Chemical chaperones improve transport and enhance stability of mutant α-glucosidases in glycogen storage disease type II

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Abstract

Glycogen storage disease type II (GSDII; Pompe disease or acid maltase deficiency) is an autosomal recessive disorder caused by lysosomal acid α-glucosidase (AβGlu) deficiency and manifests predominantly as skeletal muscle weakness. Defects in post-translational modification and transport of mutant AβGlu species are frequently encountered and may potentially be corrected with chaperone-mediated therapy. In the present study, we have tested this hypothesis by using deoxynojirimycin and derivatives as chemical chaperones to correct the AβGlu deficiency in cultured fibroblasts from patients with GSDII. Four mutant phenotypes were chosen: Y455F/Y455F, P545L/P545L, S25del/R600C and D645E/R854X. In case of Y455F/Y455F and P545L/P545L, N-(α-1-deoxynojirimycin (NB-DNJ) restored the transport, maturation and activity of AβGlu in a dose dependent manner, while it had no effect on the reference enzyme β-hexosaminidase. NB-DNJ promoted export from the endoplasmic reticulum (ER) to the lysosomes and stabilized the activity of mutant AβGlu species, Y455F and P545L, inside the lysosomes. In long-term culture, the AβGlu activity in the fibroblasts from the patients with mutant phenotypes, Y455F/Y455F and P545L/P545L, increased up to 14.0- and 7.9-fold, respectively, in the presence of 10 μmol/L NB-DNJ. However, the effect of NB-DNJ on Y455F/Y455F subsided quickly after removal of the compound. We conclude that NB-DNJ acts in low concentration as chemical chaperone for certain mutant forms of AβGlu that are trapped in the ER, poorly transported or labile in the lysosomal environment. Chemical chaperone therapy could create new perspectives for therapeutic intervention in GSDII.

Keywords: Chemical chaperone; Glycogen storage disease type II; Pompe disease; Acid maltase deficiency; Acid α-glucosidase; Deoxynojirimycin derivatives

Introduction

Glycogen storage disease type II (GSDII; Pompe disease or acid maltase deficiency; OMIM 232300) is an autosomal recessive disorder of glycogen metabolism resulting from a generalized deficiency of the lysosomal enzyme acid α-glucosidase (AβGlu; EC 3.2.1.20/3). The ensuing lysosomal accumulation of glycogen disturbs the intracellular architecture of many cell types but predominantly of skeletal muscle fibers and cardiomyocytes. There is a spectrum of disease severity. All patients suffer from progressive muscle weakness affecting their mobility and respiratory capacity. The most severely affected infants also have cardiomegaly and usually die before their first year of life due to cardio-respiratory insufficiency [1]. Numerous mutations have been identified in the AβGlu gene (GAA) [2]. Several missense mutations were described to lead to...
retention of the AαGlu precursor in the endoplasmic reticulum (ER) and degradation in prelysosomal and lysosomal compartments [2–5]. The interference of missense mutations with folding, transport and post-translational modifications of lysosomal proteins is a frequent finding in lysosomal storage disorders.

Since the first successful application of enzyme replacement therapy in type 1 Gaucher disease, intravenous administration of the missing lysosomal enzyme is applied as therapeutic intervention for some of the lysosomal storage disorders including Gaucher disease [6], Fabry disease [7,8], mucopolysaccharidosis type I [9,10], type VI [11] and type II [12], and GSDII [13]. Recently, the EMEA and FDA have approved the application of enzyme replacement therapy in all clinical forms of Pompe disease (http://www.emea.eu.int/human/docs/Humans/EPAR/myozyme/myozyme.htm). The recombinant human acid α-glucosidase is produced in genetically engineered CHO cells and marketed under the name Myozyme™. Clinical studies are still ongoing, but promising results of enzyme replacement therapy have already been published [14–16]. Enzyme replacement therapy, however, has limitations as to its broad applicability to lysosomal storage diseases. The blood brain barrier precludes the treatment of lysosomal storage disorders with central nervous system involvement. Moreover, there is the potential complication of an immune response against the infused product [17].

