



Aggressive immunotherapy combined with bortezomib and rituximab for membranous nephropathy associated with enzyme replacement therapy in Pompe disease

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Abstract

Background Pompe disease (PD) is a lysosomal glycogen storage disorder caused by a deficiency in acid α -glucosidase (GAA) activity. Various organs, including the skeletal muscle, cardiac muscle, and liver, are commonly involved. Early initiation of enzyme replacement therapy (ERT) with recombinant human α -glucosidase (rhGAA) can improve the outcome. However, some patients experience a poor clinical course despite ERT because of the emergence of anti-rhGAA antibodies that neutralize rhGAA. Treatment against anti-rhGAA antibodies is challenging.

Case–diagnosis/treatment A 14-year-old boy with late-onset PD was referred to our hospital with proteinuria detected by school urinalysis screening. He was diagnosed with PD at the age of 4 years based on muscle biopsy and decreased GAA activity. Treatment with rhGAA was initiated, but anaphylaxis occurred frequently. Anti-rhGAA antibodies were detected and immune tolerance therapy was therefore given, but his antibody titer remained high. Kidney biopsy revealed stage II membranous nephropathy. Immunohistochemistry staining revealed anti-rhGAA antibody/rhGAA immune complexes along the glomerular capillary loop. Aggressive immunotherapy combined with bortezomib and rituximab was then initiated. Serum levels of anti-rhGAA antibodies decreased significantly and his proteinuria finally resolved.

Conclusions There have been few reports of membranous nephropathy associated with ERT for PD. We clarified the cause in the current patient. Bortezomib and rituximab effectively suppressed anti-rhGAA antibody production resulting in the resolution of proteinuria and maintenance of ERT efficacy.

Keywords Child · Pompe disease · Membranous nephropathy · Enzyme replacement therapy · Bortezomib · Rituximab

Introduction

Pompe disease (PD) is an autosomal recessive congenital metabolic disorder caused by a deficiency in acidic α -glucosidase (GAA), which is a lysosomal enzyme that breaks down glycogen into glucose. Glycogen, as a substrate for GAA, accumulates in various organs, such as the skeletal muscle, cardiac muscle, and liver, causing different

symptoms in patients with PD. PD has been classified into two major categories based on the age of onset and severity of organ involvement. The infantile-onset form presents early in life and is associated with hypotonia, growth retardation, hepatomegaly, cardiomegaly, respiratory impairment, and death within 1 year if untreated. In contrast, symptoms of late-onset PD occur in children, juveniles, and adults, do not typically include severe cardiomyopathy, and progress gradually [1]. Radical therapy for PD involves enzyme replacement therapy (ERT), supplemented with recombinant human α -glucosidase (rhGAA) every 2 weeks. ERT has dramatically improved the life prognosis of patients with severe infant-type PD [2, 3] and enhanced the quality of life in late-onset PD due to improved motor and respiratory functions [4]. However, various allergic reactions and side effects have been associated with ERT for PD. In particular, the emergence of anti-rhGAA antibodies decreases the efficacy of ERT and induces hypersensitivity reactions, leading to

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difficulties in continuing ERT. Additionally, immune complexes with anti-enzyme antibodies and enzymes may cause secondary membranous nephropathy (MN) [5, 6]. We experienced a boy with late-onset PD who developed MN due to anti-rhGAA antibodies.

Here, we first report the efficacy of aggressive immunotherapy combined with bortezomib and rituximab for the treatment of MN.

