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Visions & Reflections (Minireview)

Chemical chaperone therapy for G_{M1}-gangliosidosis

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Abstract. We have proposed a chemical chaperone therapy for lysosomal diseases, based on a paradoxical phenomenon that an exogenous competitive inhibitor of low molecular weight stabilizes the target mutant molecule and restores its catalytic activity as a molecular chaperone intracellularly. After Fabry disease experiments, we investigated a new synthetic chaperone compound *N*-octyl-4-epi-β-valienamine (NOEV) in a G_{M1}-gangliosidosis model mice. Orally administered NOEV entered the brain through the blood-brain barrier, enhanced β-galactosidase activity, reduced the substrate storage, and clinically improved neurological deterioration. We hope that chemical chaperone therapy will prove useful for some patients with G_{M1}-gangliosidosis and potentially other lysosomal storage diseases with central nervous system involvement.

Keywords. Chemical chaperone therapy, G_{M1}-gangliosidosis, β-galactosidase, *N*-octyl-4-epi-β-valienamine, neurogenetic disease.

Introduction

For more than 15 years we performed molecular analyses of two genetic diseases: G_{M1}-gangliosidosis (OMIM 230500) with generalized neurosomatic dysfunctions and Morquio B disease (OMIM 253010) with generalized skeletal dysplasias without neurological involvement [1]. Both are caused by allelic mutations of the gene *GLB1* (3p21.33) coding for lysosomal β-galactosidase (EC 3.2.1.23) catalyzing hydrolysis of ganglioside G_{M1} and related glycoconjugates. Ganglioside G_{M1} accumulates in the G_{M1}-gangliosidosis-affected brain. Oligosaccharides derived from keratan sulfate or glycoproteins are detected in visceral organs and urine from G_{M1}-gangliosidosis and Morquio B patients.

At present only symptomatic therapy is available for the brain lesion in human G_{M1}-gangliosidosis patients. Enzyme replacement therapy is not possible. In 2003, we proposed chemical chaperone therapy for brain pathology in G_{M1}-gangliosidosis, using *N*-Octyl-4-epi-β-valienamine (NOEV) as a potent stabilizer of mutant β-galactosidase [2].

Concept of chemical chaperone therapy

In general, molecular events in genetic diseases are affected by various molecular processes [3]. Three possible molecular abnormalities can be listed in genetic diseases: (1) biosynthetic defect; (2) deficient catalytic activity of a mutant protein; and (3) unstable mutant protein with normal or near-normal biological activity. We tested these possibilities first in Fabry disease, and found some mutant enzyme proteins were unstable at neutral pH in the endoplasmic reticulum (ER)/Golgi apparatus, and rapidly degraded because of inappropriate molecular folding [4]. Galactose surprisingly induced a high expression of α-galactosidase A activity in cultured fibroblasts from Fabry patients [5]. We then found a commercially available compound 1-deoxygalactonojirimycin (DGJ) for stabili-

zation of the enzyme [6]. After extensive molecular analysis we came to the following conclusion.
A competitive inhibitor, a substrate of transition state analog, binds to misfolded mutant protein as a molecular chaperone in the ER/Golgi compartment of the cell, 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 conditions, the mutant enzyme remains stabilized, and its catalytic function is expressed.

NOEV: Physicochemical and biological characteristics

After using galactose and DGJ for α-galactosidase A, I came across two synthetic compounds: β-galactosidase inhibitor *N*-octyl-4-epi-β-valienamine (NOEV) and β-glucosidase inhibitor *N*-octyl-β-valienamine (NOV). NOV was the first compound synthesized as a glucocerebrosidase inhibitor, and NOEV was subsequently synthesized by epimerization of NOV [7]. NOEV experiments moved faster than NOV simply because of more experimental data and materials for β-galactosidase and G_{M1}-gangliosidosis.
NOEV is a potent inhibitor of lysosomal β-galactosidase *in vitro*. It is stable and freely soluble in methanol or DMSO. The hydrochloride salt is freely soluble in water. Its molecular weight is 287.40, and it has an IC_{50} of 0.125 μM toward human β-galactosidase [2].
NOEV is 50-fold more efficient than DGJ for expression of mutant β-galactosidase activity in G_{M1}-gangliosidosis. Our calculations suggest that at least 10% of normal enzyme activity is necessary for catalytic digestion of the storage substrate below the pathological level in lysosomal diseases. Theoretically the disease will not occur in patients expressing enzyme activity above this level during his or her lifetime. We anticipate that the effective NOEV concentrations in human cells and animal tissues are much lower than the IC_{50} for this agent *in vitro*. In fact, NOEV is effective at the IC_{50} concentration in the culture medium for enhancement of mutant enzyme activity [8]. Under the current experimental conditions, the NOEV content in the lysosomal compartment must be much lower than that in the extracellular environment.

