Brain & Development 38 (2016) 175–180

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

Chemical chaperone treatment for galactosialidosis: Effect of NOEV on β-galactosidase activities in fibroblasts

Mohammad Arif Hossain [a,1], Katsumi Higaki [b], Michiko Shinpo [a], Eiji Nanba [b], Yoshiyuki Suzuki [c], Keiichi Ozono [a], Norio Sakai [a,*,2]

[a] Department of Pediatrics, Osaka University Graduate School of Medicine, Suita, Osaka, Japan
[b] Division of Functional Genomics, Research Center for Bioscience and Technology, Tottori University, Yonago, Japan
[c] Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

Received 13 May 2015; received in revised form 14 July 2015; accepted 24 July 2015

Abstract

Introduction: Galactosialidosis is a rare lysosomal storage disease caused by a combined deficiency of G_{M1} β-galactosidase (β-gal) and neuraminidase secondary to a defect of a lysosomal enzyme protective protein/cathepsin A (PPCA) and mutation in *CTSA* gene. Three subtypes are recognized: early infantile, late infantile, and juvenile/adult. There is no specific therapy for patients with galactosialidosis at this time.

Objectives: The aim of this study was to determine the chaperone effect of *N*-octyl-4-epi-β-valienamine (NOEV) on β-gal proteins in skin fibroblasts of PPCA-deficit patients.

Methods: β-Gal and neuraminidase activities were measured for the diagnosis of the patients with galactosialidosis. Western blotting for PPCA protein and direct sequencing for *CTSA* gene were performed. Cultured skin fibroblast were treated with NOEV.

Results: We report four novel patients with galactosialidosis: one had the early infantile form and the other three had the juvenile/adult form. We found that NOEV stabilized β-gal activity in lysate from cultured skin fibroblasts from these patients. Treatment with NOEV significantly enhanced β-gal activity in cultured skin fibroblasts in the absence of PPCA.

Conclusions: Our results indicate the possibility that NOEV chaperone therapy might have a beneficial effect, at least in part, for patients with galactosialidosis.

Keywords: Galactosialidosis; Hydrops fetalis; Cherry-red spot; Chaperone; *N*-Octyl-4-epi-β-valienamine

1. Introduction

Galactosialidosis (MIM: 256540) is an autosomal recessive lysosomal storage disorder originally characterized as a combined deficiency of G_{M1} β-galactosidase (EC 3.2.1.23) and *N*-acetyl-α-neuraminidase (EC 3.2.1.18) due to a primary defect of protective protein/cathepsin A (PPCA) [1,2] that regulates their stability and activity in lysosomes through the formation of a multienzymic

Abbreviations: EEG, electroencephalogram; NOEV, *N*-octyl-4-epi-β-valienamine; PPCA, protective protein/cathepsin A; SEM, standard error of the mean; β-gal, G_{M1} β-galactosidase.

* Corresponding author at: Department of Pediatrics, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan. Tel.: +81 6 6879 3932; fax: +81 6 6879 3939.
E-mail address: norio@ped.med.osaka-u.ac.jp (N. Sakai).
[1] Present address: Genetic Division, Department of Pediatrics, King Abdulaziz Medical City, National Guard Health Affairs, Riyadh, Saudi Arabia.
[2] Present address: Division of Health Science, Osaka University Graduate School of Medicine, Suita, Osaka, Japan.

http://dx.doi.org/10.1016/j.braindev.2015.07.006
0387-7604/

complex [3,4]. To date, about 80 patients have been reported globally. More than 60% of patients have been reported in Japan and most of them have juvenile or adult onset. Most patients develop loss of vision as an initial symptom, followed by neurological abnormalities (such as action myoclonus or cerebellar ataxia), skeletal dysplasia, cherry-red spots, and angiokeratoma (juvenile/adult form) [2]. A few early infantile patients have also been reported with fetal hydrops, visceromegaly, skeletal dysplasia, and cherry-red spots [5–7]. Single-base mutations, *c.146 A > G* (*p.Q49R*) and *c.1184 A > G* (*p.Y395C*), have been reported as early infantile mutations for Japanese patients in several publications [5,6,8]. Another A > G substitution at position 3 of the 5′ donor splice site of intron 7 (*SpDEx7*) has been reported as a juvenile/adult mutation [5,9].