Case report

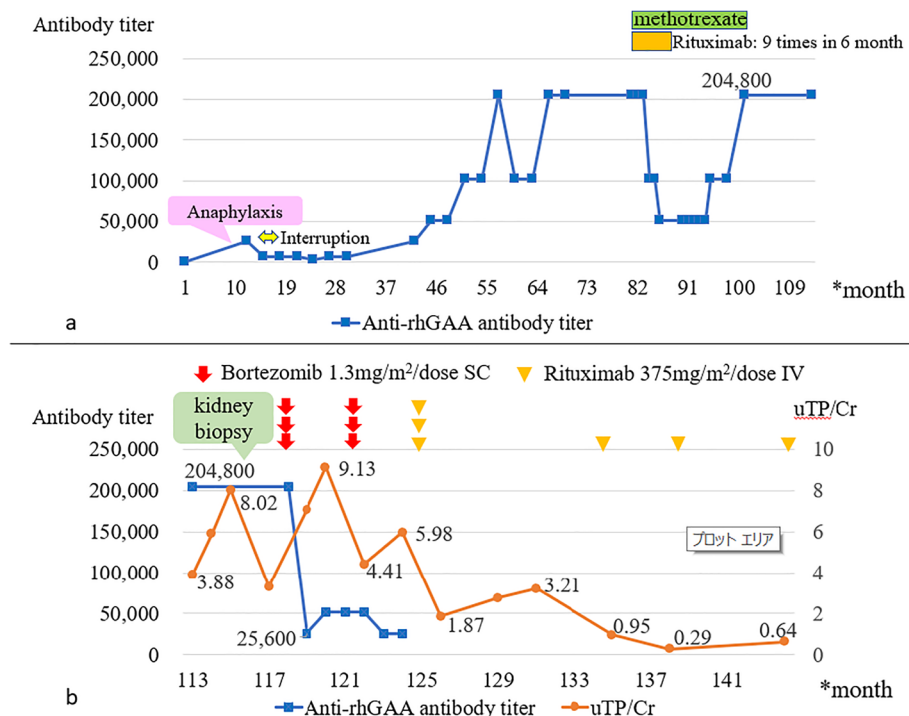
The presented case is a 14-year-old boy with PD. He started walking at 1 year and 4 months of age but was prone to falling. Additionally, his speech was slurred. At the age of 3 years, he showed delayed motor development, Gowers' sign, and elevated serum creatine kinase and aldolase. Subsequent muscle biopsy revealed low GAA activity, leading to the diagnosis of late-onset PD. Genetic analysis revealed two heterozygous GAA mutations (c.2 T>c and c.1826dupA). At 4 years, ERT with rhGAA (20 mg/kg, every 2 weeks) was started. However, allergic symptoms appeared after the third administration, and anaphylaxis reactions started to occur 1 year later. Anti-rhGAA antibodies were detected (titer 25,600). After temporary treatment interruption for 3 months, rhGAA therapy was resumed following pretreatment with an antihistamine and steroid. However, his antibody titer gradually increased to 204,800. Weekly oral methotrexate 8 mg and rituximab 375 mg/m² were administered to suppress anti-rhGAA production. His antibody titer

temporarily decreased to 51,200 but later increased again to 204,800 (Fig. 1a). Fortunately, he was able to continue ERT despite infusion reactions, including urticaria and slight respiratory distress.

Proteinuria was first detected by school urinalysis at age 14 years, and he was referred to our hospital. There was no family history of kidney disease. In addition to PD, he had atopic dermatitis, bronchial asthma, and hypercholesterolemia. He was taking carvedilol, enalapril, fexofenadine, and ezetimibe. His development, except motor development, was age appropriate. He could walk unaided, but usually used a wheelchair because of leg weakness and used continuous positive airway pressure at night. Physical exam findings were as follows: height 164 cm (50th percentile), body weight 35 kg (3rd percentile), blood pressure 117/76 mmHg, heart rate 90 beats/min, and SpO₂ 99% (room air). Myopathic face, dysarthria, and significant weakness of the proximal muscles of the upper and lower limbs were observed. Edema was not present.

Blood work revealed no hematological abnormalities (hemoglobin 15.5 g/dL, leukocytes 5630/μL, and platelets 332 × 10³/μL) and no hypoalbuminemia (albumin 4.3 g/dL). Muscle enzymes were elevated (aspartate transaminase 380 U/L, alanine transaminase 186 U/L, creatine kinase 2738 U/L, and lactic acid dehydrogenase 974 U/L). His serum creatinine (Cr) level was low (0.36 mg/dL) because of the primary disease, his kidney function was normal (cystatin C 0.89 mg/dL), and immunoglobulin and complement levels were normal. Rheumatic diseases and viral infections were ruled out by antibody titers. Urinalysis showed