In addition to enzyme replacement therapy, current efforts are directed to the use of low molecular weight chemicals to rescue misfolded or unstable proteins from ER-associated degradation, and thereby restore protein function. Chemicals with these properties are called “chemical chaperones” and include reversible competitive inhibitors (for enzymes) and agonists/antagonists (for receptors) with affinity to the target proteins. Early reports demonstrated the possibility of active-site-specific chemical chaperone therapy for Fabry disease which is caused by lysosomal α-galactosidase deficiency [18,19]. Various mutant α-galactosidases were post-translationally restored using galactose as reversible competitive inhibitor. The α-galactosidase activity in cultured lymphoblasts from patients with Fabry disease increased, and mutant α-galactosidase species were restored in transfected COS-1 cells. These observations prompted a clinical trial with galactose infusion therapy for a cardiac variant of Fabry disease, which is caused by the α-galactosidase gene mutation causing the cardiac variant of Fabry disease [21]. Derivatives of deoxygalactonojirimycin were considered as well [22]. It is generally recognized that only a small increment of residual lysosomal enzyme activity may be sufficient to reduce the rate of substrate accumulation. Therefore, chemical chaperone therapy has been tested in other lysosomal storage disorders by applying various chemical compounds: in Gaucher disease with N-(α-nonyl) deoxyojarimycin (NN-DNJ) [23,24] and with N-(α-buty1) deoxyojirimycin (NB-DNJ) [25]; in G_M1-gangliosidosis with N-octyl-l-4-epi-β-valenamine [26]; and in Tay-Sachs and Sandhoff diseases with N-acetylgalcosamine-thiazoline [27].

In this study, we investigated the effect of deoxyojirimycin (DNJ) and its derivatives as putative chemical chaperones to promote the transport and enhance the stability of mutant forms of AαGlu in fibroblasts from patients with GSDII, and in COS-7 cells transfected with mutant AαGlu cDNA constructs. We demonstrate that chemical chaperones can mobilize mutant AαGlu species that are trapped in the ER and Golgi compartments and partly restore the catalytic function.

Materials and methods

Enzymes and chemicals

To examine the kinetic properties of the inhibitors, we used two different forms of AαGlu including the mature 76 kDa form purified from human placental [3] and the 110 kDa recombinant human AαGlu precursor produced in genetically engineered Chinese hamster ovary cells [28]. DNJ (5-imino analog of 1-deoxyglucose) and four N-alkyl derivatives [NB-DNJ, NN-DNJ, N-4(7-oxadecyl)deoxyojarimycin (NO-DNJ) and N-(α-dodecyl)deoxyojirimycin (ND-DNJ)], voglibose and acarbose were purchased from Toronto Research Chemicals (North York, Canada). These chemicals were dissolved in a concentration of 10 mmol/L in water (DNJ, voglibose and acarbose), methanol (NB-DNJ and ND-DNJ) or dimethyl sulfoxide (NO-DNJ and NN-DNJ). These stock solutions were stored at −20 °C until use. 4-Methylumbelliferyl α-n-glucopyranoside, 4-methylumbelliferyl β-N-acetylgalosamme and brefeldin A (β;4-di-hydroxy-2-[4-hydroxy-1-heptenyl]-4-cyclopentanecarboxylic acid 1-lactone) were purchased from Sigma–Aldrich (St. Louis, MO). 4-Methylumbelliferyl α-n-glucopyranoside and 4-methylumbelliferyl β-N-acetylgallosame were used to assay lysosomal AαGlu and total βHex activities, respectively, as described [29]. Other chemicals were of reagent grade and from Merck (Darmstadt, Germany). The protein concentration of cell homogenates was measured using the Pierce BCA protein assay reagent kit (Rockford, IL) with bovine serum albumin as a calibrator. Fluorescence and absorbance were measured by a VARIOSKAN spectropho/spectrofluorometer (Thermo Electron Corporation, Vantaa, Finland).

Fibroblasts from GSDII patients

Six fibroblast lines, established from five patients with GSDII and one unaffected individual, were used in this study. The genotypes and phenotypes of the patients were the following: 525delI/525delL causing premature termination at codon 176, this cell-line came from a severely affected infant with the classic infantile form of GSDII and served as negative control [30,31]; Y455F/Y455F came from an affected 5 years old child homozygous for an A to T transition at nucleotide position 1364 resulting in the substitution of Tyr455Phe [2]; P545L/P545L came from an adult patient homozygous for a C to T transition at nucleotide position 1634 resulting in the substitution Pro545Leu [30]; 525delIR660C came from an affected child who died at the age of 14 years and who was identified as compound heterozygote with a 1798C > T transition besides the base pair deletion at nucleotide position 525 [23,24]; and D645E/R554X came from an adult patient identified as compound heterozygote with genotype 1935C > A/2560C > T [33]. The fibroblasts were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and antibiotics (50 kU/L penicillin and 50 mg/L streptomycin) at 37 °C in 10% CO2. The cells were passaged on reaching confluency and harvested with
Transient expression in COS-7 cells

