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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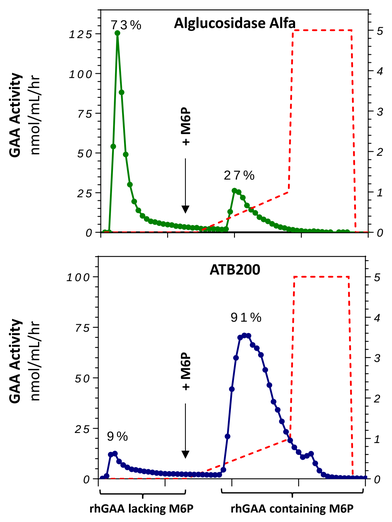
Introduction

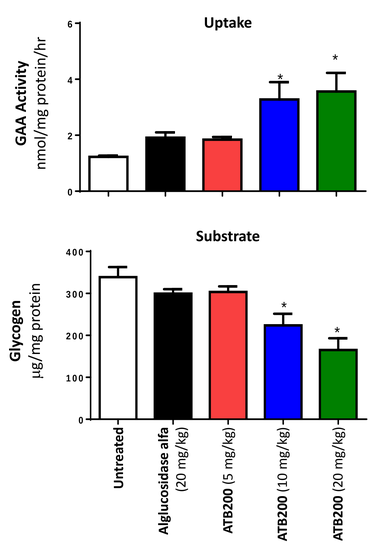
Pompe disease is an inherited lysosomal storage disorder that results from a deficiency in acid alpha-glucosidase (GAA) activity, and is characterized by progressive accumulation of lysosomal glycogen in cardiac and skeletal muscles. Enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA) 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

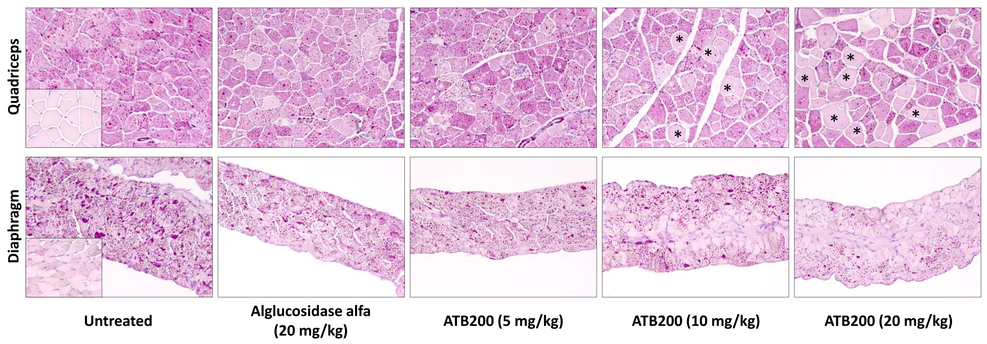
(A) CI-MPR Affinity Chromatography



(B) Quadriceps of *Gaa* KO Mice



(C) Glycogen by PAS Stain



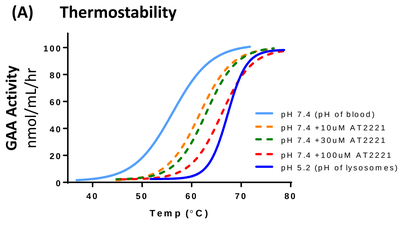
(A) Alglucosidase alfa or ATB200 was loaded onto a CI-MPR column. Only enzyme that contained M6P was retained and then eluted from the column using free M6P of increasing concentration (dotted line in red). Both unbound (flow-thru) and bound/eluted fractions were collected and assayed for GAA activity. The majority of ATB200 (91%) was bound compared to alglucosidase alfa (27%), suggesting that ATB200 has a higher M6P content, which is key to the efficient endocytosis and lysosomal targeting of rhGAA.

(B) Twelve-week-old male *Gaa* KO were administered 2 bi-weekly bolus intravenous (IV) injections of alglucosidase alfa (20 mg/kg) or ATB200 (5-20 mg/kg) via tail vein (n=6-7 per group). Quadriceps were collected 14 days post the last dose and measured for GAA activity and glycogen levels. ATB200 shows dose-dependent increases in uptake and substrate reduction. Importantly, 5 mg/kg ATB200 is comparable to 20 mg/kg alglucosidase alfa, whereas 20 mg/kg ATB200 is significantly better than alglucosidase alfa, indicating improved potency of ATB200. Bars represent mean ± SEM. * p<0.05 vs. alglucosidase alfa in 2-sided t-test.

