Review

Pharmacological chaperone therapy for Fabry disease

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Abstract: Fabry disease is an inherited lysosomal storage disorder caused by deficient α-galactosidase A activity. Many missense mutations in Fabry disease often cause misfolded gene products, which leads to their retention in the endoplasmic reticulum by the quality control system; they are then removed by endoplasmic reticulum-associated degradation. We discovered that a potent α-galactosidase A inhibitor, 1-deoxygalactonojirimycin, acts as a pharmacological chaperone to facilitate the proper folding of the mutant enzyme by binding to its active site, thereby improving its stability and trafficking to the lysosomes in mammalian cells. The oral administration of 1-deoxygalactonojirimycin to transgenic mice expressing human mutant α-galactosidase A resulted in significant increases in α-galactosidase A activity in various organs, with concomitant reductions in globotriaosylceramide, which contributes to the pathology of Fabry disease. Seventy-eight missense mutations were found to be responsive to 1-deoxygalactonojirimycin. These data indicate that many patients with Fabry disease could potentially benefit from pharmacological chaperone therapy.

Keywords: pharmacological chaperone, Fabry disease, α-galactosidase A, therapy

Introduction

Fabry disease is an X-linked lysosomal storage disorder caused by a deficiency in the activity of α-galactosidase A (α-Gal A, EC 3.2.1.22), an enzyme responsible for the catabolism of glycosphingolipids, predominantly globotriaosylceramide (Gb3).[1] The progressive accumulation of Gb3 in lysosomal and nonlysosomal compartments of cells in the skin, heart, kidney, brain, and other tissues contributes to the disease pathology.[2),3] The major clinical manifestations in patients with classic Fabry disease, that is, with no detectable α-Gal A activity, include pain in the distal extremities and acroparesthesia, angiokeratoma, hypohidrosis, corneal opacity in childhood, and progressive vasculopathy of the heart, kidney, and central nervous system.[4] In contrast, patients with mild-form Fabry disease and residual α-Gal A activity are usually asymptomatic until their late thirties. Their clinical manifestations are often limited to the heart[5),6] and kidney.[7),8]

Enzyme replacement therapy for Fabry disease has been available since 2001,[9),10] and a variety of clinical benefits in patients have been reported relating to renal function,[11),12] cardiac manifestation,[13),14] and pain-related quality of life.[10),15] However, the treatment is expensive, with an annual cost of approximately $200,000 per patient.[16] Alternative therapeutic strategies are needed to reduce the cost of clinical management. In 1999, we began to develop a new therapeutic strategy, a pharmacological chaperone therapy for Fabry disease, using synthesized chemicals.[17] A pharmacological chaperone is a small molecule that can facilitate the proper folding of a mutant protein, thereby accelerating the protein's mature processing and transport to its final cellular destination. This article focuses on the concept of this

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Abbreviations: α-Gal A: α-galactosidase A; Gb3: globotriaosylceramide; DGJ: 1-deoxygalactonojirimycin; ER: endoplasmic reticulum; ERAD: ER-associated degradation; TgM mouse: transgenic mouse expressing R301Q mutant α-Gal A; KO mouse: α-Gal A knock-out mouse; TgM/KO mouse: transgenic mouse expressing R301Q mutant α-Gal A in a murine α-Gal A knock-out background; GlcCer: glucosylceramide; LacCer: lactosylceramide.

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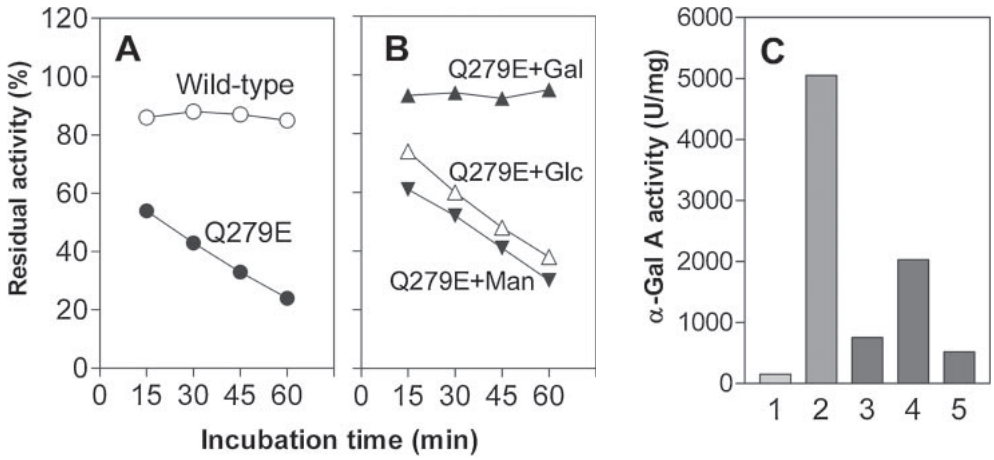

Fig. 1. Thermostability of a mutant enzyme and effect of galactose addition. In A, the thermostability of the purified wild-type and Q279E mutant proteins (10 μg/ml) was determined by incubation in 0.1 M HEPES-NaOH buffer (pH 7.0), containing 1 mg/ml bovine serum albumin, at 37°C for the times indicated. In B, the thermostability of the mutant protein was assayed in the presence of 200 mM galactose (Gal), glucose (Glc), or mannose (Man). In C, COS-1 cells expressing wild-type or mutant α-Gal A were cultured at 37°C in 5% CO_2 in Ham's F-10 medium supplemented with 10% FCS with or without 200 mM hexose. Column 1, mock transfection; column 2, wild-type α-Gal A; column 3, Q279E mutant α-Gal A; column 4, Q279E with galactose; column 5, Q279E with glucose.[21]

new therapeutic strategy and its potential for clinical application.

