Co-Administration of the Pharmacological Chaperone AT2221 with A Proprietary Recombinant Human Acid α-Glucosidase Leads to Greater Plasma Exposure and Substrate Reduction Compared to Alglucosidase Alfa

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Introduction

Pompe disease is an inherited lysosomal storage disorder that results from a deficiency in acid alpha-glucosidase (GAA) activity, and is characterized by progressive accumulation of lysosomal glycogen in cardiac and skeletal muscles. Enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA) is the only approved treatment available for Pompe disease. While rhGAA provides some clinical benefits, the infused enzyme shows insufficient uptake into key disease-relevant muscles, which is likely due to sub-optimal levels of mannose-6-phosphate (M6P), a carbohydrate that binds cation-independent M6P receptors (CI-MPR) at the cell surface resulting in enzyme internalization and lysosomal targeting. In order to increase the targeting efficiency of ERT, we have developed a proprietary mammalian cell line and purification process that yields a novel form of rhGAA (designated as ATB200) with a significantly higher M6P content compared to the alglucosidase alfa. In this study, we have examined the effects of ATB200 on tissue exposure and substrate reduction with and without the addition of a small molecule pharmacological chaperone (PC) AT2221.

1. ATB200 Has a Higher M6P Content and Results in Better Tissue Uptake and Greater Glycogen Reduction in vivo Compared with Alglucosidase Alfa

(A) AT-B200 was co-administered with a small molecule pharmacological chaperone (PC) AT2221. (B) Quadriceps of Gaa KO Mice

GAA Activity

Untreated Alglucosidase alfa ATB200 ATB200 + AT2221 WT

(C) Glycogen by PAS Stain

2. The Pharmacological Chaperone AT2221 Increases the Stability and Exposure of ATB200

(A) Thermal stability of ATB200 and ATB200 + AT2221 was assessed for 5 days at pH 7.4 and pH 5.2. (B) Plasma Exposure in NHPs

3. AT2221 Co-administration Leads to Greater ATB200-mediated Glycogen Reduction and Reduces Lysosome Proliferation in Disease-relevant Muscles of Gaa KO Mice

(A) Twelve-week-old male Gaa KO mice were administered a total of 2 bi-weekly bolus IV injections of 20 mg/kg alglucosidase alfa or ATB200. In addition, ATB200 was co-administered with various doses of AT2221 (3-30 mg/kg). Glycogen levels were determined in quadriceps collected 14 days post last dose. (Left) While ATB200 alone resulted in greater glycogen reduction compared to alglucosidase alfa, its efficacy is further improved by co-administration, mostly significantly with 10 mg/kg ATB200 (green arrow). Bars represent Mean ± SEM of 7-21 mice/group. * p<0.05 vs. alglucosidase alfa; # p<0.05 vs. ATB200 alone in 2-sided t-test. (Right) PAS staining also showed the lowest glycogen level in quadriceps with the co-administration of 10 mg/kg AT2221 with 20 mg/kg ATB200 (upper panel). Co-administration of 10 mg/kg AT2221 also led to marked further reduction in glycogen compared to ATB200 alone in heart (bottom panels) and additional tissues (data not shown). Magnification = 200x.

(B) Subsequently, the effect of co-administration of 20 mg/kg ATB200 + 10 mg/kg AT2221 was compared with 20 mg/kg alglucosidase alfa or ATB200 alone in another 2-bi-weekly-administration study in male Gaa KO mice of twelve-weeks of age. IHC examination of lysosome marker LAMP1 in quadriceps revealed a substantial up-regulation of LAMP1 in fibers of untreated animals (top panel), which is indicative of lysosomal proliferation, a hallmark of Pompe disease. Unlike alglucosidase alfa, ATB200 alone leads to a marked decrease in LAMP1 signal, whose level was lowered further still with the co-administration of AT2221, approaching that seen in WT tissues. The change in LAMP1 level closely follows the change in glycogen level in quadriceps, and is repeated in additional tissues, such as heart, diaphragm, and soleus. Magnification = 400x.

(B) Moreover, the fiber type response to ATB200 was investigated by IHC with LAMP1 antibody (top) and a type I (slow twitch) fiber-specific antibody NQG 5-4D (bottom) on adjacent sections of soleus, which has a relatively equal representation of both type I and II (fast twitch) fibers. ATB200 alone is much more effective than alglucosidase alfa, as indicated by the normalization of LAMP1 levels in most type I fibers and, significantly, a fraction of type II fibers as well, contrary to their reported resistance to alglucosidase alfa. With co-administration, a reversal of lysosomal proliferation was achieved in the majority of muscle fibers, regardless of fiber type. This result is consistent with the observed superiority of ATB200 + AT2221 compared to alglucosidase alfa in quadriceps and diaphragm (B), tissues with a predominant type II fiber content. Asterisks mark all of the type I fibers in a section, while the red triangles highlight the type II fibers with significantly reduced LAMP1 signals. Magnification = 400x.

Summary and Conclusions

- We have developed a novel rhGAA, ATB200, with a significantly higher M6P content compared to alglucosidase alfa, which resulted in greater enzyme uptake and glycogen reduction in disease-relevant tissues of Gaa KO mice, likely due to the improved endocytosis and lysosomal targeting of the exogenous recombinant enzyme mediated by the binding of M6P to its receptor CI-MPR.

- More importantly, we showed that co-administration with the optimized pharmacological chaperone AT2221 leads to further improvement of the efficacy of ATB200, possibly via binding and stabilizing ATB200 in the blood, keeping the enzyme in a properly folded, active form that is more accessible for tissue uptake and lysosomal delivery. As a result, AT2221 improves the exposures of ATB200, broadens its bio-distribution, and achieves significantly greater glycogen reduction in disease-relevant cell types/tissues that have responded poorly to alglucosidase alfa, such as type II skeletal muscle fibers and skeletal muscles with a higher content of type II fibers.

- Taken together, these preclinical data highlight the efficacy of our proprietary rhGAA, ATB200, in mice when combined with a pharmacological chaperone using our proprietary CHART platform, and thus warrant further investigation.