(C) Paraffin sections of quadriceps and diaphragm from study described in panel B were also examined for glycogen accumulation by Periodic acid-Schiff's reagent (PAS), which stains glycogen magenta. Consistent with the biochemical measurements, 20 mg/kg ATB200 appeared more effective in glycogen reduction compared to 20 mg/kg alglucosidase alfa in both tissues. Images of age-matched wild-type (WT) animal are shown in the insets. Each image is representative of 6-7 animals per group. Magnification is 200x.

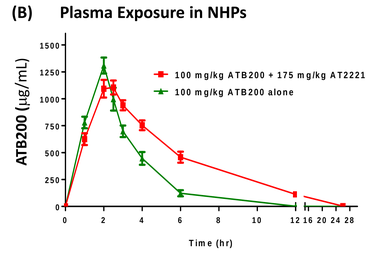
2. The Pharmacological Chaperone AT2221 Increases the Stability and Exposure of ATB200

(A) Thermostability



Test Condition	Tm (°C)
pH 7.4	56.2
pH 7.4 + 10 µm AT2221	61.6
pH 7.4 + 30 µm AT2221	62.9
pH 7.4 + 100 µm AT2221	66.0
pH 5.2	67.3

(B) Plasma Exposure in NHPs



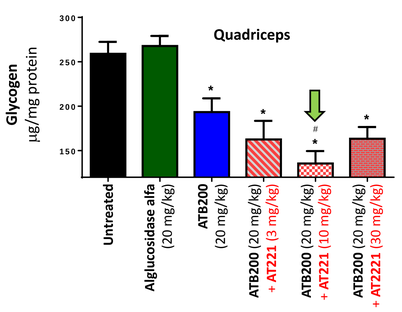
PK Measurement	ATB200	ATB200 + AT2221
AUC (µg · hr/mL)	3647	6801
$T_{½}$ (hr)	1.2	3.2
Cmax (µg/mL)	1155	1105
Tmax (hr)	2	2.5

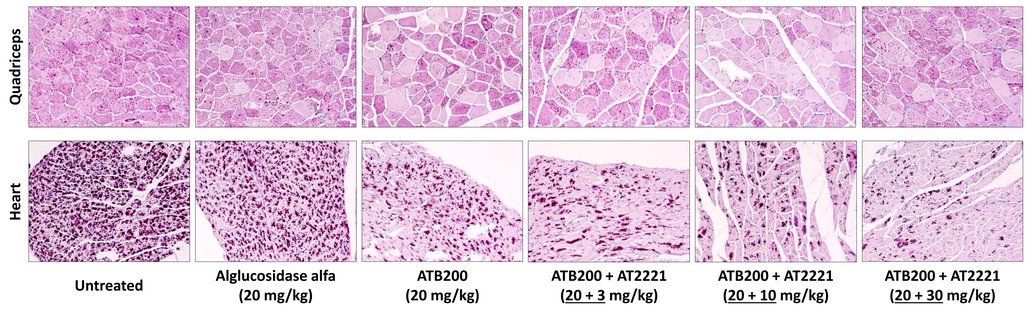
(A) The stability of ATB200 in acidic or neutral pH buffers was evaluated in a thermostability assay using SYPRO Orange. AT2221 stabilizes ATB200 at pH 7.4 in a concentration-dependent manner, to approaching the level seen at pH 5.2, a condition that mimics the acidic environment of the lysosome, as demonstrated by a nearly 10°C increase in the melting temperature (T_m) of ATB200.

(B) Cynomolgus monkeys (2-3 years of age) were administered a single 2-hour IV infusion of 100 mg/kg ATB200 alone or with oral administration of 175 mg/kg AT2221 30 minutes earlier. Plasma samples were collected over the following 24 hours and GAA activity was determined. Co-administration resulted in an approximate 2-fold increase in ATB200 exposure (AUC) and half-life ($T_{½}$), compared to administration of ATB200 alone. Each time point represents the mean ± SEM of 8 NHPs (4 males and 4 females)/group.