Characteristics of the mutant enzyme in patients with Fabry disease

More than 500 gene mutations have been identified in patients with Fabry disease (Human Gene Mutation Database, http://www.hgmd.cf.ac.uk) since the α-Gal A gene was cloned by Bishop *et al.*[18] in 1986. Large gene rearrangements, which are easily predicted to cause deficient activity, represent less than 10% of the mutations; instead, many of them are single base substitutions. In our early gene diagnosis studies,[19] we identified single amino acid substitutions in mild-form patients with Fabry disease, who have residual activity, and we proved that the mutant enzymes had low activity by transiently expressing them in mammalian cells. Although these studies confirmed that the gene mutations cause low α-Gal A activity in mammalian cells, we could not explain why given amino acid substitutions resulted in low activity.

To address this question, we expressed wild-type and Q279E mutant α-Gal A in insect cells with a baculovirus expression vector,[20] purified the recombinant proteins, and characterized their properties.[21),22] The kinetic properties of Q279E were the same as those of the wild-type enzyme; however, its thermostability was low, especially at neutral pH (Fig. 1A). We also tested the effect of substrate analogues on Q279E's stability, because enzyme substrates and their analogues are historically used as enzyme stabilizers *in vitro*, and we discovered that the mutant enzyme could be stabilized by adding galactose but not glucose or mannose (Fig. 1B). The activity of Q279E (750 U/mg protein) was lower than that of the wild-type enzyme (5050 U/mg protein) in transiently transfected COS-1 cells, and the activity of Q279E was markedly increased by the addition of 200 mM galactose (2030 U/mg protein) but not glucose (Fig. 1C). These data indicated that the low activity of the mutant α-Gal A might be caused by its low thermostability, and that the activity could be restored by adding an active-site-binding compound such as galactose.

Search for a potent pharmacological chaperone

Although galactose could function as a pharmacological chaperone, its working concentration was too high for clinical application, because the affinity of α-Gal A for galactose is low. Asano *et al.*[23)–25] reported that many glycosidases have a high affinity for iminosugars, and that iminosugars act as competitive inhibitors. The inhibitors used as pharmacological chaperones must be competitive inhibitors, because they need to dissociate from the enzyme when it reaches its final destination, such as the lysosome. We therefore searched for a potent pharmacological chaperone among iminosugars that strongly inhibited human α-Gal A. Twelve potent

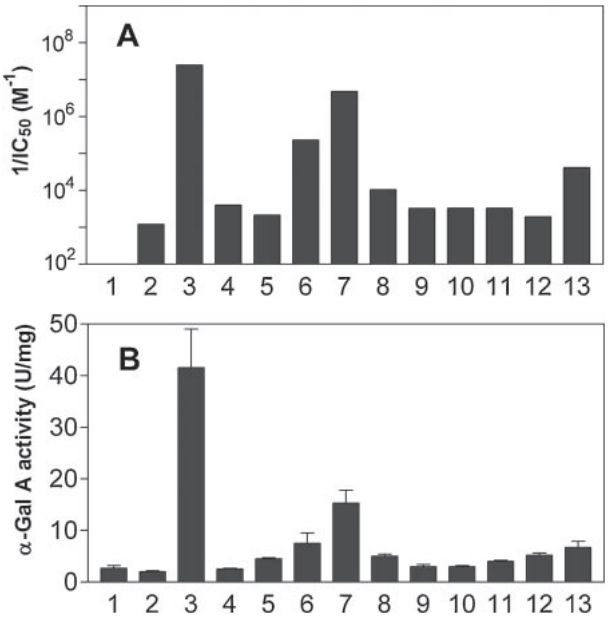

Fig. 2. *In vitro* inhibition (A) and intracellular enhancement (B) of α-Gal A by inhibitors. In A, the concentrations for 50% inhibition of α-Gal A (IC50) were determined with purified human α-Gal A. In B, a patient's lymphoblasts with the R301Q mutation were cultured in culture medium containing each inhibitor (100 µM) for 4 days. The intracellular α-Gal A activity was determined with 4MU-α-galactoside as a substrate. For both A and B: 1, no addition; 2, 1-deoxynojirimycin; 3, 1-deoxygalactonojirimycin; 4, 1,2-dideoxygalactonojirimycin; 5, α-homomannojirimycin; 6, α-homoallonojirimycin; 7, α-homogalactonojirimycin; 8, N-methyl-1-deoxygalactonojirimycin; 9, N-ethyl-1-deoxygalactonojirimycin; 10, N-propyl-1-deoxygalactonojirimycin; 11, N-butyl-1-deoxygalactonojirimycin; 12, N-hydroxyethyl-1-deoxygalactonojirimycin; 13, β-1-C-butyl-1-deoxygalactonojirimycin.[26)]

inhibitors were examined for their *in vitro* inhibitory activity and chaperone effect in patients' cells (Fig. 2). Of the iminosugars tested, 1-deoxygalactonojirimycin (DGJ) showed the strongest effect as a pharmacological chaperone.[26)]

