A major obstacle to enzyme replacement therapy (ERT) with recombinant human acid-α-glucosidase (rhGAA) for Pompe disease is the development of high titers of anti-rhGAA antibodies in a subset of patients, which often leads to a loss of treatment efficacy. In an effort to induce sustained immune tolerance to rhGAA, we supplemented the rhGAA therapy with a weekly intravenous injection of synthetic vaccine particles carrying rapamycin (SVP-Rapa) during the first 3 weeks of a 12-week course of ERT in GAA-KO mice, and compared this with three intraperitoneal injections of methotrexate (MTX) per week for the first 3 weeks. Empty nanoparticles (NP) were used as negative control for SVP-Rapa. Co-administration of SVP-Rapa with rhGAA resulted in more durable inhibition of anti-rhGAA antibody responses, higher efficacy in glycogen clearance in skeletal muscles, and greater improvement of motor function than mice treated with empty NP or MTX. Body weight loss was observed during the MTX-treatment but not SVP-Rapa-treatment. Our data suggest that co-administration of SVP-Rapa may be an innovative and safe strategy to induce durable immune tolerance to rhGAA during the ERT in patients with Pompe disease, leading to improved clinical outcomes.

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used nanoparticle-encapsulated antigen together with rapamycin, a tolerogenic immunomodulator, to induce immunological tolerance in hemophilia A mice [17]. They demonstrated that NP containing both the immunosuppressant rapamycin and an antigen (coagulation factor VIII) inhibited antigen-specific CD4+ and CD8+ T-cell activation, increased regulatory cells, induced durable B-cell tolerance, and inhibited antibody responses against coagulation factor VIII. Subsequently, two studies reported that co-administration of free antigen and SVP containing rapamycin (SVP-Rapa) induced antigen-specific and SVP-Rapa-dependent immune tolerance in mice and non-human primates [18, 19]. In this study, we demonstrate that SVP-Rapa can induce immune tolerance to rhGAA and improve efficacy of ERT in GAA-knockout (KO) mice that is superior to immunosuppression with MTX.

2. Material and methods

2.1. Drugs

The rhGAA (Myozyme®, alglucosidase alfa; manufactured by Sanofi Genzyme) was purchased from Pharmaceutical Buyers, Inc. (New Hyde Park, NY). Empty NP and SVP-Rapa were prepared and provided by Selecta Biosciences, Inc. (Watertown, MA, USA). Briefly, poly(lactic-co-glycolic acid) (PLGA), preglycated polyactic acid (PLA-PEG), and rapamycin were dissolved in dichloromethane to form an oil phase. The oil phase was then added to an aqueous solution of polyvinyl alcohol and emulsified by sonication (Branson Digital Sonifier 250A). Following emulsification, single emulsions were added to a beaker containing phosphate buffer solution (PBS) and stirred at room temperature for 2 h to allow the dichloromethane to evaporate. The resulting nanoparticle suspension was stored at 4 °C. The nanoparticles were washed twice by centrifuging at 75,600 × g for 1 h and then resuspended in buffer.

2.2. Mice and treatment

Homozygous GAA-KO mice (6Glca1-4Glca1-4Glc (Glc4), Hex4) generated by Raben and colleagues by targeted disruption of the GAA gene [20], were used in this study. A total of 15 male mice were used for ERT with weekly intravenous injections of 20 mg/kg rhGAA. For each mouse, pretreatment was performed 10 min prior to intravenous (IV) injection of rhGAA in each of week 0, 1, and 2 of ERT, as previously described [21]. All animal experiments had the lowest. The ERT largely cleared the glycogen storage in the liver and heart of all the three groups, indicated by measured glycogen content and GAA activity as described [23].