Site-directed mutagenesis was carried out as previously described [34]. The following primer sets were used: sense, 5'-CCTGCAGAGGGTTCTAGCCCTAGAC-3' and antisense, 5'-GTGCTGAGGCTGAAAGTCCGCAGG-3' for Y455F; and sense, 5'-CCCTACGTGCTTTGGGTTGTT-3' and antisense, 5'-AACACCCCCGAACGACTAGGGG-3' for P545L. Wild-type AβGlu and βHex constructs were prepared as previously described [2]. Mock transfections were carried out with empty vector. COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and antibiotics (50 kU/L penicillin and 50 mg/L streptomycin) at 37 °C in 10% CO2. The transfection was performed with 2 µg of cDNA construct per 60-mm dish using Lipofectin™ 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s instruction. After transfection, the cells were cultured for 16 h in the same medium, which was then replaced by medium containing various concentrations of NB-DNJ in the presence or absence of brefeldin A. Two days later, the cells were harvested with trypsin-EDTA, washed twice with phosphate-buffered saline and homogenized in water by sonicating on ice for immunoblot analysis or for measuring the AβGlu activity. For immunocytochemistry, COS-7 cells were co-transfected with mutant AβGlu and wild-type βHex cDNA constructs, and cultured with and without chemical chaperone for 4 days under the same condition.

Immunoblotting

The cell homogenates (60 µg protein for fibroblasts and 20 µg protein for transfected COS-7 cells) and culture medium (100 µL) were mixed with sample buffer under reducing conditions, and directly applied to SDS-polyacrylamide gel electrophoresis. After separation of the proteins by electrophoresis, they were blotted onto nitrocellulose filter (Schleicher & Schuell, Dassel, Germany). AβGlu was visualized on X-ray film after incubation of the nitrocellulose sheet with polyclonal rabbit antibodies against human placenta AβGlu in combination with ECL™ Western blotting detection reagents (Amersham Biosciences, Piscataway, NJ).

Immunocytochemistry

Immunocytochemistry on transiently transfected COS-7 cells was performed according to an established procedure [35]. AβGlu was visualized with mouse polyclonal antibodies against human placenta AβGlu [36] combined with goat anti mouse IgG conjugated to fluorescein, and βHex was visualized with rabbit polyclonal antibodies against human placenta βHex [3] combined with goat anti rabbit IgG conjugated to rhodamine.

Results

GSDII fibroblasts responsive to DNJ

To examine the functioning of DNJ as chemical chaperone, we selected four cell-lines in which the synthesis of AβGlu was normal, but the post-translational modification and transport defective. These cell-lines were derived from two children and two adults with the following mutant phenotypes: Y455F/Y455F (child), 525del/R600C (child), P545L/P545L (adult) and D645E/R854X (adult). A fifth cell-line from a patient with classic-infantile GSDII, with genotype 525del/525del and totally deficient in AβGlu synthesis, was chosen as negative control. The cells were incubated with and without 100 µmol/L DNJ in Dulbecco’s modified Eagle’s medium for 4 days under the standard condition as described in Materials and methods. They were harvested and washed with phosphate-buffered saline, whereafter the AβGlu activity was measured in the cell homogenates. Two out of these four cell-lines (Y455F/Y455F and P545L/P545L) showed a significant increase of AβGlu activity in the presence of DNJ, while the activity of β-hexosaminidase (βHex) remained unchanged (Fig. 1).

Effect of DNJ and its derivatives on mutant AβGlu

The two cell-lines (Y455F/Y455F and P545L/P545L) that were responsive to DNJ and a control cell-line from a healthy subject were cultured with various concentrations of this compound and four derivatives including NB-DNJ, NO-DNJ, NN-DNJ and ND-DNJ (Fig. 2). The cells treated with DNJ, NB-DNJ and NO-DNJ exhibited a significant increase of AβGlu activity, whereas the cells treated with ND-DNJ showed a decrease of the activity. Although NN-DNJ showed an enhancement of the activity at the low concentration (2 µmol/L), the effect disappeared at much lower concentrations.

