# Concept and Development of Chaperone Therapy for Protein Misfolding Diseases

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**Abstract.** Chaperone therapy is a new concept of molecular therapeutic approach mainly developed for lysosomal diseases, based on a paradoxical molecular interaction involving a mutant enzyme and its competitive inhibitor as an intracellular enhancer (chaperone). The misfolded mutant protein is transported safely to the lysosome as a complex with a specific chaperone. The enzyme activity is expressed after dissociation from its chaperone. The advantages of this molecular therapeutic approach can be summarized twofold; first, oral administration to individuals with intractable diseases; and second, delivery to the central nervous system for treatment of brain dysfunction. In recent years the chaperone therapy research has been widely developed for various protein misfolding diseases as well as lysosomal diseases. In this article, recent progress of chaperone therapy for lysosomal diseases is briefly reviewed, mainly focusing on $G_{M1}$-gangliosidosis and Morquio B disease (β-galactosidase deficiency) and Gaucher disease (β-glucosidase deficiency) as representative neurogenetic diseases.

**Keywords:** chaperone therapy, lysosomal disease, competitive inhibitor, $G_{M1}$-gangliosidosis, Morquio B disease, Gaucher disease.

## 1 Introduction

A large number of genetic diseases are caused by functional defect of enzymes (enzyme deficiency), resulting in diverse metabolic derangements in human somatic cells. The metabolic defect is expressed generally in various tissues and organs, but most prominently in the central nervous system (neurogenetic diseases). Among them the diseases involving the lysosome, one of the important cellular organelles, digesting various high molecular endogenous or exogenous compounds under the acidic condition, have been well recognized as classic neurogenetic diseases with

specific enzyme deficiency affecting mainly infants and young children. Cellular dysfunction caused by an excessive storage of substrates ensues, and a genetic metabolic disease (lysosomal disease) develops in humans and other animals with neurological and other somatic manifestations. Severity of enzyme deficiency is variable in individual patients. In general, severe enzyme deficiency tends to cause severe clinical manifestations in early life [1].

Since mid-1960s, attempts have been made to treat patients with lysosomal diseases. Enzyme replacement therapy was the most successful achievement by intravenous administration of the functional human recombinant enzyme. However, the effect has not been confirmed on brain pathology in patients with neurological manifestations.

## 2 Turnover of mutant enzyme protein and correlation with the age of onset

In early 1980s we found that thiol (cysteine) protease inhibitors protected degradation of endogenous human or exogenous fungal β-galactosidase, an enzyme responsible for $G_{M1}$-gangliosidosis in humans [2-3]. These results prompted us to search for a new way to rescue apparently inactive mutant enzyme proteins as a new molecular therapy of enzyme deficiency disorders.

In this connection we found a correlation between residual β-galactosidase activity and clinical onset in $G_{M1}$-gangliosidosis patients. The amount of residual enzyme activity showed positive parabolic correlation with the age of onset in various phenotypic forms of β-galactosidase deficiency disorders [4]. Based on these observations, we anticipated that at least 10% of normal enzyme activity is necessary for washout of the storage substrate in somatic cells, particularly in neuronal cells. The age of onset in patients expressing the enzyme activity above this level will be theoretically beyond the human life span [4].

## 3 Theoretical background of chaperone therapy

In the last decade of the 20th century we found that some mutant α-galactosidase A proteins were unstable and unable to express catalytic activities in somatic cells from Fabry patients [5]. Galactose and a galactose analogue compound 1-deoxygalactonojirimycin (DGJ) were effective to restore the mutant α-galactosidase A activity in Fabry cells and tissues [6-7]. Furthermore another galactose analogue N-octyl-4-epi-β-valienamine (NOEV) was found to be effective to restore the mutant β-galactosidase activity in $G_{M1}$-gangliosidosis cells and tissues [8].

After extensive gene and protein analyses of Fabry disease and $G_{M1}$-gangliosidosis, we proposed the following hypothesis [9]. A substrate analogue inhibitor binds to a mutant lysosomal misfolding protein as a kind of molecular chaperone (chemical chaperone), to induce normal molecular folding at the endoplasmic reticulum (ER)/Golgi compartment in somatic cells, resulting in formation of a stable molecular complex at neutral pH. The protein-chaperone complex is safely transported to the lysosome, where it dissociates under the acidic condition, the mutant enzyme remains stabilized in its normal folding structure, and its catalytic function is expressed [4]

Molecular pathology of inherited metabolic diseases can be generally classified into the following three major conditions related to the structure and function of mutant proteins [9].