Pharmacological chaperone assists proper folding

The proper folding of newly synthesized lysosomal proteins as well as membrane and secretory proteins is prerequisite for their export from the endoplasmic reticulum (ER). Misfolded proteins, which are folded incorrectly, are retained in the ER and ultimately degraded by ER-associated degradation (ERAD).[27)–29)] Protein misfolding has been recognized as an important pathological cause in many inherited diseases, including cystic fibrosis, α1-antitrypsin deficiency, familial hypercholesterolemia, and Alzheimer's disease.[30)–32)] The three-dimensional structure of human α-Gal A is altered by single amino acid substitutions in Fabry disease.[33),34)]

To distinguish properly folded from misfolded α-Gal A, we used trypsin treatment. The active form of α-Gal A was resistant to trypsin treatment, while an inactive form created by heating and denaturing the active protein, was completely digested to short peptides by this treatment (Fig. 3A). Cell lines (TNK or TMK2 cells) established from transgenic mice expressing human wild-type or R301Q mutant α-Gal A, respectively,[35)] were pulse-labeled with an [^{35}S]Protein labeling mix for 30 min. Cell lysate from the labeled cells was treated with trypsin, and both trypsin-treated and intact samples were immunoprecipitated with an anti-α-Gal A antibody. The newly synthesized wild-type α-Gal A was obtained as a main 50-kD band, a large amount of which remained after trypsin digestion (Fig. 3B). In contrast, the newly synthesized R301Q protein appeared as a main 50-kD band with other minor bands, and only a small amount of the 50-kD band remained after trypsin treatment. These data indicated that the R301Q mutation may cause a high frequency of misfolding, resulting in the enzyme's rapid degradation and low residual activity in mammalian cells. We then examined the folding of newly synthesized R301Q mutant in the presence of DGJ. The trypsin-resistant 50-kD band of R301Q was markedly increased by DGJ treatment, indicating that DGJ does not just stabilize the mutant α-Gal A, but rather facilitates its proper folding in the ER.

Intracellular processing of mutant α-Gal A

Wild-type α-Gal A is synthesized as a 50-kD precursor protein, and processed to a 46-kD mature protein in human fibroblasts.[36)] Similar intracellular maturation was observed in COS-7 cells expressing wild-type human α-Gal A (Fig. 4A), from which the 50-kD precursor form and 46-kD mature form were recovered in the microsomal fraction and lysosomal fraction, respectively, after Percoll density gradient centrifugation.[37)] In contrast to the wild-type enzyme, the R301Q mutant α-Gal A was degraded in these cells without undergoing maturation. Since only properly folded proteins can be transported out of the ER to the Golgi apparatus by the ER quality control system,[27)] misfolded and incompletely assembled proteins are retained in the ER and eventually degraded by the ERAD. We observed that R301Q bound to DGJ was processed from a 50-kD band to a 46-kD band, presumably owing to the increase in properly folded protein. Furthermore,

