Subcutaneous Administration of Recombinant Human Acid α-Glucosidase Co-formulated with the Pharmacological Chaperone AT2220 Leads to Lysosomal Uptake of rhGAA and Glycogen Reduction in Disease-relevant Tissues of Pompe Mice

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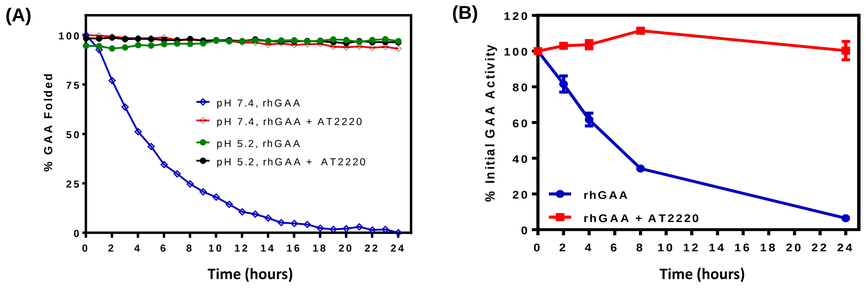
Introduction

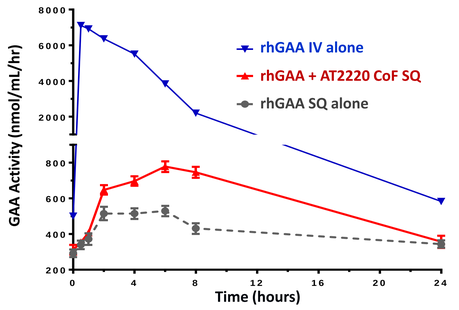
Pompe disease is an inherited lysosomal storage disease that results from deficiency in acid α-glucosidase (GAA) activity, and is characterized by progressive accumulation of lysosomal glycogen in heart and skeletal muscles. Enzyme replacement therapy using recombinant human GAA (rhGAA) is the only approved treatment available for Pompe disease. While rhGAA provides some clinical benefits, the infused enzyme tends to be unstable at neutral pH/body temperature, shows insufficient uptake in key tissues, and can elicit immune responses that affect tolerability and efficacy. We have shown previously that oral pre-administration or co-formulation of the pharmacological chaperone AT2220 (1-deoxynojirimycin HCl, duvoglustat HCl) improves the pharmacological properties of IV rhGAA via binding and stabilization in the blood, leading to increased enzyme uptake and glycogen reduction in *Gaa* knock-out (KO) mice. In this study we compared the effects of subcutaneous (SQ) administration of co-formulated rhGAA and AT2220 (denoted hereafter as "co-formulation", or "rhGAA + AT2220 CoF") to that of IV administration of rhGAA alone.

1. AT2220 Prevents rhGAA Denaturation and Loss of Activity *in vitro and ex vivo*



A. The stability of rhGAA (1 μM) ± AT2220 (50 μM) was measured as a function of time in buffers of neutral and acidic pH at 37 ºC in a thermal denaturation assay, using the environment-sensitive dye SYPRO® Orange which binds to exposed hydrophobic residues when proteins denature. **B**. The activity of rhGAA (0.5 μM) ± AT2220 (50 μM) was also measured in human blood (~ pH 7.4) at 37 ºC in a 4-MUG assay. While rhGAA remained stable at pH 5.2 (a condition mimicking the acidic environment of the lysosome), the protein quickly unfolded at pH 7.4 ($t_{1/2}$ ≈ 3 hr) and lost its activity by the end of a 24-hr period. Co-incubation with AT2220 stabilized rhGAA, preventing its pH-, temperature-, and time-dependent denaturation and inactivation, thus allowing the protein to retain its structural integrity and activity over a 24-hr period.

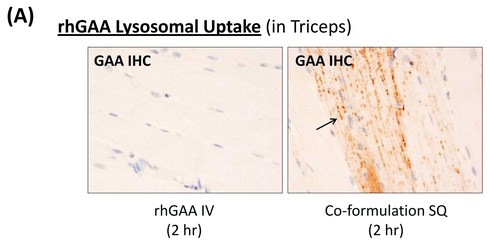
2. Co-formulation Followed by SQ Administration Increases the Circulating Levels of Active rhGAA in Rats