The human *CTSA* gene encoding PPCA protein is composed of 15 exons and 480 amino acids [10]. The glycosylated precursor (54 kDa) is synthesized in the endoplasmic reticulum and dimerizes [11]. After synthesis, the precursor undergoes a protease-mediated maturation process. The mature and functional protein present in lysosomes consists of two subunits (32 and 20 kDa) [12–14]. It has been reported that the primary defect in galactosialidosis is the absence or deficiency of the 32/20-kDa PPCA and its 54-kDa precursor. It has also been postulated that this protein is needed to protect β-gal against rapid proteolytic degradation in the lysosomal environment and is required to unite β-gal monomers and neuraminidase into a complex [1].

To date, there are no available therapies for human galactosialidosis. However, there has been one published report on the utility of bone marrow transplantation in a murine galactosialidosis model [15]. We have identified a low molecular weight compound, *N*-octyl-4-epi-β-valienamine (NOEV), as a potent chemical chaperone for mutant β-gal proteins [16–18]. We have also demonstrated its effectiveness in a murine G_{M1}-gangliosidosis model [19,20].

In this study, we report four new Japanese patients with galactosialidosis. We then determined the chaperone effect of NOEV on β-gal protein in skin fibroblasts of these PPCA-deficient patients.

2. Materials and methods

2.1. Patients

The study included four Japanese patients with newly diagnosed galactosialidosis, who have not been previously reported. Their diagnosis was based on clinical signs, symptoms, and laboratory and radiological findings. Their low β-gal and neuraminidase activities in blood lymphocytes and/or skin fibroblasts are reported in Table 1.

2.2. DNA analysis by direct sequencing

After informed consent, cDNA was prepared for patients 1 and 2. PCR amplification and direct sequencing were conducted for the *CTSA* gene as previously reported [5]. We also determined mutations by direct genomic DNA sequencing for all four patients.

2.3. Cell culture and NOEV treatment

For one healthy subject and patients 1, 2, and 4, skin fibroblasts were grown to 80–90% confluence in 3.5-cm dishes on the day prior to NOEV treatment in standard culture medium of Dulbecco's Modified Eagle Medium (Life Technologies Co., Carlsbad, CA) with 10% fetal

Table 1
Clinical information for the patients with galactosialidosis.

Case	Age at onset	Clinical presentation	β-Gal activity[a]	Neuraminidase activity[b]	Mutation[c]
Pt. 1	38 yrs	• Cherry-red spots • Cerebellar ataxia • Myoclonus	56 (SF)	2 (SF)	*[SpDEx7];[SpDEx7]*
Pt. 2	Intra-uterine	• Hydrops fetalis • Generalized edema • Massive hepato-slpenomegaly • Died on day 17 with renal failure	64 (SF)	0 (SF)	*p.[Q49R;Y395C]*
Pt. 3	17 yrs	• Visual impairment • Cherry-red spots	23 (LYM)	2 (LYM)	*[SpDEx7];[SpDEx7]*
Pt. 4	4–5 yrs	• Cherry-red spots • Cerebellar ataxia • Myoclonic seizure • Severe bone deformity	64 (SF)	0.03 (SF)	*c.[**478_480delTTC**];[?]*

SF, skin fibroblast; LYM, lymphocyte.
[a] Normal β-gal activity is ~300 and ~150 nmol/h/mg protein in SF and LYM, respectively.
[b] Normal neuraminidase activity is ~50 and ~25 nmol/h/mg protein in SF and LYM, respectively.
[c] "?" indicates no mutation was found in the second allele and bold indicates a novel mutation.

calf serum. NOEV treatment was performed for 96 h at 37 °C as previously reported [18].

2.4. Measurement of lysosomal enzyme activities

β-Gal and neuraminidase activities were measured as previously reported [21]. In short, cultured skin fibroblasts were washed with phosphate-buffered saline and trypsinized. After centrifugation, cell pellets were sonicated in deionized distilled water. Protein concentrations were measured by Lowry's method [22]. Cell lysates were incubated with 4 MU substrates at 37 °C for 30 min. Fluorescence (excitation at 365 nm/emission at 450 nm) was measured with a microplate reader. Enzyme activities were calculated as nmol/h/mg protein.