3. Results

3.1. Immune tolerance induction against rhGAA

Co-administration of SVP-Rapa with the first three doses of rhGAA effectively prevented anti-rhGAA antibody development throughout the 12-week study period except for ERT week 12 (Fig. 1). After 12 weeks on ERT, two of the five mice in the SVP-Rapa group showed an increase of anti-rhGAA antibody, while the remaining three animals showed no sign of antibody formation. The empty NP co-treatment did not show any suppressive effect on anti-rhGAA antibody response, as the kinetics of anti-rhGAA antibody in the Empty NP group was similar to that in GAA-KO mice on ERT with rhGAA only as reported previously [21, 25]. Mice treated with MTX at 0, 24, and 48 h after the first three injections of rhGAA started developing anti-rhGAA antibody from ERT week 6, and the overall antibody titers in the MTX group were lower than those in the Empty NP group, but higher than those of the SVP-Rapa group except at week 12.

3.2. Effects of adjunct treatments on rhGAA uptake and glycogen clearance

Liver had extremely high GAA activity (533–729 mmol/h/mg) in all three groups of mice on ERT compared with basal activity in GAA-KO mice measured in our laboratory (3 mmol/h/mg), and GAA activity in heart (21–38 mmol/h/mg) was also significantly higher than basal level (2 mmol/h/mg), while uptake of rhGAA by skeletal muscles was poor (Fig. 2A). Among the three groups, the Empty NP group surprisingly demonstrated the highest GAA activities in all tissues despite developing the highest anti-rhGAA antibodies, while the MTX group had the lowest. The ERT largely cleared the glycogen storage in the liver and heart of all the three groups, indicated by measured glycogen...
content (~0.1 μmol Glc/mg in liver and 0.05–0.1 μmol Glc/mg in heart) (Fig. 2B), compared with ~2.8 μmol Glc/mg in liver and ~1.5 μmol Glc/mg in heart of untreated 3-month-old GAA-KO mice observed in our laboratory (shown in Ref. value). In skeletal muscles, glycogen clearance by ERT was most efficient in the SVP-Rapa group and least effective in the Empty NP group. The higher ERT efficiencies of the SVP-Rapa group in muscles coincided with the lowered tendency of developing anti-rhGAA antibody response (Figs. 1 and 2B), but it is surprising that the glycogen clearance did not correlate with GAA activities measured in these tissues (Fig. 2A, B). It should be noted that the glycogen clearance data reflects the cumulative activity of rhGAA over the 12 weeks of therapy, whereas the GAA activity data reflects residual GAA activity from the last dose of rhGAA.

3.3. Physical and clinical outcomes

Appropriate and steady weight gain is a health indicator in growing animals. A positive effect was observed in the SVP-Rapa group throughout the course of ERT (Fig. 3). In contrast, the MTX-co-treatment exerted a negative effect on growth as indicated by weight loss during the three weeks when MTX was administered (Fig. 3). Improvement in Rota-rod performance (percent increase in fall latency) after 4 weeks on ERT in the SVP-Rapa group was statistically greater than that of the Empty NP group (Fig. 4A). Urinary Hex4 levels were significantly reduced in all three groups after ERT, regardless of the adjunct treatment (Fig. 4B).

4. Discussion

Enzyme replacement therapy is currently the only effective treatment in patients with Pompe disease (1–3). However, inevitable immune response to ERT with development of HSAT has been a limitation to the injection of the recombinant protein, especially in
CRIM negative patients [8,26,27]. Several studies have reported that use of immunosuppressant drugs, such as cyclophosphamide, mycophenolate mofetil, belimumab, rituximab, bortezomib, and MTX can lead to successful induction of immune tolerance in GAA-deficient mice and in humans with infantile Pompe disease [9–13,21,27]. Although no serious side effects have been noted in these regimens, concerns about compromised safety due to systemic immunosuppression, reduced cost effectiveness, and the need of long-term treatment still remain.

