

## **The Pharmacological Chaperone Isofagomine Increases L444P GCCase Levels in Mice with a Gaucher Disease-like Phenotype**

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Gaucher disease is a lysosomal storage disorder caused by a deficiency in acid  $\beta$ -glucosidase (GCCase) activity. This disease has been broadly classified into two forms on the basis of central nervous system involvement: a non-neuronopathic form (Type I) and the more severe neuronopathic form, with infantile or juvenile onset (Types II and III, respectively). Gaucher patients homozygous for a common GCCase missense mutation (L444P) typically present with a neuronopathic form of the disease. In addition to the CNS manifestations, these patients also have typical Gaucher-related pathologies, including hepatosplenomegaly, bone crises, pulmonary disease, thrombocytopenia and anemia. The use of small molecule pharmacological chaperones to increase the activity of less stable mutant proteins is a promising new therapeutic approach, in part because they are orally available and can cross the blood-brain barrier. To better understand the effects of the GCCase-selective pharmacological chaperone isofagomine (IFG), we have used a knock-in mouse model expressing murine L444P GCCase. Compared to humans with the same mutation, these mice have an attenuated phenotype, characterized by reduced tissue GCCase activity, moderately increased liver and spleen weights, and elevated plasma markers (IgG and chitin III) compared to isogenic wild type mice. Oral administration of IFG to L444P mice resulted in a dose-dependent increase in GCCase levels (2 to 5-fold) in liver, lung, spleen, skin and, importantly, brain, with a minimum effective dose of 3 mg/kg. In separate studies, IFG was detected in rat brain and monkey CSF, consistent with the increased GCCase activity seen in the brain of L444P mice. The IFG-mediated increase in L444P GCCase levels was selective, as the activities of the lysosomal hydrolases  $\alpha$ -galactosidase A,  $\alpha$ -glucosidase,  $\beta$ -glucuronidase, and  $\beta$ -galactosidase were not altered by IFG treatment in any tissue examined. Concomitant with increased GCCase activity, IFG also lowered plasma IgG (15%) and chitin III (33%) levels. Similarly, treatment for 3 to 6 months with IFG significantly decreased spleen (22%) and liver (20%) weights in these mice. In line with previously published data, fibroblasts generated from the L444P mice showed no increase in GCCase activity after IFG treatment *in vitro*. However, primary liver macrophage cultures isolated from L444P mice did show an increase in GCCase activity in response to *ex vivo* treatment with IFG. Collectively, these data indicate that administration of IFG to L444P mice increases tissue GCCase activity and results in an improvement of the Gaucher-like phenotype. Thus, the pharmacological chaperone IFG merits further evaluation for the treatment of neuronopathic forms of Gaucher disease.

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