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

(A) AT2221 Dose Range Determination



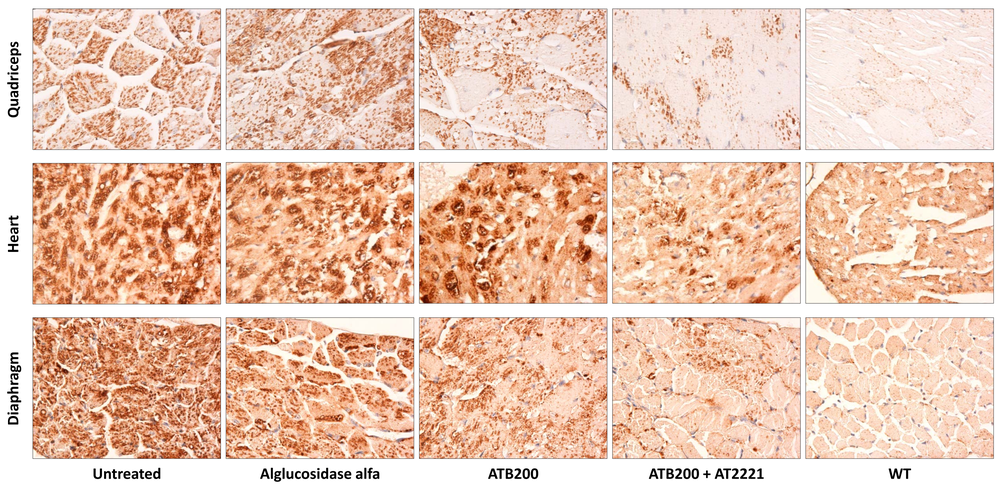


(A) Twelve-week-old male *Gaa* KO mice were administered a total of 2 bi-weekly bolus IV injections of 20 mg/kg alglucosidase alfa or ATB200. In addition, ATB200 was co-administered with various doses of AT2221 (3-30 mg/kg). Glycogen levels were determined in quadriceps collected 14 days post-last dose. (**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 AT2221 (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 panels). Co-administration of 10 mg/kg AT22221 also led to marked further reduction in glycogen compared to ATB200 alone in heart (bottom panels) and additional tissues (data not shown). Magnification = 200x.

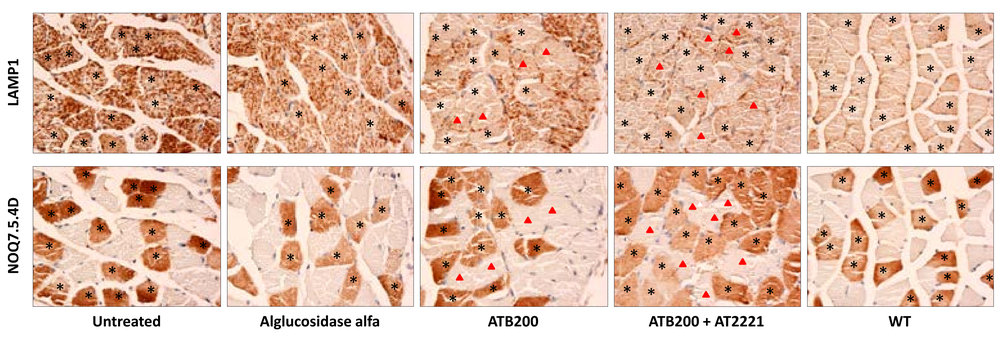
(B) Subsequently, the effect of co-administration of 20 mg/kg ATB200 + 10 mg/kg AT2221 was compared with 20 mg/kg alglucosidase alfa or ATB200 alone in another 2-biweekly-administration study in male *Gaa* KO mice of twelve-weeks of age. IHC examination of lysosome marker LAMP1 in quadriceps revealed 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.

(C) Moreover, the fiber type response to ATB200 was investigated by IHC with LAMP1 antibody (top) and a type I (slow twitch) fiber-specific antibody NOQ7.5.4D (bottom) on adjacent sections of soleus, which has a relative equal representation of both type I and type 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 the type I fibers in a section, while the red triangles highlight the type II fibers with significantly reduced LAMP1 signals. Magnification = 400x.

(B) IHC Examination of LAMP1 in Disease-Relevant Muscles of *Gaa* KO Mice



(C) IHC Examination of Fiber Type Response to ATB200 in Soleus



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 lysosome 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.