Genetic Protein Misfolding Disorders: Development of new pharmaco-therapeutic strategies

1. Summary

Single genetic diseases are rare (orphan diseases), but the whole group of disorders may affect one in 100 individuals. Recent advances in the field of functional genomics revealed that a surprisingly high number of genetic diseases is caused by protein misfolding and can be treated by small molecules that restore correct protein folding acting as "pharmacological chaperones".

2. Goals of the Project

In 2002, we had shown that the small molecule 6[R]-L-erythro-5,6,7,8-tetrahydrobiopterin (BH₄) rescues the biochemical phenotype in a significant share of patients suffering from phenylketonuria (PKU), the most frequent genetic disorder of amino acid metabolism. The project aimed to elucidate the molecular basis of PKU and the mode of action of the small molecule exerting its effect in the presence of several different genotypes. Moreover, we wanted to establish PKU as a model disease for a larger number of disorders showing the molecular phenotype of protein misfolding with loss-of-function potentially correctable by the oral administration of small molecules.

3. Results

To elucidate the molecular basis of functional impairment in PAH deficiency, we first investigated the impact of PAH missense mutations on function and structural conformation of the purified recombinant PAH protein (Figure 1 and Figure 2). Residual enzyme activity was generally high, but allostery was disturbed in almost all cases and pointed to altered protein conformation. This was confirmed by reduced proteolytic stability, impaired tetramer assembly or aggregation, increased hydrophobicity, and accelerated thermal unfolding observed in most variants. Three-dimensional modeling revealed the involvement of functionally relevant amino acid networks that communicate misfolding throughout the protein. Our results substantiated the view that PAH deficiency is a protein misfolding disease in which global conformational changes hinder molecular motions essential for physiological enzyme function. At this time, PKU had evolved from a model of a genetic disease that leads to severe neurological impairment to a model of a treatable protein misfolding disease with loss-of-function. In 2007 (FDA) and 2008 (EMA) sapropterin dihydrochloride, the synthetic form of BH₄, was approved – also on the basis of our scientific work – as an orphan drug for the treatment of PKU. This initiated a paradigm change in the therapy of monogenetic diseases. However, at the time of approval the mode of action of the new drug was still not understood. To address this issue, we identified a mouse model showing the specific phenotype of BH₄-responsive PAH deficiency, the Pahtncl mouse, carrying two mild mutations in the PAH gene (Figure 3). We showed that loss of function in this disease results from loss of PAH, a consequence of misfolding, aggregation, and accelerated degradation of the enzyme. BH₄ attenuates this triad by conformational stabilization augmenting the effective amount of the PAH protein in the cell. This leads to rescue of the biochemical phenotype and enzyme function in vivo defining BH₄ as the first pharmacological chaperone on the market. This data provided new molecular-level insights into the mechanisms underlying protein misfolding with loss-of-function and supported a general model of pharmacological chaperone-induced stabilization of protein conformation to correct this intracellular pathology. In future, Pahtncl will be essential for pharmaceutical drug optimization and to design individually-tailored therapies. To address the fact...
Future prospects and possible economic impact

If the general molecular concept of protein misfolding linked to virtually all missense mutations holds true, and if we take into account that the overall frequency of monogenetic diseases is one in 100 with a total of 100,329 disease-associated single amino acid substitutions described, a systematic approach to detect genetic protein misfolding disorders and to develop pharmacological chaperone therapies for these diseases may be promising. This concept will define a new and rapidly expanding class of disorders and open the door to the development of next generation orphan drugs for patients with inborn errors of metabolism, for other monogenetic diseases, for polygenetic diseases, and potentially also for nongenetic multifactorial diseases. The possible economic impact arises from the frequency of the candidate disorders and from the general principle of life-long treatment. This is demonstrated by a market potential of 1 Billion Euro per year for Kuvan® (Merck Serono) based on disease incidence and price underscoring the commercial opportunities linked to drug development for orphan diseases.

Selected publications