Fig. 1. Effect of DNJ on AβGlu and βHex activities in cultured fibroblast lines from five patients with GSDII. The fibroblasts with different combinations of AβGlu gene mutations were maintained in standard culture medium in the presence (hatched bars) or absence (open bars) of 100 µmol/L DNJ for 4 days. They were then harvested and homogenized and the activities of AβGlu (left panel) and βHex (right panel) were measured. AβGlu activity in the fibroblasts from healthy individual was 84.5 nmol/h/mg protein.
concentration (data not shown). The control cell-line treated with these compounds showed a decrease of the AβGlu activity depending on dose and type of compound. The five chemical compounds had approximately the same IC₅₀ values (expressed in μmol/L) as measured with precursor and mature forms of wild-type AβGlu using 4-methylumbelliferyl α-D-glucopyranoside as substrate (DNJ, 1.3 and 1.2 for precursor and mature, respectively; NB-DNJ, 1.0 and 1.0; NO-DNJ, 1.3 and 1.3; NN-DNJ, 1.0 and 1.0; ND-DNJ, 1.7 and 1.7). In addition to these compounds, we tested voglibose and acarbose, which are α-glucosidase inhibitors used for the treatment of diabetes mellitus. Voglibose showed higher inhibitory effect (IC₅₀ 3.4 and 3.7 μmol/L) than acarbose (59 and 81 μmol/L). Although the AβGlu activity increased slightly (Y455F/Y455F, 1.4-fold; and P545L/P545L, 1.9-fold) in the presence of 50 μmol/L acarbose, voglibose did not show this chaperone feature.

Maturation of mutant AβGlu restored with NB-DNJ

Fig. 3 illustrates the effect of various concentration of NB-DNJ on the maturation of mutant Y455F/Y455F and P545L/P545L AβGlu species as analyzed by SDS-polyacrylamide gel electrophoresis followed by immunoblotting. Fibroblasts from a healthy individual served as control. In control cells, the 95 kDa processing intermediate and the 76 kDa mature form of AβGlu were observed regardless of the concentration of NB-DNJ employed while very little 110 kDa precursor was visible. By contrast, the precursor was clearly present in the mutant cell-lines, while intermediate and mature forms were missing in the absence of NB-DNJ, signifying a block in intracellular transport and maturation. With increasing concentrations of NB-DNJ, the mature and intermediate forms were formed and the precursor vanished.

Brefeldin A blocks the corrective effect of NB-DNJ

Because the post-translational modifications of AβGlu occur in conjunction with transport, the observed maturation of the mutant AβGlu species in the presence of NB-DNJ is likely effectuated by chaperone-mediated export from the ER/Golgi complex. We investigated this hypothesis in a following experiment in which we used brefeldin A to inhibit transport from ER/Golgi to the lysosomes [37]. To this end, COS-7 cells were transiently transfected with wild-type, Y455F or P545L AβGlu cDNA construct and were then harvested and homogenized, and the cell homogenates were subjected to immunoblotting with rabbit polyclonal antibodies against human AβGlu as described in Material and methods.

Fig. 2. Effect of DNJ and four derivatives on the AβGlu activity in cultured fibroblasts from a healthy individual (control) and two GSDII patients. The patients were homozygote for Y455F and P545L, respectively. The fibroblasts were exposed to various doses of DNJ or derivatives (0, 2, 10, 50 μmol/L) in standard culture medium for 4 days. They were then harvested and homogenized and the activity of AβGlu was measured in the homogenates. The symbols stand for: closed squares, NB-DNJ; closed triangles, NO-DNJ; closed circles, DNJ; open squares, NN-DNJ; open triangles, ND-DNJ. Open circles and broken lines present the AβGlu activities in the absence of DNJ and DNJ derivatives, respectively.

Fig. 3. Maturation of AβGlu in cultured fibroblasts is induced by various doses of NB-DNJ. Fibroblasts from a healthy individual (control) and from two GSDII patients homozygous for Y455F and P545L, respectively, were exposed to different doses of NB-DNJ (0, 2, 10, 50 μmol/L) in standard culture medium for 4 days. They were then harvested and homogenized, and the cell homogenates were subjected to immunoblotting with rabbit polyclonal antibodies against human AβGlu as described in Material and methods.
cultured for 2 days with various concentrations of NB-DNJ and in the presence or absence of 10 mg/L brefeldin A. The cell homogenates and the culture media were subjected to SDS-polyacrylamide gel electrophoresis and immunoblotting as described (Fig. 4). In the absence of brefeldin A, the same effects were observed in these transfected COS-7 cells as in the patient fibroblasts. The maturation process of mutant Y455F and P545L AαGlu species was restored with NB-DNJ in a dose dependent manner. The over-expression in COS-7 cells also allowed the analysis of the secreted 110 kDa precursor form of AαGlu. In the absence of NB-DNJ, this precursor appeared in the medium as an abnormally broad band due to excessive sialylation [2], but normalized with increasing concentrations of NB-DNJ (lower panel in Fig. 4). In the presence of brefeldin A, the corrective effect of NB-DNJ was completely lost.