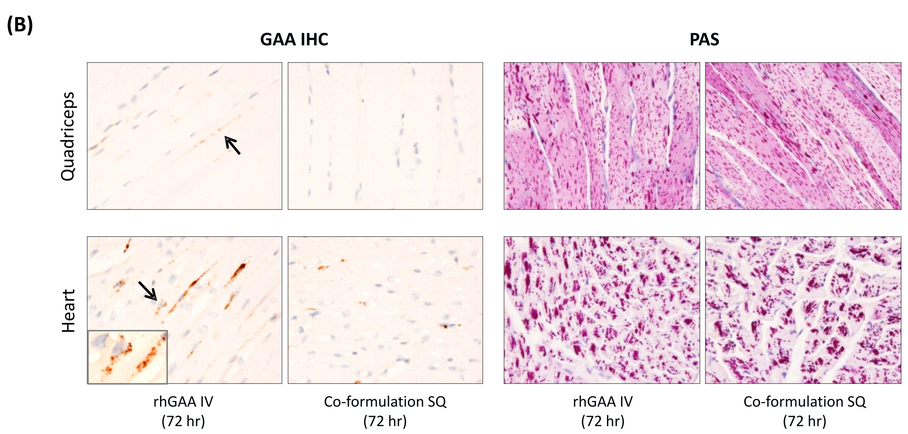
Plasma PK	rhGAA IV	Co-formulation SQ	rhGAA SQ
AUC (nmol/mL)	61594	14053	10016
Cmax (nmol/mL/hr)	7110	778	529
Tmax (hr)	0.5	6.0	6.0

Eight-week-old male Sprague Dawley rats (n=3/group) were given a single administration of either rhGAA (20 mg/kg) alone via IV bolus tail vein injection or rhGAA ± co-formulated AT2220 (30 mg/kg) via SQ injection between the shoulder blades. Plasma samples were taken at various time points and rhGAA PK was determined as measured by GAA activity. The exposure (AUC) and maximal plasma levels (Cmax) of the enzyme form rhGAA SQ alone were 16% and 7%, respectively, of the values from rhGAA IV alone. Co-formulation followed by SQ administration increased the AUC and Cmax values of rhGAA both by up to 1.5 fold compared to rhGAA SQ alone. The PK profile of SQ administration of co-formulated rhGAA indicates a slow absorption into blood (Tmax=6.0 hr), which could be attributed to a substantial retention and/or subsequent cellular uptake of rhGAA in tissues close to the injection site.

3. Single SQ Administration of Co-formulated rhGAA Leads to Greater Tissue Uptake and Glycogen Reduction in *Gaa* KO Mice

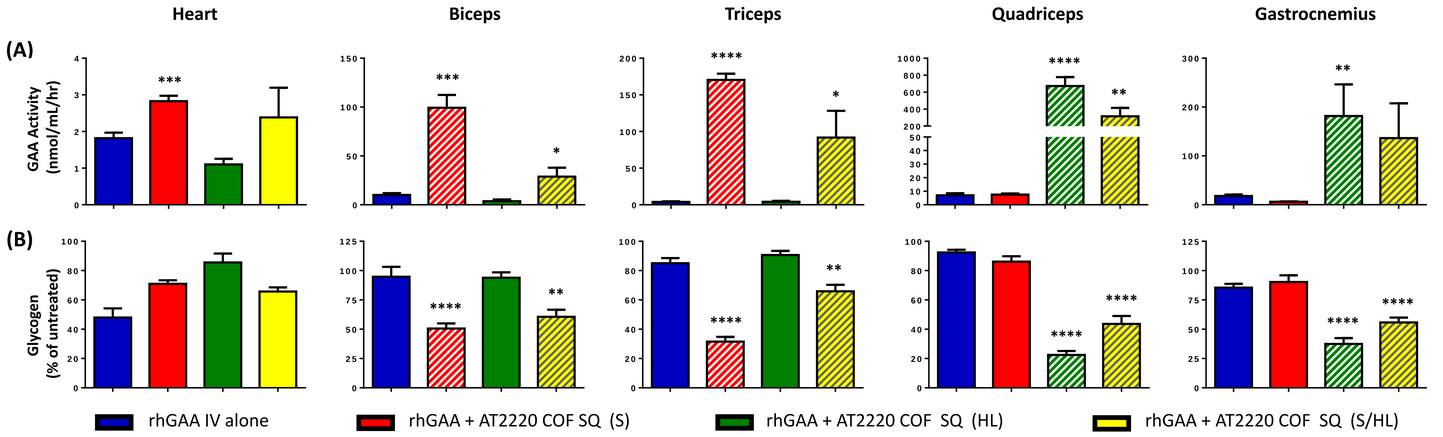


Eight-week-old male *Gaa* KO mice (n=3/group) were given either a single administration of rhGAA alone (20 mg/kg) via IV bolus tail vein injection or co-formulated rhGAA + AT2220 (30 mg/kg) via SQ injection between the shoulder blades. Tissues were collected 2 and 72 hours post-injection and analyzed for rhGAA uptake (by IHC) and glycogen levels (by PAS). A significant increase in rhGAA uptake into skeletal muscle fibers (thin arrow) was observed in triceps with co-formulation 2 hours post-SQ injection (top). The IHC pattern of GAA resembled the vesicular staining of LAMP1 (data not shown), a lysosomal marker, suggesting that rhGAA was correctly folded, processed, and taken into lysosomes. At 72 hours post-SQ injection, individual fibers with rhGAA uptake also showed reduced LAMP1 level, whose elevation in *Gaa* KO mouse tissues signifies lysosomal proliferation, a hallmark of Pompe disease (middle). In addition, increased uptake in triceps translated to a decrease in glycogen levels (bottom). ** marks a muscle fiber with rhGAA uptake and reduced LAMP1 on adjacent sections. Fibers with significant glycogen reduction are labeled with #.