(a) Biosynthetic defect of the protein in question.
    Mutant enzyme is not synthesized, and accordingly rescue of the protein is not possible.
(b) Defect of biological activity.
    In spite of normal biosynthesis, the protein does not maintain biological activity because of its drastic structural abnormality. There is no possibility to restore the biological activity of this molecule.
(c) Unstable mutant protein with normal or near-normal biological activity.

The mutant protein has normal biological function in its mature form under normal folding. However, it is unstable because of misfolding and rapidly degraded or aggregated immediately after biosynthesis.

In the third case, the protein function is expected to be restored if the molecule is somehow stabilized and transported to the cellular compartment where it is expected to exhibit biological activity; the lysosome in the case of lysosomal enzyme. This is the principle of chaperone therapy to restore the enzyme activity by low molecular inhibitors with appropriate molecular structure fitting in the enzyme molecule. A similar concept was presented for cystic fibrosis [10-11], and therapeutic experiments have been reported.

This approach is particularly important for correction of brain pathology if they are delivered to the central nervous system through the blood-brain barrier [4]. Chaperone therapy is theoretically effective in 30-60% of patients with Fabry disease and $G_{M1}$-gangliosidosis patients [12-13].

## 4 Fabry disease, $G_{M1}$-gangliosidosis/Morquio B disease, and Gaucher disease

Fabry disease is an inherited generalized vasculopathy caused by α-galactosidase A deficiency, resulting in involvement of the brain, heart, and kidneys after the middle ages, with increasing storage of globotriaosylceramide in the vascular endothelium. In this disease the p.Q279E mutant enzyme was low in catalytic activity, and unstable at relatively high temperature and also at low pH [5]. It was rapidly degraded because of molecular misfolding. A high dose of galactose in the culture medium induced a high expression of catalytic activity in Fabry lymphoblasts and mutant enzyme-expressing COS-1 cells [6]. However, we thought that galactose was not an ideal candidate for restoration of the mutant enzyme in human tissues, as galactose is rapidly metabolized in cells and tissues, and the continuously high galactose concentration in somatic cells could cause direct

galactose intoxication such as galactosemia, and result in pathological osmolarity higher than that in the human blood under the physiological condition, causing significant dehydration, shrinkage and dysfunction of somatic cells. A long-term treatment with galactose at this high dose would not be realistic, although a human experiment of intravenous galactose infusion every other day for 2 years was reported, achieving the beneficial effect on hypertrophic cardiomyopathy in a Fabry patient [14]. DGJ showed the chaperone effect mainly on mutant α-galactosidase A. It is currently used for human studies.

$G_{M1}$-gagliosidosis is a relatively rare lysosomal disease, presenting clinically with progressive neurological deterioration mainly in infancy and childhood with various somatic manifestations [1]. We started the chaperone experiment on β-galactosidase immediately after the experiments on α-galactosidase A. The first report was published, with a newly synthesized organic compound valienamine derivative NOEV as a chemical chaperone for β-galactosidase toward genetically engineered $G_{M1}$-gangliosidosis model mice [8]. It is a potent competitive inhibitor of β-galactosidase in vitro, and restores mutant enzyme activities in somatic cells from patients with $G_{M1}$-gangliosidosis. Pharmacokinetic analysis revealed rapid intestinal absorption and renal excretion after oral administration of NOEV [15]. It was delivered to the central nervous system through the blood-brain barrier to achieve high expression of the apparently deficient β-galactosidase activity in the GM1-gangliosidosis model mice. NOEV treatment starting at the early stage of disease resulted in remarkable arrest of neurological progression within a few months with prolongation of survival time [15]. Recently a new chaperone compound MTD118, a bicyclic DGJ derivative, has been found to show a chaperone spectrum complementary to that of NOEV for some mutant genes [13]. Thus, combination of NOEV and MTD118 will cover 60-70% of patients with $G_{M1}$-gangliosidosis, and hopefully those with Morquio B disease.