Fig. 1 **a** Time course of anti-rhGAA IgG antibody titer from ERT initiation to first visit to our hospital. **b** Time courses of antibody titer and urine protein/creatinine ratio from first referral to our hospital. *Months from initiation of ERT. uTP, urinary total protein; Cr, creatinine; SC, subcutaneous injection; IV, intravenous injection



proteinuria (urinary protein creatinine ratio 3.38 g/gCr) and slight hematuria (red blood cells 1–4/high-power field). Abdominal ultrasonography showed no abnormality. Kidney biopsy was performed for further investigation. Light microscopy showed diffuse thickening of the glomerular capillary wall, spike formation, and a bubble-like appearance. Immunofluorescence showed that the capillary wall was positive for IgG, IgM, and C3. Electron microscopy showed subepithelial electron dense deposits, with some deposits located in transmembrane and mesangial regions. The diagnosis was secondary MN stage II. Anti-rhGAA antibodies were strongly suspected as the cause of MN and we therefore performed immunohistochemistry for rhGAA. The glomerular capillaries were positively stained for rhGAA (Fig. 2). The distribution of rhGAA and IgG was consistent. He was finally diagnosed with MN caused by rhGAA and anti-rhGAA antibodies.

Suppressing antibody production may have been a fundamental treatment for his MN; however, treatment with methotrexate and rituximab had previously failed. Bortezomib, a proteasome inhibitor targeting plasma cells, was thus administered to eliminate mature plasma cells. It was given for two courses 4 months apart. Each course lasted 2 weeks and included 1.3 mg/m² bortezomib subcutaneous injection three times on days 1, 4, and 10. His antibody titer rapidly decreased to 25,600 and urinary protein significantly decreased in parallel with the reduction in antibody titer.

Rituximab (375 mg/m²) was also administered to prevent the antibody titer from increasing again. His proteinuria disappeared after 8 months of bortezomib treatment (Fig. 1b), but we were unable to perform a second kidney

biopsy or ongoing anti-rhGAA antibody titer assessments. He remained in remission for 2 years with normal kidney function. At the last follow-up, his treatment consisted of oral enalapril and rituximab when his CD19-positive cell count recovered. Monitoring included CD19-positive cell counts, urinary protein for early detection of recurrent MN, and creatine kinase to evaluate PD.

Discussion

ERT has been used to treat some lysosomal storage diseases caused by a deficiency or decreased activity of enzymes and has significantly improved the life expectancy and clinical outcomes of patients, including those with PD. However, the supplemental enzymes are homologous antigens and may thus trigger an immune response because of absent or reduced levels in the patient [5]. Anti-supplementary enzyme antibodies commonly develop during the early course of ERT but are usually asymptomatic; however, they may lead to severe infusion reactions and diminished therapeutic efficacy in some patients.

In patients with PD, the effectiveness of rhGAA treatment depends on several factors, including age, disease stage at the start of treatment, and anti-rhGAA IgG antibody development [7]. Some patients with infantile-onset PD show high sustained anti-rhGAA IgG antibody titers (HSAT; defined as $\geq 1:51,200$ on two occasions ≥ 6 months from the start of ERT), which have been shown to have a negative impact on clinical outcomes [7, 8]. Most patients with HSAT do not produce native GAA enzymes and are designated