2.5. Stabilization of β-gal in vitro with NOEV

Heat stabilization of β-gal *in vitro* with NOEV was performed as previously reported [23]. In short, skin fibroblast cell lysates from the healthy subject and patients 1, 2, and 4 were incubated in citrate phosphate buffer (pH 7.0) at 48 °C for specified times with increasing concentrations of NOEV and then placed on ice to terminate incubation. β-Gal activity was measured as described above.

2.6. Western blotting for PPCA protein

Western blotting was performed as previously reported [23]. In short, cell lysates from patients 1 and 2 (skin fibroblasts) and patients 3 and 4 (lymphocytes) were mixed with sample buffer containing protease inhibitor and SDS. Samples (20–30 μg protein per lane) were applied to a 16% polyacrylamide gel. After SDS–PAGE, protein was transferred to a 0.45-μm PVDF membrane (Merck-Millipore) followed by blocking with 5% skim milk solution. Rabbit polyclonal anti-CTSA antibody (cat. No. AP10476a, Abgent.com) was used at 1:1000 dilution as the primary antibody followed by the secondary antibody conjugated with horse radish peroxidase at 1:5000 dilution. Detection was performed by chemical luminescence (SuperSignal West Dura; Thermo Fisher Scientific Inc., Tokyo, Japan).

2.7. Statistical analysis

Experiments were performed three times each in duplicate. Data are presented as mean ± standard error (SEM). Comparisons was conducted using the Student's *t*-test. Results were considered significant for $P < 0.05$.

2.8. Ethical approval

Ethical approval for this study was obtained from the Institutional Review Board of Osaka University School of Medicine.

3. Results

3.1. Mutation analysis for galactosialidosis patients

Seven mutant alleles were detected among the four Japanese patients with galactosialidosis. Patients 1 and 3 had a splice mutation *IVS7ds + 3A > G* (*SpDEx7*) in a homozygous state, which has been reported as a common late-onset mutation [5]. Patient 2 had two compound heterozygous mutations *c.146 A > G* (*p.Q49R*) and *c.1184 A > G* (*p.Y395C*), which have been reported as infantile mutations [6,8]. We could detect only one mutant allele for patient 1, which was *c.478_480delTTC*, a novel mutation (Table 1).

3.2. PPCA expression for galactosialidosis patients

To determine PPCA expression whole cells lysates from lymphocytes or skin fibroblasts were used for Western blotting. We found very thin or no PPCA precursor (54 kDa) for samples from the healthy subject or the patients. However, the mature fragment (32 kDa) was only detected for the sample from the healthy subject (Fig. 1). We could not detect 20 kDa fragments

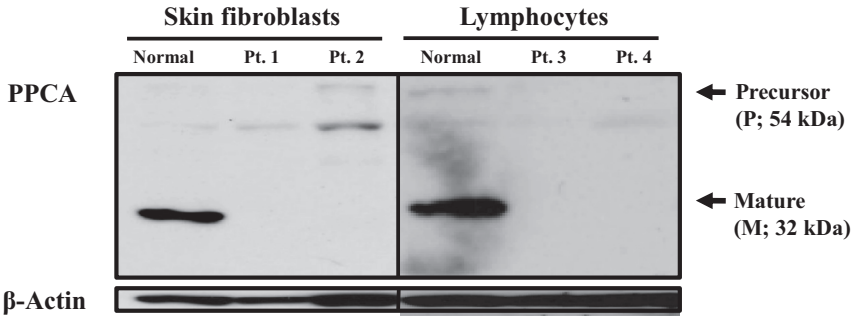

Fig. 1. PPCA expression. Cell lysates of skin fibroblasts or lymphocytes were subjected to Western blotting (20–30 μg protein per lane) in 16% SDS–PAGE. Anti-rabbit polyclonal antibody was used as first antibody at 1:1000 dilution. The precursor form of PPCA (54 kDa) is faint or absent and the mature protein (32 kDa) is only observed in cells for a normal control subject.

as the antibody we used is an *N*-terminal antibody that cannot recognize 20 kDa fragments.

