A New Approach to the Treatment of Gaucher Disease: Mechanism of Enhancement of N370S GCase Activity

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Gaucher Disease is a lysosomal storage disorder caused by a deficiency in acid β-glucosidase (glucocerebrosidase or GCase), the lysosomal enzyme responsible for the catabolism of the glycolipid glucosylceramide. Certain mutations, including the common N370S GCase variant, have been shown to cause impaired GCase exit from the ER with a significant fraction eliminated by ER-associated degradation (ERAD) (Schmitz et al., 2005; Ron & Horowitz, 2005). Using pulse-chase experiments, we have shown that approximately 70% of the newly synthesized N370S GCase protein is degraded via ERAD in Gaucher patient fibroblasts. By contrast, when these cells are incubated with the iminosugar isofagomine (IFG), a reversible inhibitor of GCase, the total amount of newly synthesized N370S GCase exported out of the ER is significantly increased as shown by increased N-linked oligosaccharide processing. These data indicate that IFG functions as a pharmacological chaperone by stabilizing N370S GCase in the ER and facilitating its release from this compartment. The improved protein stability and trafficking translates into an extended cellular half-life, which in turn provides significant gains in GCase levels in the lysosomes as shown by confocal immunofluorescence microscopy and subcellular fractionation experiments. Once in the lysosome, the rescued N370S GCase persists for more than 3 days and is catalytically active after drug washout as measured by an *in situ* enzyme activity assay. Interestingly, IFG treatment also increases the specific activity of the rescued N370S GCase enzyme, possibly due to a shift in the pH optimum to 5.2, the optimal pH for the wild type enzyme. The specificity of IFG was determined by evaluating its effect on other glucosidases (e.g., intestinal disaccharidases and N-glycan processing enzymes) and glucosylceramide synthase and we found that IFG is much more specific than the related iminosugar *N*-butyl-deoxynojirimycin (NB-DNJ, also known as miglustat). These findings suggest that the small molecule pharmacological chaperone IFG may be a useful treatment for Gaucher disease.

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