in mice. Echocardiography 2007;24:83-9.
28. Scherrer-Crosbie M, Kurtz B. Ventricular remodeling and function: Insights using murine echocardiography. J Mol Cell Cardiol 2010;48:512-17.
29. Scherrer-Crosbie M, Thibault HB. Echocardiography in translational research: of mice and men. J Am Soc Echocardiogr 2008;21:1083-92.
30. Reddy AK, Amador-Noguez D, Darlington GJ, et al. Cardiac function in young and old little mice. J Gerontol A Biol Sci Med Sci 2007;62:1319-25.
31. Sheppard MN, Cane P, Florio R, et al. A detailed pathologic examination of heart tissue from three older patients with Anderson-Fabry disease on enzyme replacement therapy. Cardiovasc Pathol 2010;19:293-301.
32. Owens CL, Russell SD, Halushka MK. Histologic and electron microscopy findings in myocardium of treated Fabry disease. Hum Pathol 2006; 37:764-68.
33. Ohshima T, Murray GJ, Swaim WD, et al. Alpha-galactosidase A deficient mice: a model of Fabry disease. Proc Natl Acad Sci USA 1997;94:2540-4.
34. Thurberg BL, Fallon JT, Mitchell R, et al. Cardiac microvascular pathology in Fabry disease: evaluation of endomyocardial biopsies before and after enzyme replacement therapy. Circulation 2009;119:2561-7.