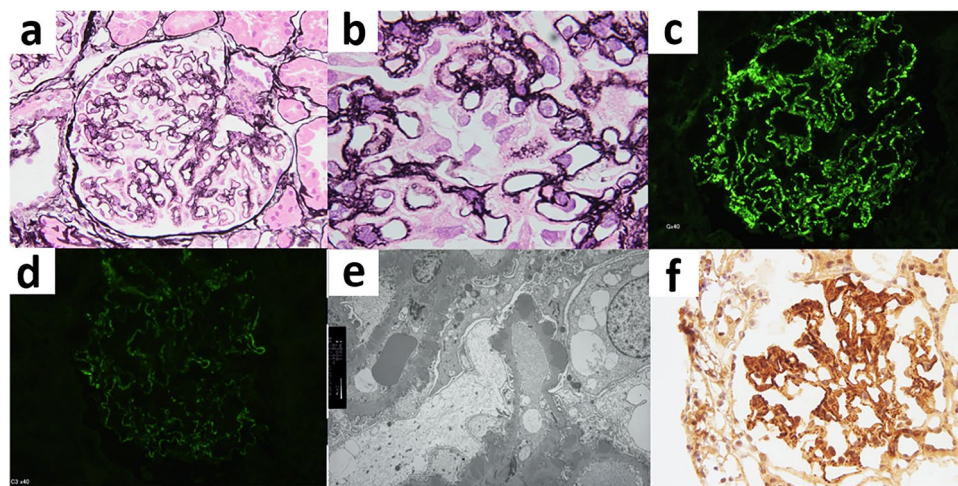


Fig. 2 Histologic features of kidney biopsy tissue. **a, b** Periodic acid methenamine silver staining (**a** $\times 400$, **b** $\times 1000$) showed diffuse thickening of glomerular capillary walls, formation of spikes, and bubbling appearance of basement membrane. No mesangial cell proliferation, extraluminal lesions, or glomerular sclerosis were observed. (**c, d**) Diffuse and fine granular deposits (**c** IgG, **d** C3) were positively

stained along the outer surface of capillary walls. **e** Electron microscopy showed subepithelial electron dense deposits. Some deposits were transmembranous. Deposits were also found in some mesangial regions. **f** Capillary walls were positive for rhGAA, as shown by immunofluorescence staining using an rhGAA-specific antibody

as cross-reactive immunological material (CRIM)-negative [9]. In the HSAT group, strong immunotherapy is recommended to maintain the efficacy of ERT and avoid complications [10, 11]. High antibody titers may not be associated with clinical signs in late-onset PD, and immunotherapy is recommended in the event of clinical deterioration or infusion reactions [12]. The usefulness of immunotherapy with methotrexate, rituximab, and intravenous immunoglobulins has been reported [13].

MN associated with ERT is a rare complication with limited reports and no specific treatment to date. Hunley et al. reported a case of MN associated with ERT for infantile PD [6]. They attempted the temporary discontinuation or reduction of ERT. Although the antibody titer remained high, edema improved within 1 month of drug adjustment, and proteinuria and hypoalbuminemia normalized over the next 4 months. A case of MN associated with ERT for mucopolysaccharidosis type IV (Maroteaux–Lamy syndrome) has also been reported [5].

In this report, the discontinuation of ERT resulted in decreased proteinuria after 6 months. However, this resulted in worsening of the primary disease, hepatosplenomegaly, and repeated upper respiratory tract infections. Immunotherapy with low-dose corticosteroids, rituximab, intravenous immunoglobulins, and methotrexate was thus administered and lower-dose ERT was restarted 8 weeks later, leading to markedly improved symptoms of the primary disease. Although temporary interruption of treatment may reduce anti-supplemental enzyme antibodies, stopping ERT may have serious consequences for the patient. To the best of our knowledge, this is the first report of a case in which ERT-associated MN was successfully treated without interruption of ERT.

Corticosteroids and combination therapy with methotrexate and rituximab failed to decrease the antibody titer in our patient. Long-lived plasma cells, in contrast to B cells and short-lived plasma cells, do not express CD20 or respond to rituximab [13]. We therefore presumed that long-lived plasma cells actively produced anti-rhGAA antibodies in our patient.

Bortezomib is a proteasome inhibitor targeting plasma cells, used to treat multiple myeloma and mantle cell lymphoma. Bortezomib may also be used as a desensitizing treatment to prevent severe rejection due to anti-donor-specific antibodies in kidney transplant patients [14].

CRIM-negative PD patients, as a high-risk group for anti-rhGAA antibodies, have recently been treated with immune tolerance induction therapy, including bortezomib and rituximab, to prevent antibody production before starting ERT [15]. The current patient's treatment was based on this immunotherapy. Although our patient did not experience any serious adverse events with bortezomib and rituximab, immunosuppression may increase the risk of respiratory

infections in PD patients with complicated respiratory impairment, and careful observation is thus required.

