

# Studying Eu-labeled $\alpha$ -Gal A uptake in pathologically relevant cell models of Fabry disease

Fabry disease is a rare disorder which can cause fatal renal, cardiac and cerebrovascular complications. It results from the accumulation of glycosphingolipids, mainly globotriasylceramide (Gb3), in lysosomes due to a deficiency in  $\alpha$ -Galactosidase A ( $\alpha$ -Gal A). Two  $\alpha$ -Gal A enzyme replacement therapies are currently licensed worldwide; Fabrazyme® (Genzyme Corporation, a Sanofi Subsidiary) and Replagal® (Shire Human Genetic Therapies). The effects of both therapies on reversing lysosome storage varies between tissue and cell types, and is ineffective in many cases.

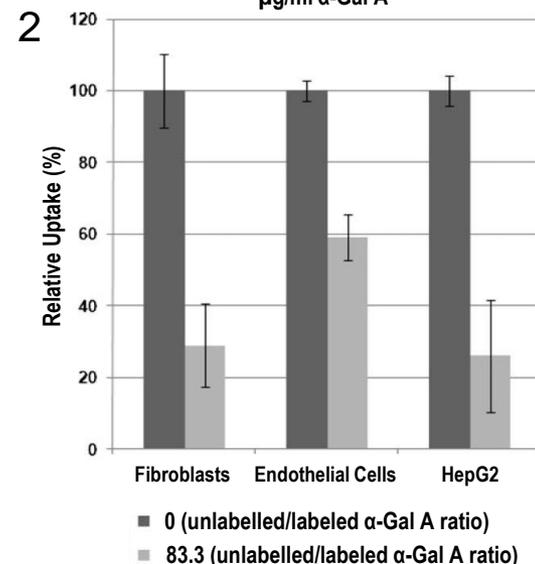
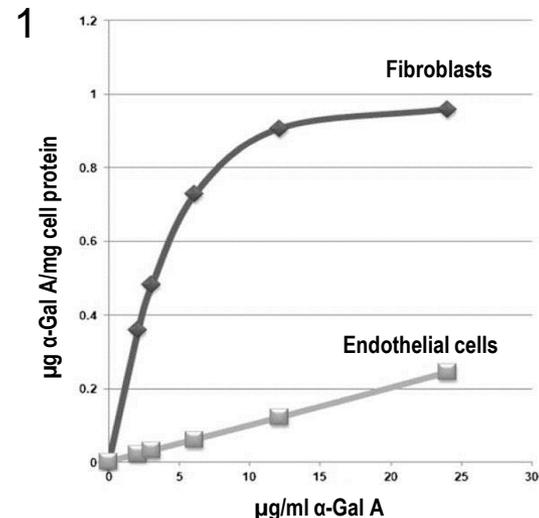
Current knowledge of  $\alpha$ -Gal A uptake mechanisms and clearance of lysosomal storage in pathologically relevant tissues is incomplete. Many studies have been conducted using mouse models and human fibroblasts, where it is well documented that uptake occurs via a mannose-6-phosphate (M6P) receptor-mediated recapture pathway. It is assumed that  $\alpha$ -Gal A uptake is similar in all tissues, however, the authors of this study hypothesised that more pathologically relevant cells do not share the effective uptake of  $\alpha$ -Gal A seen in fibroblasts. In order to understand the effectiveness of Fabry disease treatments, they set out to investigate  $\alpha$ -Gal A uptake in models of tissues and organs affected by storage and pathology.

Researchers used time-resolved fluorescence (TRF) to follow the uptake of Europium (Eu)-labeled therapeutic  $\alpha$ -Gal A in different cell types. The naturally fluorescent lanthanide Eu has high sensitivity and very low background, making it highly amenable to TRF detection. Labeling of  $\alpha$ -Gal A was performed using a DELFIA® Eu-labeling kit, and TRF detection was measured using the VICTOR™ 3 Multilabel Plate Reader.

For both Fabrazyme® and Replagal® formulations of  $\alpha$ -Gal A, time resolved fluorescence detection showed that uptake in fibroblasts was saturable (figure 1) and inhibited by excess unlabeled  $\alpha$ -Gal A (figure 2) and M6P. Lysosomal precipitation experiments and confocal microscopy revealed that 70 % of the labeled  $\alpha$ -Gal A was associated with lysosomes. Together, these results are consistent with the expected M6P receptor-mediated uptake mechanism in fibroblasts.

In contrast,  $\alpha$ -Gal A uptake in vascular endothelial cells was found to be non-saturable (figure 1), inhibited to a much lesser extent by excess  $\alpha$ -Gal A (figure 2) and M6P, and only 5 % was associated with lysosomes. These results suggest that endothelial cell uptake of  $\alpha$ -Gal A is independent of the M6P receptor pathway.

The results highlight the importance of studying uptake in pathologically relevant cells. The findings may also contribute to an understanding of the reported ineffectiveness of  $\alpha$ -Gal A replacement in Gb3 lysosomal clearing in some cell types.



**Figure 1:** TRF detection of Eu-labeled  $\alpha$ -Gal A uptake in fibroblasts and blood outgrowth endothelial cells after 3 h incubation at increasing enzyme concentration.

**Figure 2:** Degree of  $\alpha$ -Gal A uptake inhibition in fibroblasts, human pulmonary arterial endothelial cells and HepG2 cells by 83-fold excess of unlabeled  $\alpha$ -Gal A. Experiments were carried out comparing Fabrazyme® with Replagal® and the results were essentially the same.