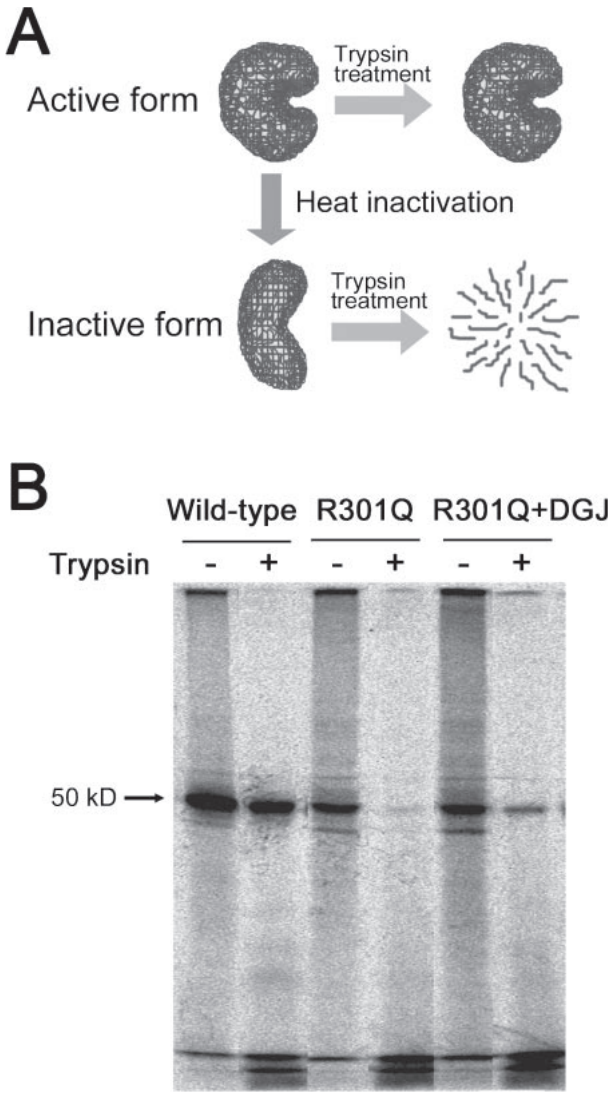

Fig. 3. Identification of active and inactive forms of α-Gal A. A shows a schematic representation of the method for identifying the active form of α-Gal A by trypsin treatment. Purified α-Gal A (1 µg/ml) was incubated with 100 µg/ml trypsin for 10 min at 37°C. Heat-inactivated α-Gal A (95°C, for 5 min) is completely digested by this treatment, while the active form of α-Gal A does not change. In B, TNK or TMK2 cells established from transgenic mice expressing human wild-type or R301Q mutant α-Gal A, respectively, with and without DGJ treatment were labeled with an [^{35}S]Protein labeling mix for 30 min. Cell lysates extracted from the labeled cells were incubated with or without 100 µg/ml trypsin for 10 min at 37°C, and immunoprecipitated with an anti-α-Gal A antibody. An aliquot was analyzed by SDS-polyacrylamide gel electrophoresis and visualized by fluorography (Hamanaka, R. *et al.*, unpublished data).

immunoelectron microscopic examination clearly revealed that R301Q without DGJ was retained in the ER and was not present in lysosomes (Fig. 4B and 4C), but in the presence of DGJ, R301Q was transported out of the ER and reached the lysosomes (Fig. 4D and 4E).[38]

Concept of pharmacological chaperone therapy

Human α-Gal A consists of 429 amino acids, and after removal of the 31-residue signal sequence, its 50-kD precursor form is synthesized in the lumen of the ER.[33),39)] For the wild-type enzyme, most newly synthesized protein is properly folded and transported out of the ER. In contrast, many mutant proteins with a single amino acid substitution, which comprise more than half the mutations detected in patients with Fabry disease, show misfolding characterized by low thermostability at neutral pH,[37)] as well as abnormal binding with BiP[40)] and rapid degradation by the ERAD.[37),38)] The concept of pharmacological chaperone therapy is based on the observation that certain small molecules that bind to the active site of α-Gal A can facilitate the proper folding of its mutant forms (Fig. 5).[41)]

Indeed, separate studies have shown that enzyme activity in DGJ-treated, isolated lymphocytes from Fabry patients is markedly increased, because of the increase in properly folded protein,[17)] that the mutant enzyme in DGJ-treated cells reaches the lysosomes,[38)] and that the mutant enzyme in DGJ-treated cells degrades accumulated Gb3.[42)] Although treatment with DGJ at effective concentrations (lower than 100 µM) can increase mutant α-Gal A activity,[26)] treatment with excessive concentrations (higher than 1 mM) significantly inhibits it, resulting in increased Gb3 accumulation in mammalian cells.[43)] Thus, for effective and safe treatment using this pharmacological chaperone, the appropriate dose of DGJ needed to be determined in animal models.

Mouse model for pharmacological chaperone therapy

For the preclinical study of the efficacy and safety of DGJ, we prepared a transgenic mouse (TgM mouse) expressing the R301Q mutant α-Gal A.[44)] Although a mouse model for Fabry disease was previously established by disrupting the murine α-Gal A gene (KO mouse),[45)] this mouse model is not suitable for the study of pharmacological chaperone therapy, which requires the expression of a human mutant enzyme. To exclude the mouse endogenous α-Gal A activity, the TgM mouse was crossed with the KO mouse, to generate a transgenic mouse expressing the R301Q mutant α-Gal A in a murine α-Gal A knock-out background (TgM/KO mouse).[35)]