3.3. In vitro NOEV effects on β-gal stabilization

To evaluate the *in vitro* effects of NOEV on enzyme stabilization, skin fibroblast whole cells lysates from the healthy subject and patients 1, 2, and 4 were incubated with increasing concentrations of NOEV and were subjected to heat inactivation (48 °C) at pH 7.0. In the absence of NOEV, the activities from both the healthy subjects and the patients decreased to 0–10%. After 15 min incubation, NOEV induced effective stabilization for normal and patients' β-gal proteins in a dose-dependent manner (Fig. 2). This also showed that β-gal stability in adult galactosialidosis (patient 1) was better than infantile galactosialidosis (patients 2 and 4), with statistical significance for incubation with 2 μM NOEV. We did not have skin fibroblasts for patient 3 SF to determine the effects of NOEV.

3.4. Chaperone effects of NOEV in cultured human skin fibroblasts

To determine the chaperone effect of NOEV in cultured human skin fibroblasts, cells were cultured with increasing concentrations of NOEV for 96 h. Then, β-gal and neuraminidase activities were measured with 4 MU substrates and PPCA expression was determined by Western blotting using cell lysates. We found a chaperone effect with NOEV for β-gal in all three patients. β-Gal activity was measured as 172–284 nmol/h/mg protein with 0.2 μM or 2 μM concentrations of NOEV (Fig. 3). However, neuraminidase activity and PPCA expression remained unchanged (data not shown).

4. Discussion

With respect to the diagnosis of juvenile/adult galactosialidosis, bilateral cherry-red spots are a common sign [2,5]. Patients 1, 3, and 4 all had this sign at presentation. Major neurologic signs for adult galactosialidosis include cerebellar ataxia and myoclonus [5,24], which were present in patients 1 and 4. Fetal hydrops has been reported in many cases of early infantile galactosialidosis [6,7,25,26]. This was detected by ultrasonography in patient 2 at 26 weeks of gestation. We diagnosed all four patients with low β-gal and neuraminidase activities and low PPCA expression.

When the *p.Q49R* or *p.Y395C* mutation is present in a homozygous form in a patient, the phenotype becomes the early infantile subtype of the disease [6,8]. In patient 2 both mutations were present in a compound

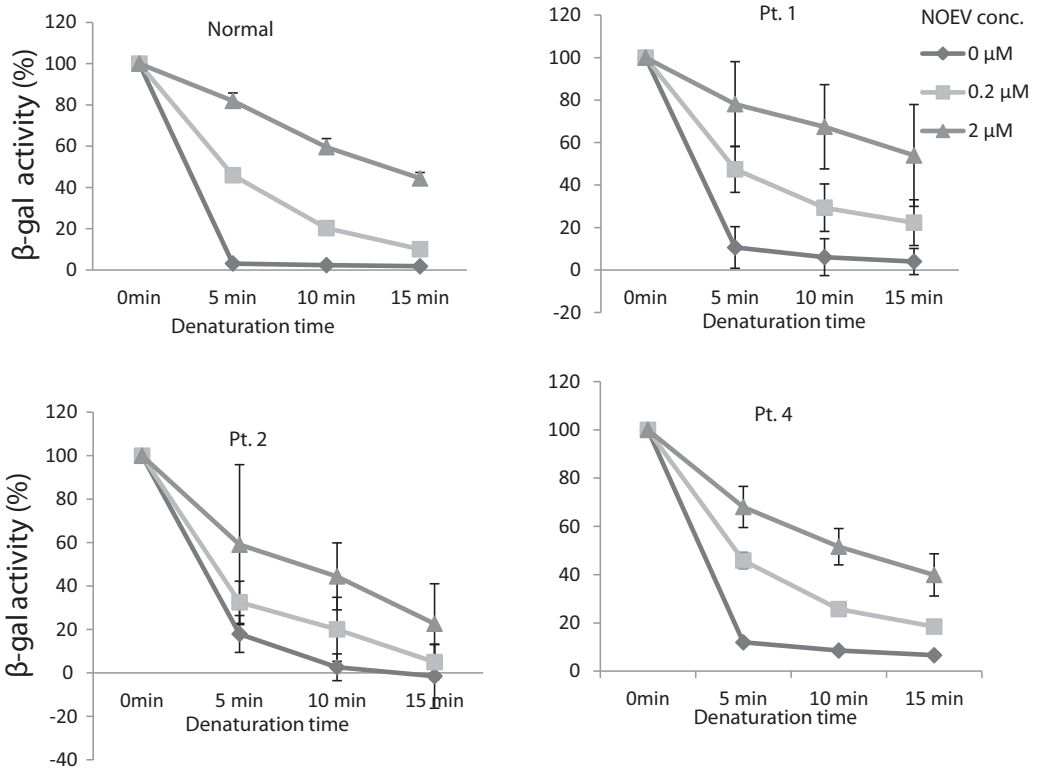

Fig. 2. Heat stabilization of β-gal with NOEV. Cell lysates were incubated at pH 7.0 and 48 °C for the indicated time and β-gal activity was measured. Each point represents means of triplicates obtained in at least three independent experiments. Values were expressed as relative activity in the absence of NOEV.