Consistent with its low plasma exposure, SQ administration of co-formulated rhGAA showed less efficient uptake in quadriceps and heart, two tissues that are more distant to the injection site, compared to rhGAA IV alone. Nevertheless, a single IV administration of rhGAA alone did not yield greater glycogen reduction in these two tissues compared to a single SQ administration of co-formulated rhGAA + AT2220. Arrows point to the skeletal myofiber or cardiac myocyte positive for GAA IHC signals.

4. Repeat SQ Administration of Co-formulated rhGAA Leads to Greater Enzyme Uptake and Glycogen Reduction in Multiple Tissues of *Gaa* KO Mice



Twelve- or fourteen-week-old male *Gaa* KO mice were administered co-formulated rhGAA (20 mg/kg) + AT2220 (30 mg/kg) via SQ twice a week (Mondays and Thursdays) for a total of four injections. The injections were given between the shoulder blades (S), by the left upper hind limb (HL), or alternating between the aforementioned two locations (S/HL). In comparison, *Gaa* KO mice were administered rhGAA (20 mg/kg) alone via IV bolus tail vein injection biweekly for a total of four injections. Tissues were analyzed for GAA activity (panel A) and glycogen levels (panel B, using amylo-glucosidase digestion) 3 and 14 days post-last injection, respectively. The data supported the notion that the effectiveness of SQ administration is largely determined by the proximity of the tissues to the injection site. Repeat SQ administration of co-formulated rhGAA at a single site (S or HL) led to significant rhGAA uptake and glycogen reduction in the nearby limb muscles that exceeded what was achieved with repeat IV administration of rhGAA alone, and a similar effect was seen in more muscles when the injections were alternated between the shoulder (Mondays) and the hind limb (Thursdays). Taken together, the results suggest that by using multiple injection sites, the overall biodistribution of co-formulated rhGAA followed by SQ administration is expanded and mimics that of rhGAA IV alone. It is also worth noting that lethargy or mortality, the adverse events typically associated with repeat IV administration of rhGAA alone, were not observed in mice that received repeat SQ injections of co-formulated rhGAA + AT2220, even though four times the amount of rhGAA was given within the same time span. Therefore, it may be possible to conduct future repeat SQ CoF studies with a longer duration to further optimize the dose, dosing frequency, and injection site location. Each bar represents mean ± SEM of 5-7 mice/group, and the hatched bars represent the tissues with proximity to the SQ injection site(s). Asterisks mark the groups with significantly greater rhGAA uptake or glycogen reduction compared to the rhGAA IV alone group. (*, p ≤ 0.05; **, p ≤ 0.01; ***, p ≤ 0.0005; ****, p ≤ 0.0001)

Summary and Conclusions

- AT2220 increases the physical stability of rhGAA at neutral pH and body temperature *in vitro* and *ex vivo*.
- In rats, SQ administration of co-formulated rhGAA + AT2220 increased the total plasma exposure of rhGAA up to 1.5-fold, although the maximal rhGAA plasma levels achieved were significantly lower than those seen following IV administration.
- Histological studies indicated that in *Gaa* KO mice, a single SQ administration of co-formulated rhGAA + AT2220 led to increased lysosomal uptake of rhGAA and greater glycogen reduction in skeletal muscle compared to IV rhGAA alone.
- In the majority of the tissues, repeat SQ administration of co-formulated rhGAA + AT2220 to *Gaa* KO mice (four administrations over two weeks) at alternating sites resulted in rhGAA uptake and glycogen reduction that was greater compared to the extent seen following four IV administrations of rhGAA alone (every other week), without the deaths associated with repeat IV administration. The magnitude of rhGAA uptake and glycogen reduction was directly related to the proximity of the tissue to the SQ injection site.
- Collectively, these data suggest that AT2220 directly binds and stabilizes rhGAA, protecting the enzyme in a properly folded form that is accessible for tissue uptake and active once taken up into lysosomes. As a result, greater glycogen reduction is achieved in disease-relevant tissues when rhGAA is co-formulated. The effect is even more profound when the co-formulated rhGAA is delivered via the SQ route compared to IV administration of rhGAA alone. Taken together, these preclinical data highlight the potentially beneficial effects of co-formulated rhGAA + AT2220 delivered via the SQ routes, thus warranting further investigation.