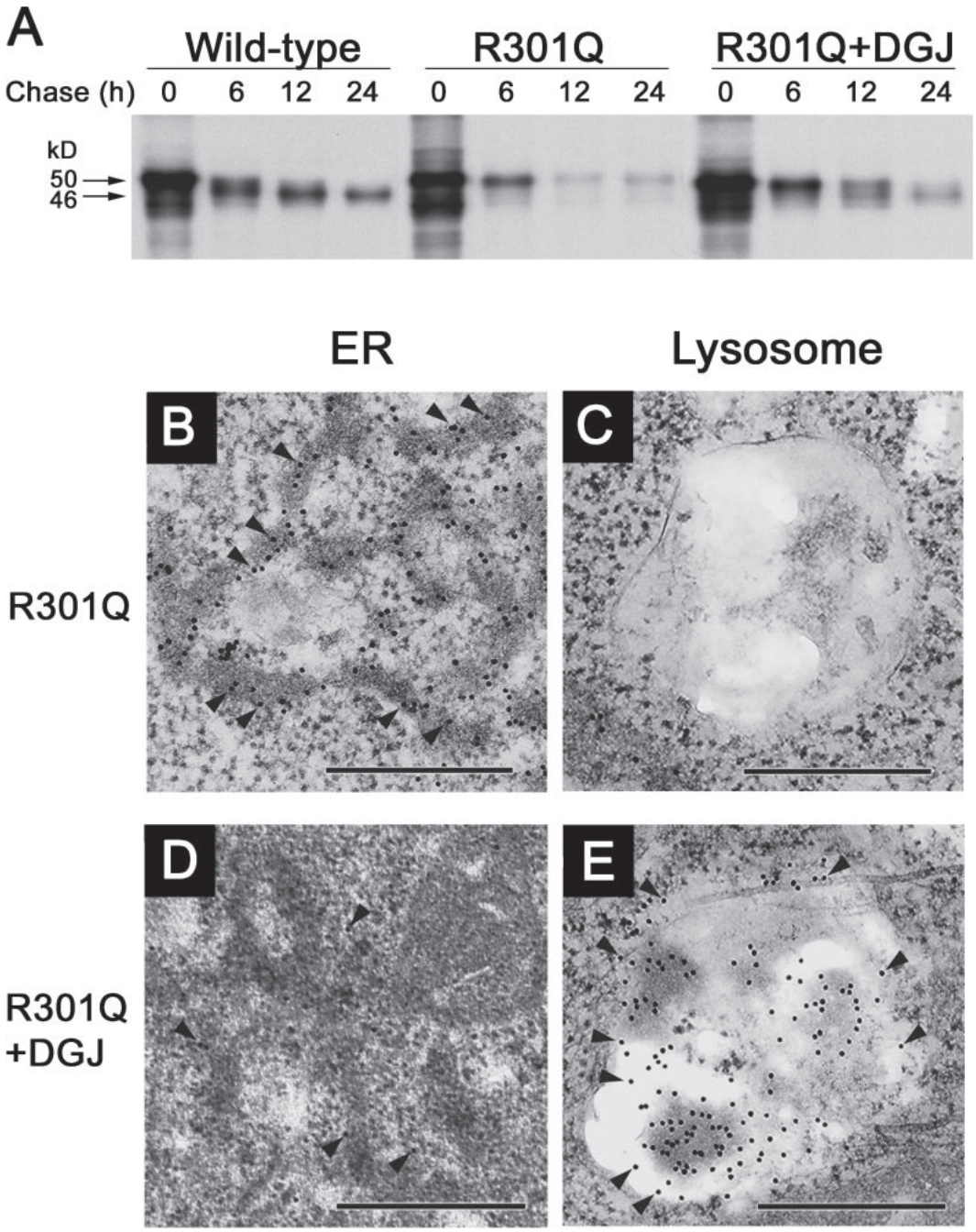

Fig. 4. Intracellular maturation and transport of R301Q mutant α-Gal A in the presence of DGJ. In A, COS-7 cells expressing wild-type or R301Q mutant α-Gal A were cultured in the absence or presence of DGJ, and pulse labeled with an [^{35}S]Protein labeling mix. After replacing the culture medium, the labeled proteins were chased for the indicated period. Following immunoprecipitation with an anti-α-Gal A antibody, an aliquot was analyzed by SDS-polyacrylamide gel electrophoresis and visualized by fluorography.[37] In B–E, the intracellular localization of R301Q in COS-7 cells in the absence (B and C) or presence (D and E) of DGJ was analyzed by immunoelectron microscopy. Ultrathin sections were incubated with an anti-α-Gal A antibody followed by immunogold labeling, and examined by transmission electron microscopy. Scale bars represent 500 nm, and typical gold particles are indicated by arrowheads.[38]

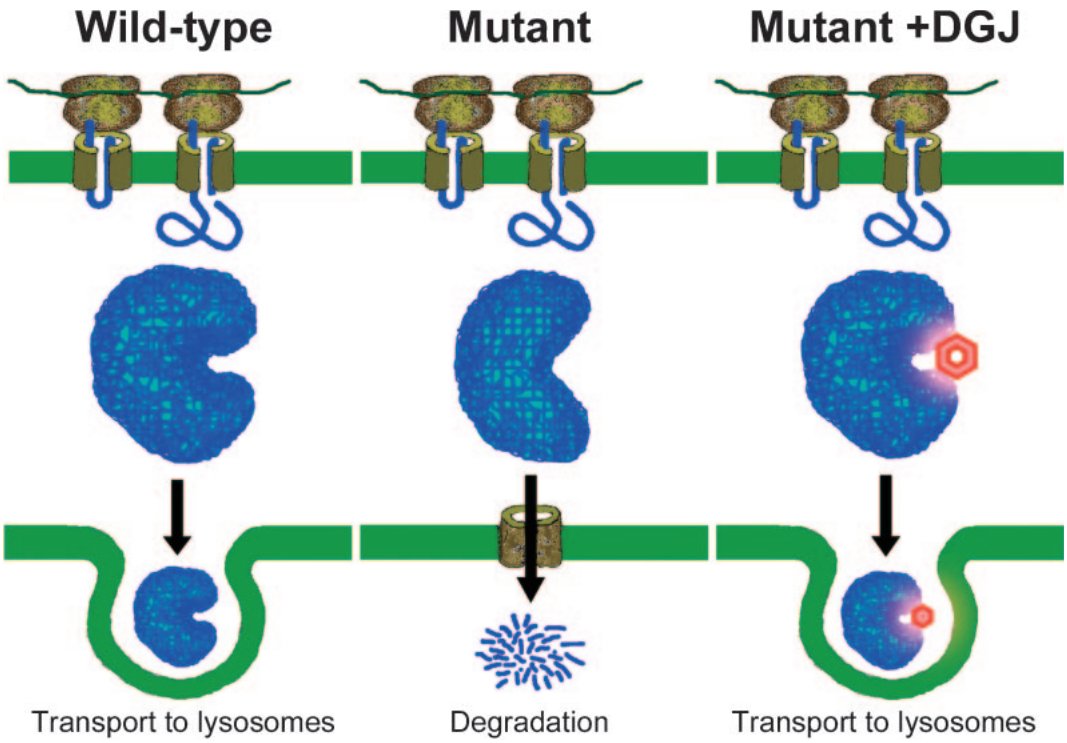

Fig. 5. Schematic representation of the effect of DGJ on mutant α-Gal A in the ER. After the signal peptide is clipped off during translation, the newly synthesized protein is immediately released into the lumen of the ER. A single amino acid substitution often causes misfolding, because the folding information of a protein chain is contained in its amino acid sequence. The misfolded mutant α-Gal A is retained in the ER by the quality control system, and degraded by the ERAD. In the presence of DGJ, proper folding of the mutant enzyme is facilitated by DGJ's binding to its active site. Appropriately folded α-Gal A molecules are transported out of the ER, and reach the lysosomes, their final destination.