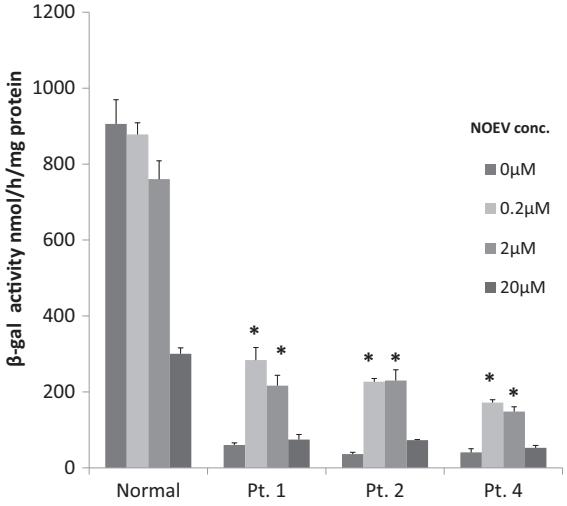

Fig. 3. β-Gal activity of NOEV-treated skin fibroblasts. Skin fibroblasts were incubated in the absence or presence of NOEV for 96 h and β-gal activity was measured in lysates. Each bar represents the mean ± SEM of three determinations each done in duplicate. $^{*}P < 0.001$ (Student *t*-test) compared to values in the absence of NOEV.

heterozygous state and showed a very severe phenotype. Patients 1 and 3 had the homozygous mutation *SpDEx7*, which is reported as an adult mutation [9] and shows a very mild phenotype.

Several lysosomal diseases such as G_{M1}-gangliosidosis [16–20], G_{M2}-gangliosidosis [27], Gaucher disease [28], and Fabry disease [29] are treated with small molecules to impact conformational folding of mutant enzymes and prevent their early degradation by endoplasmic reticulum-associated degradation. These small molecules are known as proteostasis regulators [30]. In a recent study, we evaluated the proteostatic effect of NOEV for GALC protein *in vitro* and found the strong stabilization of GALC protein in Krabbe disease [23]. In the current study, we also determined the proteostatic effect of NOEV for β-gal and found the strong stabilization of β-gal protein in PPCA-deficient skin fibroblasts *in vitro*. This suggests that NOEV binds to β-gal protein in neutral medium and prevents endoplasmic reticulum-associated degradation. When we determined the intracellular effects of NOEV, we found normal β-gal activity for NOEV-treated skin fibroblasts. This suggests that NOEV transports the β-gal precursor to lysosomes and helps multimerization in the absence of matured PPCA. Effectively matured β-gal proteins have normal activities.

Due to absence of functional PPCA, β-gal activity is partially affected in galactosialidosis patients and in murine models [15]. Previous studies also identified several substrates that accumulate in galactosialidosis including sialyloligosaccharides, G_{M1}, G_{M2}, G_{M3}, G_{D1a}-ganglioside [31–34]. The clinical relevance of β-gal deficiency with respect to galactosialidosis phenotype is still controversial. All of our experiments should be performed *in vitro* to determine the effect on β-gal activity and accumulation of G_{M1} in astrocyte or brain tissue. To examine the effect of NOEV as a treatment for galactosialidosis, experiments are warranted in a murine model of galactosialidosis for our next study [20]. It might be possible to obtain additive chaperone effects on both β-gal and neuraminidase deficiencies in galactosialidosis using combination therapy with a neuraminidase inhibitor [35] and NOEV.

In conclusion, this is the first report regarding galactosialidosis treatment using a low molecular weight chemical chaperone. NOEV may be considered as a potential therapy for galactosialidosis for partial improvement of the phenotype.

Acknowledgements

This work was supported by Grant-in-Aid for Scientific Research (C) (25461543) and Health Labour Sciences Research Grant (H22-Nanji-Ippan-002). We thank Mrs Tsujimoto for collaborative support in the various assays.