Gaucher disease is a group of diverse clinical manifestations involving both the

central nervous system and extraneural visceral organs, caused by β-glucosidase deficiency, resulting in massive storage of glucosylceramide. Clinically it is classified into three major phenotypes: chronic non-neuronopathic (adult), acute neuronopathic (infantile), and subacute neuronopathic (juvenile). Enzyme replacement therapy is available for non-neuronopathic patients, and the clinical effect has been well documented [16]. However, neurological manifestations have not been controlled by this therapeutic approach. We tried to develop chaperone compounds for Gaucher disease. NOV (N-octyl-β-valienamine), an epimer of NOEV, was the first chaperone compound for this disease [17]. Although its effectiveness was confirmed in the cell culture system, animal studies have not been carried out since appropriate animal models are not available as yet. Recently ambroxol hydrochloride, a commercially available expectorant drug, was reported to be an excellent chaperone candidate for Gaucher disease [18]. It was found by a systematic screening of 1040 FDA approved drugs. This is a pH-dependent mixed-type inhibitor of β-glucosidase. The p.N370S and p.F213I mutant enzyme activities were enhanced by ambroxol in Gaucher fibroblasts. Subsequently we tried to treat neuronopathic Gaucher patients with p. N188S mutation by long-term oral administration of ambroxol hydrochloride. They showed remarkable neurological improvements, particularly oculomotor dysfunction and myoclonus. Further studies are in progress [Narita et al, unpublished data].

## 5 Expanding concept of chaperone therapy

The trial of chaperone therapy was started mainly for lysosomal diseases together with some studies on a few other diseases [10-11, 19]. The intralysosomal environment is uniquely acidic. We were able to utilize the differential pH conditions at the two compartments, ER/Golgi and the lysosome. A competitive enzyme inhibitor (chaperone) binds to the active site of the target enzyme at ER

under the neutral condition to form a stable complex, which is transported to the lysosome, where the complex dissociates under the acidic condition, due to less strong molecular binding, in the lysosome. The free mutant enzyme remains stable and becomes catalytically active [4, 20].

This is the concept of inhibitory chaperone therapy. An in vitro inhibitor acts as an intracellular enhancer at low concentrations. We should select an appropriate concentration of the chaperone compound in question in order to attain a chaperone effect without adverse (inhibitory) reaction to the cell. Chaperone therapy will be more safely conducted if a non-inhibitory compound is available for restoration of an inactive protein caused by misfolding.

Recently some trials have been made to overcome this problem. New chaperones have been identified and called non-inhibitory chaperones [21], non-competitive chaperones [22], or allosteric chaperones [23]. They are non-substrate-like compounds that exhibit allosteric chaperone activities, not necessarily binding to the active site of the enzyme. The research in this direction will reveal a new scope for chaperone therapy in future. In fact in a lecture at an international congress, the title "chaperone therapies" was proposed, suggesting diverse approaches to find new types of chaperone compounds with different molecular actions toward misfolding proteins [ Muntau, 12th International Congress of Inborn Errors of Metabolism, Barcelona, 2013].

## 6 Conclusion

Chaperone therapy has been proposed mainly as a new therapeutic approach to lysosomal diseases, particularly those with central nervous system involvement. Currently enzyme replacement therapy is widely used for extraneural tissue pathology, with successful achievements [16]. The effect on non-neural tissues has been well documented, but two major disadvantages are present: intravenous

administration for life at regular intervals, and poor effect to the central nervous system.

The second clinical approach has been proposed to reduce the storage substrates by inhibition of glucosyltransferase: substrate reduction therapy [24]. This new approach is meant to diminish glucosylceramide in the cell, the first step product of glycosphingolipid synthesis. In fact this trial has been reported not only for Gaucher disease with glucosylceramide storage but also for Niemann-Pick C disease, Sandhoff disease and other diseases with substrate storage of other types. However, this approach inevitably deprives somatic cells of biologically active glycosphingolipids to some extent, possibly ensuing dysfunction of various types of somatic cells. In fact clinical side effects have been recorded at therapeutic dose levels even in healthy individuals, particularly headache and diarrhea. This is the most important issue when this therapeutic approach is discussed for future clinical practice.

Chaperone therapy, originally proposed as chemical chaperone therapy, has been also called pharmacological chaperone therapy or enzyme enhancement therapy at present. Advantage of this new trial is non-invasive drug administration to achieve normal metabolic turnover and enhancement of missing enzyme activity in somatic cells and tissues. It is a mutation-specific drug therapy, and we admit that not all patients under diagnosis of a single genetic disease can be treated by one chaperone drug, although at least one-third to half of patients can be the target of this therapeutic trial. In addition combination of two or more chaperone compounds will reach a broader chaperone spectrum at least to two-thirds of patients. A combination therapy with enzyme replacement may be useful. Clinical effectiveness has been confirmed for $G_{M1}$-gangliosidosis model mice (NOEV) and for human Gaucher patients (ambroxol). No clinically recognizable adverse effects have been observed so far at the effective doses in mice and humans. Further experimental confirmation will be possible for this new therapeutic concept.

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