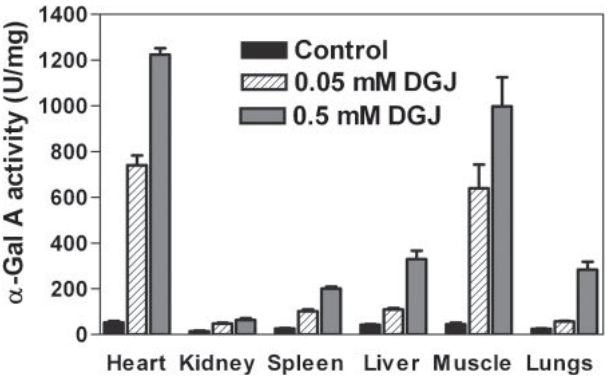

Fig. 6. DGJ administration increases the α-Gal A activity in TgM/KO mice. TgM/KO mice were given DGJ ad libitum in the drinking water for 2 weeks at the indicated doses. The α-Gal A activity was then assayed in lysates from the heart, kidney, spleen, liver, muscle, and lungs.[46] The level of α-Gal A activity in tissues from wild-type mice was 3–50 U/mg protein.

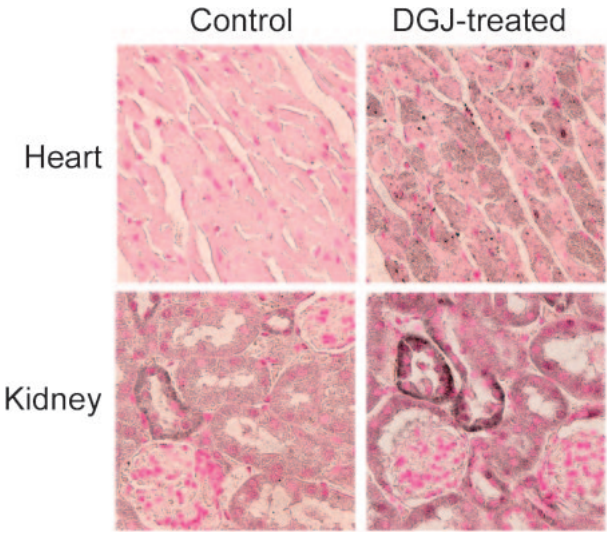

Fig. 7. Immunohistochemistry of the heart and kidney of TgM/KO mice treated with 0.5 mM DGJ for 2 weeks. Paraffin sections were stained with a polyclonal anti-α-Gal A antibody and goat anti-rabbit IgG gold (particle size, 5 nm). Sections were counter-stained with nuclear fast red.[46]

After DGJ was orally administered at different concentrations to TgM/KO mice, by adding it to their drinking water for 2 weeks (Fig. 6), a dose-dependent increase in α-Gal A activity was observed in all the major organs. At the highest DGJ dose (0.5 mM), α-Gal A activity in the TgM/KO mice after the 2-week treatment was increased 22.8-fold in the heart, 4.4-fold in the kidney, 7.8-fold in the spleen, 7.7-fold in the liver, 21.9-fold in the muscle, and 11.3-fold in the lungs. Because the heart and kidney are two major organs affected by Fabry disease, these organs from the DGJ-treated TgM/KO mouse were examined immunohistochemically (Fig. 7).[46] No immunoreactive mutant α-Gal A could be detected in the heart of control TgM/KO mice. However, granular immunostaining appeared throughout the cell matrix of cardiomyocytes of TgM/KO mice treated with DGJ. In the kidney, a marked increase in the intensity of α-Gal A staining was observed in the distal convoluted tubules, and a slight increase was seen in the proximal convoluted tubules. These data indicated that DGJ is easily delivered to the cardiomyocytes and distal convoluted tubules, where the decomposition of Gb3 is hard to achieve by enzyme replacement therapy.[47]

To elucidate how the increase in α-Gal A activity leads to a decrease in accumulated Gb3, the neutral glycosphingolipids were extracted from the kidneys of three mice treated with 0.05 mM DGJ for 4 weeks, and were subjected to TLC analysis (Fig. 8).[46] Following the DGJ treatment, a 46% reduction in the Gb3 content of the kidney was observed, but the amounts of GlcCer or LacCer did not change detectably. This result clearly indicated that the administration of DGJ at a dosage of approximately 3 mg/kg body weight/day reduces Gb3 storage in the kidney. Treatment with DGJ at higher concentrations may cause an inhibition of α-Gal A activity and an increase in the accumulation of Gb3; however, even animals given DGJ at 300 mg/kg body weight/day did not show any increase in Gb3 content.[48] Moreover, treatment with DGJ for 9 weeks at 30 mg/kg body weight/day, which is 10-fold higher than the effective dose, did not cause any abnormality in the mice, indicating that DGJ is well tolerated by these animals.[46]