NOEV effect on human and mouse fibroblasts expressing mutant human enzyme

About one-third of cultured fibroblasts from G_{M1}-gangliosidosis patients, mainly with the juvenile and some of the infantile forms, responded to NOEV. The effect was mutation specific [8]. The R457Q mutant cells responded to NOEV maximally at 0.2 μM, and the R201C/R201H mutant cells at 2 μM. The knockout (KO) mouse fibroblasts expressing mutant human β-galactosidase [9] showed essentially the same results [2]. Addition of ganglioside mixture in the culture medium increased intracellular G_{M1} in the R201C cells causing juvenile G_{M1}-gangliosidosis. This storage was almost completely prevented by NOEV.

Chemical chaperone therapy on genetically engineered G_{M1}-gangliosidosis model mice

For animal studies, we developed a KO mouse strain with complete deficiency of β-galactosidase [10], and then a transgenic (Tg) strain based on KO, expressing the human R201C mutation (4% normal β-galactosidase activity in the brain). Both strains showed neurological deterioration that differed in severity. Life-span was 7–10 months for KO and 12–18 months for Tg. Neuropathology corresponded to the clinical severity [2]. Short-term oral NOEV administration resulted in significant enhancement of the enzyme activity in all the R201C mouse tissues examined, including the brain [2]. Immunohistochemistry revealed an increase in β-galactosidase activity and decrease in G_{M1} and G_{A1} storage.
An early NOEV treatment study with the R201C mouse showed an increase of the NOEV content in the brain after starting oral administration, its rapid disappearance after withdrawal, parallel increase of β-galactosidase activity, and decrease of G_{M1} storage [11]. In this study we tried a new scoring system for neurological assessment [12]. Treatment at the very early clinical stage (2 months) resulted in a positive clinical effect within a few months, although complete arrest or prevention of disease progression was not achieved under this experimental condition. The latency before a clinical effect was longer if the therapy was started in the late symptomatic stage (6 months). We concluded that NOEV treatment at the early stage of disease is mandatory for prevention of the brain damage.
This result indicated the following sequence of events in the brain (Fig. 1). After oral administration, NOEV goes directly into the bloodstream without intestinal breakdown, is delivered to the mouse brain through the blood-brain barrier, and enhances the mutant β-galactosidase activity, resulting in substrate digestion and clinical improvement. No specific adverse effects have been observed for at least 6 months of continuous oral administration.

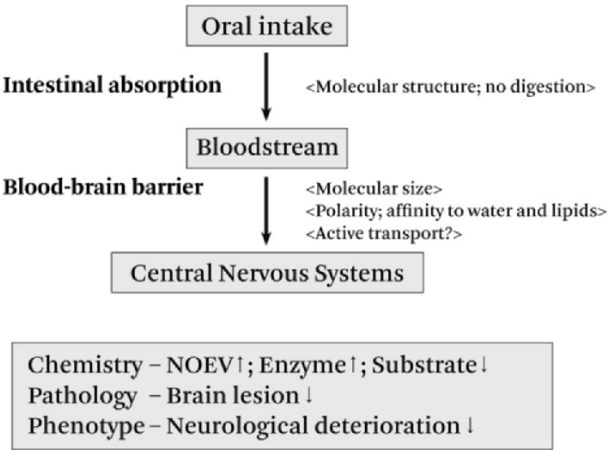

Figure 1. *N*-octyl-4-epi-β-valienamine (NOEV) goes directly from the gastrointestinal tract into the bloodstream without intestinal digestion, and into the brain through the blood-brain barrier. The catalytic activity of the mutant β-galactosidase is enhanced and the substrates are digested to improve phenotypic manifestations.

Future prospects

This new therapeutic strategy is in principle applicable to all lysosomal storage diseases if a specific compound is developed for each enzyme in question. We have already confirmed the effect in Fabry disease [5, 6], G_{M1}-gangliosidosis [2, 11], and Gaucher's disease [13, 14]. Our study started with a lysosomal disease, and is currently being expanded to a few related diseases. Further, there may be other genetic diseases that could be considered, provided that the molecular pathology in somatic cells has been studied and is understood in detail. I hope that studies in this direction will disclose a new aspect of molecular therapy for inherited metabolic diseases with central nervous system involvement in future.

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