SVP-Rapa has been demonstrated in several disease models to successfully induce durable antigen-specific immune tolerance and improve functional outcomes [18,19]. Encapsulation of rapamycin by SVP minimizes its systemic exposure and enhances its uptake by antigen presenting cells, and hence promotes the induction of tolerogenic dendritic cells while avoiding systemic immunosuppression [17–19]. Here, we evaluated the possibility of adoption of SVP-Rapa as an innovative solution in patients with Pompe disease treated with ERT to induce immune tolerance to rhGAA. The self-assembling, biocompatible, and biodegradable SVP used in this study was made with a synthetic polymer, PLGA, which has been used in a variety of marketed drugs and medical devices [17]. SVP-Rapa has been produced under good manufacturing practice (GMP) conditions and is currently being evaluated in clinical studies in combination with pegviscits, a highly immunogenic pegylated uricase enzyme for the treatment of refractory gout [28]. Rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), blocks T-cell activation, inhibits dendritic cell maturation, and selectively allows for stimulation of antigen-specific Fop3 + regulatory T-cells [29,30]. Moreover, in GAA-KO mice, rapamycin reduces the accumulation of glycogen via mTOR complex 1 inhibition and increases phosphorylation of glycogen synthase in skeletal muscle [31]. Our study revealed that co-administration of SVP-Rapa with rhGAA has a long-lasting effect on the suppression of anti-rhGAA antibody responses in GAA-KO mice. While three treatments with SVP-Rapa induced durable immune tolerance to ERT, two of the mice developed anti-rhGAA antibodies at 12 weeks after nine challenge injections of rhGAA (Fig. 1). A previous study with coagulation factor VIII (FVIII) in hemophilia A mice have demonstrated that five co-injections of SVP-Rapa with FVIII provided better durability than three co-injections, with tolerance being maintained for at least five months after treatment [17]. Further studies assessing additional co-administrations of SVP-Rapa or a different dose will be required to optimize the regimen for rhGAA.

MTX treatment was used as a positive control in this study because it has been demonstrated that a short-term, low-dose MTX therapy with rhGAA can induce long-lasting immune tolerance to rhGAA in the GAA-KO mouse model [13,21]. MTX showed good immunomodulatory activity in this study, but four of the five mice showed elevation of anti-rhGAA antibody titers starting from week 6 on ERT. SVP-Rapa has previously been shown to induce more durable induction of immune tolerance than MTX to keyhole limpet hemocyanin (KLH), a highly immunogenic antigen [18].

It has been generally known that the anti-drug antibodies (ADA), when produced in high amounts, could lead to the rapid clearance, degradation, and/or neutralization of enzyme [32,33]. However, it seems that the anti-rhGAA antibody does not affect the mannose-6-phosphate receptor (M6PR)-mediated enzyme uptake by the liver and muscle cells of GAA-KO mice because our study did not show an enhancement of rhGAA uptake in mice treated with SVP-Rapa or MTX (Fig. 2A). In fact, the GAA activities were higher but glycogen clearance was less efficient in skeletal muscles of the Empty NP treatment group than that of the SVP-Rapa group (Fig. 2A, B). It is possible that the total enzyme activity in the muscles of the empty NP-treated mice is partially contributed by the phagocytic cells (e.g., mast cells, monocytes, and macrophages) in these tissues during the process of Fcy receptor-mediated endocytosis of the rhGAA-antibody immune complexes [33,34]. Therefore, the effective GAA activity in muscle cells of the Empty NP-treated mice might be actually lower than that of the SVP-Rapa–treated mice.

Suppression of glycogen synthesis by rapamycin treatment could have contributed to the significantly lower glycogen load in muscles as previously seen in GAA-KO mice and GSD III dogs [31,35], and this adds to the benefits of using SVP-encapsulated rapamycin as an adjunct treatment. As this study used a mouse model that can be vastly different from humans, clinical investigations will be needed to assess the efficacy of this combined treatment in human patients with Pompe disease.

In summary, our data suggest that co-administration of SVP-Rapa may be an innovative and safe strategy to induce durable immune tolerance to rhGAA during the ERT in patients with Pompe disease.

Conflict of interest

TKK is an employee and shareholder of Selecta Biosciences. The other authors declare no conflict of interest.

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