**NB-DNJ restores abnormal localization of mutant AαGlu in transfected COS-7 cells**

COS-7 cells were transfected with wild-type or mutant Y455F-AαGlu cDNA construct to examine the localization of AαGlu immunocytochemically. Co-transfection with a wild-type βHex cDNA construct was performed to mark the lysosomes. The cells were cultured in the absence or presence of 50 μmol/L NB-DNJ for 4 days under standard conditions with double labeling for AαGlu (green fluorescent dye) and βHex (red fluorescent dye) (Fig. 5). The labeling pattern of the mutant AαGlu without NB-DNJ was typical for ER/Golgi localization (G-I, in Fig. 5) but that with NB-DNJ was typical for lysosomal localization, coinciding with βHex staining (J-L, in Fig. 5).

**Long term and transient effects of NB-DNJ**

The two mutant cell-lines, Y455F/Y455F and P545L/P545L, were cultured for 12 days in the presence of 10 μmol/L NB-DNJ and then continued on medium without NB-DNJ for 8 days. Over the whole 20 days period, the medium was refreshed every fourth day. The cells were harvested at the indicated time points and the AαGlu activity was measured. Fig. 6 shows that the AαGlu activity in both mutant cell-lines increased continuously up to 15.4 nmol/h/mg protein (equivalent to 14.0-fold increase of the initial activity without NB-DNJ) for Y455F/Y455F and up to 20.7 nmol/h/mg protein (equivalent to 7.9-fold) for P545L/P545L. After removal of NB-DNJ, the Y455F/Y455F cell-line lost most of the gained AαGlu activity within 4 days while the activity persisted for a longer period in the P545L/P545L cell-line.

**Discussion**

In mammalian cells, the processing of newly synthesized glycoproteins is monitored by a quality control system, the ER-associated degradation pathway, which removes misfolded and/or aggregated proteins from the ER lumen [38]. Proper folding is normally assured by the intrinsic properties of the amino acid sequence and supported by the cellular environment with its various molecular chaperones. In lysosomal and other genetic disorders, glycoproteins with missense mutations are frequently degraded due to misfolding of one or more domains despite the correct folding of others. It has been demonstrated that certain misfolded proteins can be rescued with low molecular weight compounds with affinity for these proteins, so-called chemical chaperones [39]. These chaperones can be ligands for receptors or substrate analogues for enzymes. In the past, we have identified various GSDII patients with missense mutations leading to premature degradation of the ER/Golgi-located AαGlu precursor. Our results show that chemical chaperone therapy may be applicable in some of these cases.

In this study, we started investigating the effect of DNJ as chemical chaperone because DNJ was known to inhibit AαGlu and other α-glucosidases in a competitive
and reversible manner [40,41]. Two out of four cell-lines with typical defects in post-translational processing of AαGlu, Y455F/Y455F and P545L/P545L, responded to DNJ treatment with elevation of AαGlu activity (Fig. 1). The activity of β-hexosaminidase, an arbitrary other lysosomal enzyme, did not change indicating that DNJ did not have a random effect on all lysosomal proteins. Using these two mutant cell-lines, we compared the chaperone function of DNJ and several of its derivatives and found that NB-DNJ was the most effective compound for restoring the AαGlu activity whereas the long-alkyl-chain derivatives NN-DNJ and ND-DNJ caused significant loss of activity despite the similar IC₅₀ values (Fig. 2). Of note, all compounds had an inhibitory effect on the normal AαGlu. Mellor et al. reported that DNJ and derivatives thereof enter cells rapidly (<1 min), independent of the alkyl-chain length, but that an increase in length causes persistent intracellular retention [42,43]. This could explain the inhibitory effect of NN-DNJ and ND-DNJ.
Fig. 3 demonstrates that NB-DNJ normalizes the post-translational processing of the mutant AβGlu species in a way that the amount of 110 kDa precursor diminishes and the amounts of 95 kDa processing intermediate and 76 kDa mature enzyme increase. It suggests that NB-DNJ facilitates transport of the mutant enzyme species from ER to lysosomes. The compound has no such effect on the normal enzyme. The fact that brefeldin A prevents the exit of proteins from the ER/Golgi complex [44] and blocks the effect of NB-DNJ at the same time supports our conclusion (Fig. 4). It is of interest to note that the mutant Y455F and P545L intracellular precursors have a decreased mobility in SDS-polyacrylamide gel electrophoresis because they are over-sialylated [2]. The same holds for the mutant precursors that are secreted into the medium (lower panels in Fig. 4). NB-DNJ also corrects this over-sialylation. Since sialyl transferase is located in the trans-Golgi compartment we conclude that NB-DNJ acts as chaperone along the entire transport route from the ER to Golgi onwards to the lysosomes. The results obtained with immunocytochemistry leave no doubt that the mutant enzymes are transported to the lysosomes with the help of NB-DNJ (Fig. 5).

