Molecular Genetics and Metabolism Reports 13 (2017) 18–22

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

A pilot study on using rapamycin-carrying synthetic vaccine particles (SVP) in conjunction with enzyme replacement therapy to induce immune tolerance in Pompe disease

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ARTICLE INFO

Article history:
Received 21 March 2017
Accepted 21 March 2017
Available online xxxx

Keywords:
Pompe disease
Acid alpha-glucosidase
Enzyme replacement therapy
Tolerogenic nanoparticles
Rapamycin

ABSTRACT

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.

1. Introduction

Pompe disease (glycogen storage disease type II, OMIM 232300) is a lysosomal storage disorder caused by a deficiency of lysosomal enzyme acid-α-glucosidase (GAA; acid maltase; EC 3.2.1.20), and characterized by progressive structural disruption and cell dysfunction of muscle tissues due to lysosomal accumulation of glycogen [1]. Without treatment in classic infantile Pompe disease, which represents the most severe end of the disease spectrum, death secondary to cardiorespiratory failure typically occurs within the first 1–2 years of life [2,3]. The availability of intravenous enzyme replacement therapy (ERT) with recombinant human acid-α-glucosidase (rhGAA, alglucosidase alfa, Myozyme®) has dramatically improved overall survival and daily activities for patients with Pompe disease [4,5]. However, the development of high and sustained antibody titer (HSAT) against the therapeutic rhGAA occurs in cross-reactive immunologic material negative (CRIM-) patients and a subset of CRIM+ patients, which severely compromises the safety and efficacy of the ERT [6,7]. Patients with HSAT respond poorly to ERT and need an additional immunomodulation therapy to prevent ongoing disease progression [6,8]. A broad range of agents have been evaluated for immune tolerance induction, among which rituximab (monoclonal anti-CD 20), rapamycin, mycophenolate mofetil, cyclophosphamide, belimumab (anti-B-cell activating factor; anti-BAFF), Methotrexate (MTX), intravenous immunoglobulin (IVIG), and bortezomib have been shown to be capable of modulating the anti-rhGAA antibody response [9–13]. However, these universal immunosuppressant agents induce systemic immune suppression and may cause side effects such as bone marrow and gastrointestinal toxicities with the possibility of opportunistic infections and tumorigenesis, and chronic administration is often needed in those with an established immune response [10,11,14].

For immune tolerance induction in diseases treated with immunogenic drugs, it would be desirable to transiently target the immunosuppressant's effects to dendritic cells and other antigen-presenting cells at the time of antigen encounter. Dendritic cells play a key role in antigen presentation to helper T-cells and control of the immune response [15]. Synthetic vaccine particles (SVP™), also called nanoparticles (NP), effectively deliver antigen and drug to antigen-presenting cells in a similar way as a virus [16]. Recently, Maldonado et al.

Abbreviations: ERT, enzyme replacement therapy; rhGAA, recombinant human acid-α-glucosidase; CRIM, cross-reactive immunologic material; HSAT, high and sustained antibody titer; MTX, methotrexate; SVP-Rapa, synthetic vaccine particles carrying rapamycin; NP, empty nanoparticles.

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http://dx.doi.org/10.1016/j.ymgmr.2017.03.005

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 polylactic 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 NP were washed twice by centrifuging at 75,600*g* and 4 °C followed by re-suspension of the pellet in PBS. Each SVP-Rapa injection consisted of ~50 μg of rapamycin. Methotrexate was purchased from Calbiochem (San Diego, CA, USA). Diphenhydramine was purchased from Baxter Healthcare Corporation (Deerfield, IL, USA).

2.2. Mice and treatment

Homozygous GAA-KO mice ($6^{neo}/6^{neo}$), 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 with 15 mg/kg diphenhydramine by intraperitoneal (IP) injection was performed 10–15 min prior to intravenous (IV) administration of rhGAA to prevent anaphylactic reactions [21]. The ERT was initiated at age of 10 weeks (set as ERT week 0) and ended at age of 22 weeks (ERT week 12) and mice received 13 injections of rhGAA in total. These mice were randomly divided into 3 groups (n = 5 each) for different adjunct treatments as described below. Group 1 (Empty NP group): 4 ml/kg empty NP was mixed with rhGAA for injection in ERT weeks 0, 1, and 2; Group 2 (SVP-Rapa group): 4 ml/kg SVP-Rapa was mixed with rhGAA for injection in ERT weeks 0, 1, and 2. Group 3 (MTX group): 3 consecutive IP injections of MTX (10 mg/kg) were given at 0, 24, and 48 h after IV injection of rhGAA in each of week 0, 1, and 2 of ERT, as previously described [21]. All animal experiments were approved by the Institutional Animal Care and Use Committee of Duke University, and following local and national guidelines and regulations.

2.3. Sample collection and analyses

Plasma samples were obtained every two weeks 4–6 days following rhGAA administration and stored at −80 °C for later analysis of anti-rhGAA antibody titer. Urine samples were collected prior to ERT and after 12 weeks of ERT. Total urinary hexose tetrasaccharide (Glca1-6Glca1-4Glca1-4Glc (Glc_4), Hex_4) tests were performed for therapeutic responses by liquid chromatography-stable isotope dilution tandem mass spectrometry (LC-MS/MS) as described [22]. Rota-rod tests were performed every 4 weeks to determine motor balance, strength, and coordination [23]. Mice were euthanized 48 h after the last rhGAA injection following overnight fasting. All tissues were kept frozen for evaluating glycogen content and GAA activity as described [23].

2.4. Measurement of anti-rhGAA IgG antibody

The anti-rhGAA antibody titer was measured by enzyme linked immunosorbent assay (ELISA) as described [24]. Briefly, 96-well plates (Corning Inc., Corning, NY, USA) were coated overnight at 4 °C with 100 μl per well 5 μg/ml rhGAA. Following washing with 0.05% Tween 20 in PBS, 100 μl per well diluted serum (1:200) were added in duplicates to rhGAA-coated plates and incubated at 37 °C for 1 h. The plates were washed, and alkaline phosphatase-conjugated goat anti-mouse IgG secondary Ab (Cat # 115-055-205, Jackson ImmunoReasearch Laboratory Inc., West Grove, PA, USA) was added and allowed to incubate for 1 h at 37 °C. Following a final wash, 4-Nitronphenyl phosphate disodium salt hexahydrate (Sigma-Aldrich Co., St. Louis, MO, USA) was added and allowed to develop for 20 min at room temperature. Absorbance at 405 nm was read on a VICTOR X Multilabel Plate Reader (PerkinElmer Corporation, Waltham, MA, USA).

2.5. Statistical analysis

One-way ANOVA with post hoc test (Tukey) was performed to analyze the differences among the three groups. If the data did not meet the Shapiro-Wilk test for normality, the Kruskal-Wallis test and Mann-Whitney *U* test were performed for nonparametric data. Data in graphs were presented as mean ± standard deviation (SD) or standard errors of mean (SEM) as indicated. The urinary Hex_4 levels prior to and post ERT were compared using paired *t*-test. Data analyses were conducted using SPSS version 20.0 for Windows (IBM Corp, Armonk, NY, USA), and $p < 0.05$ was considered significant.

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 each of 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