References

[1] D'Azzo A, Hoogeveen A, Reuser AJJ, Robinson D, Galjaard H. Molecular defect in combined beta-galactosidase and neuraminidase deficiency in man. Proc Natl Acad Sci USA 1982;79:4535–9.
[2] D'Azzo A, Andria G, Bonten E, Annunziata I. The online metabolic and molecular basis of inherited disease (OMMBID). Galactosialidosis. doi:http://dx.doi.org/10.1036/ommbid.183 [Chapter 152].
[3] Hoogeveen AT, Verheijen FW, Galjaard H. The relation between human lysosomal β-galactosidase and its protective protein. J Biol Chem 1983;258:12143–6.
[4] Verheijen FW, Palmeri S, Hoogeveen AT, Galjaard H. Human placental neuraminidase. Activation, stabilization and association with β-galactosidase and its 'protective' protein. Eur J Biochem 1985;149:315–21.
[5] Shimmoto M, Fukuhara Y, Itoh K, Oshima A, Sakuraba H, Suzuki Y. Protective protein gene mutations in galactosialidosis. J Clin Invest 1993;91:2393–8.
[6] Matsumoto N, Gondo K, Kukita J, Higaki K, Paragison RC, Nanba E. A case of galactosialidosis with a homozygous Q49R point mutation. Brain Dev 2008;30:595–8.
[7] Groener J, Maaswinkel-Mooy P, Smit V, Mvd Hoeven, Bakker J, Campos Y, et al. New mutations in two Dutch patients with early infantile galactosialidosis. Mol Genet Metab 2003;78:222–8.
[8] Itoh K, Shimmoto M, Utsumi K, Mizoguchi N, Miharu N, Ohama K, et al. Protective protein/cathepsin A loss in cultured cells derived from an early infantile form of galactosialidosis patients homozygous for the A 1184-G transition (Y395C mutation). Biochem Biophys Res Commun 1998;247:12–7.
[9] Shimmoto M, Takano T, Fukuhara Y, Oshima A, Sakuraba H, Suzuki Y. Japanese-type adult galactosialidosis: a unique and common splice junction mutation causing exon skipping in the protective protein/carboxypeptidase gene. Proc Jpn Acad B 1990;66:217–22.