In addition to the treatment strategy, the early detection of proteinuria by school urinalysis may have been associated with a better prognosis in our patient. MN associated with ERT is a rare complication, but early detection by regular urinalysis may be important.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Kishnani PS, Steiner RD, Bali D, Berger K et al (2006) Pompe disease diagnosis and management guideline. *Genet Med* 8:267–288. <https://doi.org/10.1097/01.gim.0000218152.87434.f3>
2. Kishnani PS, Corzo D, Nicolino M, Byrne B et al (2007) Recombinant human acid α -glucosidase: major clinical benefits in infantile-onset Pompe disease. *Neurology* 68:99–109. <https://doi.org/10.1212/01.wnl.0000251268.41188.04>
3. Chien YH, Lee NC, Thurberg BL, Chiang SC et al (2009) Pompe disease in infants: improving the prognosis by newborn screening and early treatment. *Pediatrics* 124:e1116–e1125. <https://doi.org/10.1542/peds.2008-3667>
4. Toscano A, Schoser B (2013) Enzyme replacement therapy in late-onset Pompe disease: a systematic literature review. *J Neurol* 260:951–959. <https://doi.org/10.1007/s00415-012-6636-x>
5. Hanna D, Vassili V, Olivia B, Laure-Helene N et al (2014) Allo-immune membranous nephropathy and recombinant aryl sulfatase replacement therapy: a need for tolerance induction therapy. *J Am Soc Nephrol* 25:675–680. <https://doi.org/10.1681/ASN.2013.030290>
6. Hunley TE, Corzo D, Dudek M, Kishnani PS et al (2004) Nephrotic syndrome complicating alpha-glucosidase replacement therapy for Pompe disease. *Pediatrics* 114:e532–e535. <https://doi.org/10.1542/peds.2003-0988-L>
7. Banugaria SG, Prater SN, Ng YK, Kobori JA et al (2011) The impact of antibodies on clinical outcomes in diseases treated with therapeutic protein: lessons learned from infantile Pompe disease. *Genet Med* 13:729–736. <https://doi.org/10.1097/GIM.0b013e3182174703>
8. Banugaria SG, Patel TT, Mackey J, Das S et al (2012) Persistence of high sustained antibodies to enzyme replacement therapy despite extensive immuno-modulatory therapy in an infant with Pompe disease: need for agents to target antibody-secreting plasma cells. *Mol Genet Metab* 105:677–680. <https://doi.org/10.1016/j.ymgme.2012.01.019>
9. Kishnani PS, Goldenberg PC, DeArme SL, Heller J et al (2010) Cross-reactive immunologic material status affects treatment

- outcomes in Pompe disease infants. *Mol Genet Metab* 99:26–33. <https://doi.org/10.1016/j.ymgme.2009.08.003>
10. Markic J, Polic B, Stricevic L, Metlicic V et al (2014) Effects of immune modulation therapy in the first Croatian infant diagnosed with Pompe disease: a 3-year follow-up study. *Wien Klin Wochenschr* 126:133–137. <https://doi.org/10.1007/s00508-013-0475-3>
 11. Rohrbach M, Klein A, Kohli-Wiesner A, Veraguth D et al (2010) CRIM-negative infantile Pompe disease: 42-month treatment outcome. *J Inherit Metab Dis* 33:751–757. <https://doi.org/10.1007/s10545-010-9209-0>
 12. van Kooten HA, Ditters IAM, Hoogeveen-Westerveld M, Jacobs EH (2022) Antibodies against recombinant human alpha-glucosidase do not seem to affect clinical outcome in childhood onset Pompe disease. *Orphanet J Rare Dis* 17:31. <https://doi.org/10.1186/s13023-022-02175-2>
 13. Mendelsohn NJ, Messinger YH, Rosenberg AS, Kishnani PS (2009) Elimination of antibodies to recombinant enzyme in Pompe's disease. *N Engl J Med* 360:194–195. <https://doi.org/10.1056/NEJMc0806809>
 14. Everly MJ, Everly JJ, Susskind B, Brailey P et al (2008) Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. *Transplantation* 86:1754–1761. <https://doi.org/10.1097/TP.0b013e318190af83>
 15. Stenger EO, Kazi Z, Lisi E, Gambello MJ, Kishnani PS (2015) Immune tolerance strategies in siblings with infantile Pompe disease—advantages for a preemptive approach to high-sustained antibody titers. *Mol Genet Metab Rep* 4:30–34. <https://doi.org/10.1016/j.ymgmr.2015.05.004>

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