In the presence of a low concentration of NB-DNJ (10 μmol/L), the AβGlu activity in cell-lines Y455F/Y455F and P545L/P545L showed a continuous increase and reached 15–20% of the average normal value after 8 days. This gain of activity is substantial since most patients with less than 1–2% residual activity have a life expectancy of less than 2 years whereas the majority of patients with 15–20% residual activity live into adulthood [45]. However, the AβGlu activity in the cell-lines of the two patients with these mutations dropped after removal of NB-DNJ, in the case of Y455F/Y455F with a t1/2 of approximately 2 days and in the case of P545L/P545L with a t1/2 of 4 days (Fig. 6). Since normal AβGlu has an estimated half-life of 10 days this suggests that NB-DNJ not only enters the ER/Golgi complex but also the lysosomes where it acts as stabilizer of the fully processed mutant lysosomal AβGlu species.

The effect of NB-DNJ is clearly mutation specific so that chaperone therapy in GSDII can only be applied on a mutation specific and patient specific basis. Moreover, NB-DNJ also acts as inhibitor of AβGlu in the concentration dependent manner (Fig. 2). Therefore, in vivo investigations are necessary to examine whether the compounds increase the AβGlu activity sufficiently enough to prevent lysosomal glycogen storage.

Apart from being an inhibitor of AβGlu, NB-DNJ is also an inhibitor of ceramide-specific glucosyltransferase that catalyses the first step in the glycosphinolipid biosynthetic pathway. Hence, NB-DNJ is applied under the name Zavesca (OGT 918) to limit the formation of glucocerebroside in Gaucher disease [46]. However, effective inhibition of the substrate biosynthesis in cultured cells required a high concentration of NB-DNJ in the medium; 500 μmol/L with complete inhibition; 100 μmol/L with partial inhibition; and 10 μmol/L without apparent inhibition [47]. Interestingly, other studies have shown that the derivatives of DNJ also act as chemical chaperones for mutant forms of glucocerebrosidase [23–25]. Thus, NB-DNJ has a dual function as inhibitor of glycosphingolipid biosynthesis and as chaperone for mutant forms of both glucocerebrosidase [25] as well as AβGlu, as described in this paper. A third function of NB-DNJ was recently reported in that the compound was demonstrated to promote transport of the defat508 variant of CFTR from the ER to the plasma membrane. Inhibition of the interaction between mutant CFTR and calnexin was proposed as possible mechanism [48].

As to the safety of NB-DNJ and derivatives, it is notable that NB-DNJ, in a concentration of 50 μmol/L, does not interfere with the N-glycan processing pathway of cultured cells via inhibition of the ER-glucosidase I and II [49]. Further, NB-DNJ was applied in humans for substrate reduction therapy in a dose of three times 100 mg/kg orally per day giving rise to plasma concentrations up to 10 μmol/L. The most frequent adverse effect was diarrhea [46]. Mice treated with high doses of NB-DNJ (2400 mg/kg/day) exhibited weight loss, diarrhea, lymphoid organ shrinkage, interference with glycogen catabolism in the liver and male infertility [50,51]. A low dose of 15 mg/kg per day caused reversible infertility of male mice [52].

Our data demonstrate the rational for further investigating the possibilities of chemical chaperone therapy in a subset of patients with GSDII where mutations do not interfere with AβGlu synthesis and catalytic function but with transport and stability. For these patients chaperone therapy may be a useful supplement to the recently introduced enzyme replacement therapy. Like for Gaucher disease, Fabry disease and other genetic disorders, efforts should be directed to finding chemical chaperones working at the lowest concentration with the least side effects.

References