[10] Galjart NJ, Gillemans N, Harris A, van der Horst GTJ, Verheijen FW, Galjaard H, et al. Expression of cDNA encoding the human "protective protein" associated with lysosomal beta-galactosidase and neuraminidase: homology to yeast proteases. Cell 1988;54:755–64.
[11] Zhou XY, Galjart NJ, Willemsen R, Gillemans N, Galjaard H, d'Azzo A. A mutation in a mild form of galactosialidosis impairs dimerization of the protective protein and renders it unstable. EMBO J 1991;10:4041–8.
[12] Galjart NJ, Morreau H, Willemsen R, Gillemans N, Bonten EJ, d'Azzo A. Human lysosomal protective protein has cathepsin A-like activity distinct from its protective function. J Biol Chem 1991;266:14754–62.
[13] Bonten EJ, Galjart NJ, Willemsen R, Usmany M, Vlak JM, d'Azzo A. Lysosomal protective protein/cathepsin A. Role of the "linker" domain in catalytic activation. J Biol Chem 1995;270:26441–5.
[14] Rudenko G, Bonten E, d'Azzo A, Hol WGJ. Three-dimensional structure of the human "protective protein": structure of the precursor form suggests a complex activation mechanism. Structure 1995;3:1249–59.
[15] Zhou XY, Morreau H, Rottier R, Davis D, Bonten E, Gillemans N, et al. Mouse model for the lysosomal disorder galactosialidosis and correction of the phenotype with over-expressing erythroid precursor cells. Genes Dev 1995;9:2623–34.
[16] Ogawa S, Matsunaga YK, Suzuki Y. Chemical modification of the β-glucocerebrosidase inhibitor N-octyl-b-valienamine: synthesis and biological evaluation of 4-epimeric and 4-O-(β-D-galactopyranosyl) derivatives. Bioorg Med Chem 2002;10:1967–72.
[17] Iwasaki H, Watanabe H, Iida M, Ogawa S, Tabe M, Higaki K, et al. Fibroblast screening for chaperone therapy in β-galactosialidosis. Brain Dev 2006;28:482–6.
[18] Higaki K, Li L, Bahrudin U, Okuzawa S, Takamuram A, Yamamoto K, et al. Chemical chaperone therapy: chaperone effect on mutant enzyme and cellular pathophysiology in β-galactosidase deficiency. Hum Mutat 2011;32:843–52.
[19] Matsuda J, Suzuki O, Oshima A, Yamamoto Y, Noguchi A, Takimoto K, et al. Chemical chaperone therapy for brain pathology in GM1-gangliosidosis. Proc Natl Acad Sci USA 2003;100:15912–7.
[20] Suzuki Y, Ichinomiya S, Kurosawa M, Matsuda J, Ogawa S, Iida M, et al. Therapeutic chaperone effect of N-octyl 4-epi-β-valienamine on murine G_{M1}-gangliosidosis. Mol Genet Metab 2012;106:92–8.
[21] Hossain MA, Otomo T, Saito S, Ohno K, Sakuraba H, Hamada Y, et al. Late-onset Krabbe disease is predominant in Japan and its mutant precursor protein undergoes more effective processing than the infantile-onset form. Gene 2014;534:144–54.
[22] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265–75.
[23] Hossain MA, Higaki K, Saito S, Ohno K, Sakuraba H, Nanba E, et al. Chaperone therapy for Krabbe disease: potential for late-onset GALC mutations. J Hum Genet 2015. doi:http://dx.doi.org/10.1038/jhg.2015.61 [Epub ahead of print].
[24] Suzuki Y, Nanba E, Tsuji A, Yang RC, Okamura-Oho Y, Yamanaka T. Clinical and genetic heterogeneity in galactosialidosis. Brain Dysfunct 1988;1:285.
[25] Zammarchi E, Donati MA, Morrone A, Donzelli GP, Zhou, d'Azzo A. Early-infantile galactosialidosis: clinical biochemical, and molecular observations in a new patient. Am J Med Genet 1996;64:453–8.
[26] Itoh K, Miharu N, Ohama K, Mizoguchi N, Sakura N, Sakuraba H. Fetal diagnosis of galactosialidosis (protective protein/cathepsin A deficiency). Clin Chim Acta 1997;266:75–82.
[27] Maegawa GH, Tropak M, Buttner J, Stockley T, Kok F, Clarke JT, et al. Pyrimethamine as a potential pharmacological chaperone for late onset forms of GM2 gangliosidosis. J Biol Chem 2007;282:9150–61.
[28] Maegawa GH, Tropak MB, Buttner JD, Rigat BA, Fuller M, Pandit D, et al. Identification and characterization of ambroxol as an enzyme enhancement agent for Gaucher disease. J Biol Chem 2009;284:23502–16.
[29] Ishii S. Pharmacological chaperone therapy for Fabry disease. Proc Jpn Acad B 2012;88:18–30.
[30] Mu TW, Ong DS, Wang YJ, Balch WE, Yates JR, Segatori L, et al. Chemical and biological approaches synergize to ameliorate protein-folding diseases. Cell 2008;134:769–81.
[31] van Pelt J, Kamerling JP, Vliegenthart JF, Hoogeveen AT, Galjaard H. A comparative study of the accumulated sialic acid-containing oligosaccharides from cultured human galactosialidosis and sialidosis fibroblasts. Clin Chim Acta 1988;174:325–35.
[32] van Pelt J, Bakker H, Kamerling J, Vliegenthart J. A comparative study of sialyloligosaccharides isolated from sialidosis and galactosialidosis urine. J Inherit Metab Dis 1991;14:730–40.
[33] Yoshino H, Miyashita K, Miyatani N, Ariga T, Hashimoto Y, Tsuji S, et al. Abnormal glycosphingolipid metabolism in the nervous system of galactosialidosis. J Neurol Sci 1990;97:53–65.
[34] Miyatake T, Atsumi T, Obayashi T, Mizuno Y, Ando S, Ariga T, et al. Adult type neuronal storage disease with neuraminidase deficiency. Ann Neurol 1979;6:232–44.
[35] Kim CU, Lew W, Williams MA, Liu H, Zhang L, Swaminathan S, et al. Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. J Am Chem Soc 1997;119:681–90.