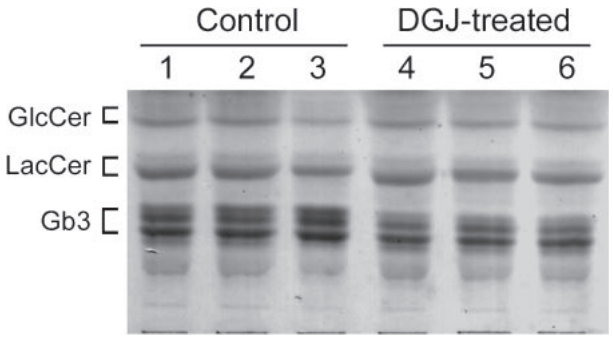

Fig. 8. Effect of DGJ administration on Gb3 storage in the kidney of TgM/KO mice. Heterozygous TgM/KO mice were treated with DGJ (0.05 mM) in their drinking water for 4 weeks. TLC analysis of the neutral glycosphingolipids extracted from the kidney of each mouse was performed using a solvent system of chloroform/methanol/water {60:35:8 (v/v/v)} and visualized by spraying with orcinol/sulfuric acid reagent. GlcCer, glucosylceramide; LacCer, lactosylceramide.[46]

Clinical application of pharmacological chaperone therapy

The first clinical trial of pharmacological chaperone therapy for Fabry disease was performed by Frustaci *et al.* using galactose.[49] Galactose (1 g per kg of body weight, every other day) was administered intravenously to a patient with a cardiac variant of Fabry disease, who had residual α-Gal A activity as the result of a missense mutation (G328R). He had severe myocardial disease and was a candidate for cardiac transplantation. After three months of treatment, marked improvements in cardiac function were observed, and the left ventricular-wall thickness was reduced from 16 mm to 14 mm. The patient returned to full-time work after 2 years of treatment.

DGJ can be given orally, because it has a 120,000-fold greater affinity for human α-Gal A than galactose. Currently, phase 3 clinical trials for DGJ (Amigal^{TM}) are being conducted with male and female Fabry disease patients (http://www.amicustherapeutics.com). Results from the phase 2 studies indicate that Amigal is safe and well tolerated. The treatment resulted in increased levels of α-Gal A in the white blood cells and kidney, and reduced levels of Gb3 in renal interstitial capillary cells, obtained from kidney biopsies, and in the urine.

Pharmacological chaperone therapy is not applicable to all patients with Fabry disease; it can only be effective in patients with misfolding mutations. Table 1 summarizes the studies in which the mutations responding to DGJ treatment were screened. Seventy-eight amino acid substitutions have been found to be responsive to DGJ treatment. These results indicate that pharmacological chaperone therapy could be of therapeutic benefit to many patients with Fabry disease.

Table 1. DGJ-responsive Mutations. Summary of mutations that showed a significant increase in α-Gal A activity or a reduction of lysosomal Gb3 storage upon treatment with DGJ, in the indicated reports. DGJ-responsive mutations were screened either by an expression study or by using fibroblasts or lymphoblasts established from patients with Fabry disease

Mutation	Determined by expression study	Assayed with patients' cells
A20P	Ishii *et al.*[37]	
N34S		Benjamin *et al.*[60]
P40S		Benjamin *et al.*[60]
T41I	Wu *et al.*[61]	Shin *et al.*,[62] Benjamin *et al.*[60]
M42V	Shimotori *et al.*,[63] Park *et al.*[64]	
R49C		Shin *et al.*[62]
R49L		Benjamin *et al.*[60]
M51I	Spada *et al.*,[65] Ferri *et al.*[66]	
M51K	Wu *et al.*[61]	Shin *et al.*,[62] Benjamin *et al.*[60]
E59K	Ishii *et al.*,[37] Wu *et al.*[61]	Ishii *et al.*[37]
E66G	Spada *et al.*[65]	
E66Q	Ishii *et al.*,[37] Wu *et al.*[61]	Ishii *et al.*,[37] Benjamin *et al.*[60]
M72V	Ishii *et al.*[37]	
A73V	Spada *et al.*[65]	
M76T	Shimotori *et al.*[63]	
I91T	Ishii *et al.*,[37] Park *et al.*,[64] Wu *et al.*[61]	Ishii *et al.*,[37] Benjamin *et al.*[60]
W95S		Benjamin *et al.*[60]
A97V	Ishii *et al.*,[37] Wu *et al.*[61]	Ishii *et al.*,[37] Shin *et al.*,[67] Shin *et al.*,[62] Benjamin *et al.*[60]
R100K		Benjamin *et al.*[60]
R112C	Park *et al.*,[64] Wu *et al.*[61]	Shin *et al.*,[67] Shin *et al.*,[62] Benjamin *et al.*[60]
R112H	Ishii *et al.*,[37] Shimotori *et al.*,[63] Wu *et al.*[61]	Ishii *et al.*,[37] Shin *et al.*,[67] Shin *et al.*,[62] Benjamin *et al.*[60]
F113I	Ishii *et al.*,[37] Spada *et al.*[65]	Ishii *et al.*[37]
F113L	Park *et al.*,[64] Wu *et al.*[61]	Benjamin *et al.*[60]
G128E	Wu *et al.*[61]	
A143T	Spada *et al.*,[65] Wu *et al.*[61]	Shin *et al.*,[67] Shin *et al.*,[62] Benjamin *et al.*[60]
G144V	Wu *et al.*[61]	Benjamin *et al.*[60]
S148N	Wu *et al.*[61]	Benjamin *et al.*[60]
A156V	Ishii *et al.*[37]	
L166V	Ishii *et al.*[37]	
D170V		Benjamin *et al.*[60]
C172Y		Benjamin *et al.*[60]
G183A	Filoni *et al.*[68]	Filoni *et al.*[68]
G183D	Wu *et al.*[61]	Benjamin *et al.*[60]
G183S	Wu *et al.*[61]	
T194I		Yam *et al.*[42]
S201F		Shin *et al.*[62]
P205R		Benjamin *et al.*[60]
P205T	Shimotori *et al.*,[63] Wu *et al.*[61]	Shin *et al.*,[62] Benjamin *et al.*[60]
Y207C		Benjamin *et al.*[60]
Y207S	Wu *et al.*[61]	Benjamin *et al.*[60]
N215S	Ishii *et al.*,[37] Spada *et al.*,[65] Wu *et al.*[61]	Ishii *et al.*,[37] Shin *et al.*,[62] Benjamin *et al.*[60]
Y216C	Filoni *et al.*[68]	Filoni *et al.*[68]
H225R	Wu *et al.*[61]	
S235C		Benjamin *et al.*[60]
S235F	Shimotori *et al.*[63]	
D244N	Wu *et al.*[61]	Benjamin *et al.*[60]

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Mutation	Determined by expression study	Assayed with patients' cells
G258V	Shimotori *et al.*[63]	
P259R	Wu *et al.*[61]	Shin *et al.*[62]
G260A	Shimotori *et al.*[63]	
N263S	Wu *et al.*[61]	Benjamin *et al.*[60]
S276G	Wu *et al.*[61]	Shin *et al.*,[62] Benjamin *et al.*[60]
Q279E	Ishii *et al.*,[37] Wu *et al.*[61]	Fan *et al.*,[17] Ishii *et al.*,[37] Benjamin *et al.*[60]
T282A	Shimotori *et al.*[63]	
W287C	Wu *et al.*[61]	Benjamin *et al.*[60]
A288P	Wu *et al.*[61]	Benjamin *et al.*[60]
I289F	Wu *et al.*[61]	Benjamin *et al.*[60]
M290L	Ferri *et al.*[66]	
F295C	Wu *et al.*[61]	Shin *et al.*[62]
M296I	Ishii *et al.*,[37] Wu *et al.*[61]	Ishii *et al.*,[37] Benjamin *et al.*[60]
M296V	Ishii *et al.*,[37] Wu *et al.*[61]	Ishii *et al.*,[37] Benjamin *et al.*[60]
L300P	Wu *et al.*[61]	Shin *et al.*,[67] Shin *et al.*,[62] Benjamin *et al.*[60]
R301Q	Ishii *et al.*,[37] Wu *et al.*[61]	Fan *et al.*,[17] Ishii *et al.*,[37] Shin *et al.*,[67] Shin *et al.*,[62] Benjamin *et al.*[60]
K308N	Shimotori *et al.*[63]	
Q312R	Shimotori *et al.*[63]	
N320Y	Wu *et al.*[61]	Benjamin *et al.*[60]
G325D	Wu *et al.*[61]	Benjamin *et al.*[60]
G328A	Wu *et al.*[61]	Shin *et al.*,[62] Benjamin *et al.*[60]
R342Q		Benjamin *et al.*[60]
R356W	Wu *et al.*[61]	Benjamin *et al.*[60]
E358A	Wu *et al.*[61]	Benjamin *et al.*[60]
E358K		Benjamin *et al.*[60]
R363C	Wu *et al.*[61]	Benjamin *et al.*[60]
R363H	Wu *et al.*[61]	Benjamin *et al.*[60]
G373D	Ishii *et al.*[37]	
G373S	Ishii *et al.*[37]	
V390fsX8		Yam *et al.*[42]
L403S	Shimotori *et al.*[63]	
P409A	Wu *et al.*[61]	Benjamin *et al.*[60]

Porto *et al.*[50] have recently reported that the incorporation of recombinant α-Gal A into fibroblasts from a patient with Fabry disease was markedly increased by the addition of DGJ. In the preclinical studies by Amicus Therapeutics, they announced that co-administration of the chaperone with enzyme replacement therapy resulted in prolonged half-life of recombinant enzyme in the circulation, increased enzyme activity in cells and greater Gb3 reduction in tissues compared to that seen with enzyme replacement therapy alone. Currently, phase 2 clinical trials of the combination therapy of pharmacological chaperone and enzyme replacement therapies are being conducted for Fabry disease (http://www.amicustherapeutics.com).

Conclusion

Pharmacological chaperone strategy is unique, because it involves using a competitive inhibitor to increase the intracellular activity of a mutant enzyme. The idea of using this technology to treat Fabry disease was inspired by a study on the mechanism of mutant enzyme loss.[17),21] The inhibitor facilitates the proper folding of mutant enzymes in the ER, resulting in maturation of the protein and its transport to lysosomes. Evidence suggests that this therapeutic strategy will be applicable to other lysosomal storage disorders[51)–55)] and other conformational diseases.[56)–59)]

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Profile

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