Recombinant human acid α -glucosidase enzyme therapy for infantile glycogen storage disease type II: Results of a phase I/II clinical trial

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Purpose: Infantile glycogen storage disease type II (GSD-II) is a fatal genetic muscle disorder caused by deficiency of acid α -glucosidase (GAA). The purpose of this study was to investigate the safety and efficacy of recombinant human GAA (rhGAA) enzyme therapy for this fatal disorder. **Methods:** The study was designed as a phase I/II, open-label, single-dose study of rhGAA infused intravenously twice weekly in three infants with infantile GSD-II. rhGAA used in this study was purified from genetically engineered Chinese hamster ovary (CHO) cells overproducing GAA. Adverse effects and efficacy of rhGAA upon cardiac, pulmonary, neurologic, and motor functions were evaluated during 1 year of the trial period. The primary end point assessed was heart failure-free survival at 1 year of age. This was based on historical control data that virtually all patients died of cardiac failure by 1 year of age. **Results:** The results of more than 250 infusions showed that rhGAA was generally well tolerated. Steady decreases in heart size and maintenance of normal cardiac function for more than 1 year were observed in all three infants. These infants have well passed the critical age of 1 year (currently 16, 18, and 22 months old) and continue to have normal cardiac function. Improvements of skeletal muscle functions were also noted; one patient showed marked improvement and currently has normal muscle tone and strength as well as normal neurologic and Denver developmental evaluations. Muscle biopsies confirmed that dramatic reductions in glycogen accumulation had occurred after rhGAA treatment in this patient. **Conclusions:** This phase I/II first study of recombinant human GAA derived from CHO cells showed that rhGAA is capable of improving cardiac and skeletal muscle functions in infantile GSD-II patients. Further study will be needed to assess the overall potential of this therapy. **Genetics in Medicine, 2001;3(2):132–138.**

Key Words: glycogen storage disease type II, Pompe disease, enzyme replacement therapy

Glycogen storage disease type II (GSD-II), also known as Pompe disease, is a fatal genetic muscle disorder caused by a deficiency of acid α -glucosidase (GAA).¹ This enzyme defect results in lysosomal glycogen accumulation in multiple tissues, with cardiac and skeletal muscles being the most seriously affected.

Clinically, GSD-II encompasses a range of phenotypes. Infantile GSD-II is uniformly lethal. Affected infants present in the first few months of life with hypotonia, generalized muscle

weakness, and a hypertrophic cardiomyopathy followed by death from cardiac failure by 1 year of age.^{1,2} Juvenile and adult onset forms are characterized by a lack of severe cardiac involvement and a later age of onset, but eventual respiratory and limb muscle involvement results in significant morbidity and mortality. The combined incidence of all three forms of GSD-II is estimated to be 1:40,000.^{3,4}

At present there is no treatment for GSD-II. Recent efforts have focused on producing recombinant GAA for use as an

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The cell line used to generate the enzyme (rhGAA) used in this clinical trial was constructed in Dr. Y.-T. Chen's laboratory at Duke University, and subsequently, Duke licensed the cell line and technology to Synpac (North Carolina), Inc., who provided the GMP-grade rhGAA and financial support for the clinical trial. Dr. Chen does not have stock ownership, other equity interest, or a consulting relationship with Synpac. Genzyme subsequently obtained the licensing right from Synpac, and the IND held by Duke University was transferred to Genzyme on August 25, 2000. Dr. Chen is currently a consultant for Genzyme to assist Genzyme in its clinical program/trial design for Pompe disease. If this therapy for Pompe disease proves successful commercially, Duke University and inventors (including Y.-T. Chen) may benefit financially pursuant to the University's Policy on Inventions, Patents, and Technology Transfer.

enzyme replacement therapy, in a manner similar to that used for other lysosomal disorders.^{5–7} Recombinant human GAA (rhGAA) has been produced in Chinese hamster ovary (CHO) cell cultures^{8,9} and in transgenic mouse and rabbit milk,^{10,11} and its therapeutic effects have been demonstrated in animal models.^{11,12} We now report the results of the first 12 months of an ongoing clinical study using rhGAA purified from CHO cells in three infantile GSD-II patients. The infantile phenotype was chosen for this initial study because the infants will benefit most from any potentially effective therapy, and clinical improvement should be clearly identifiable in a relatively short time period. Historically, survival after diagnosis in untreated infants is merely 3.5 to 4.7 months (Table 1).

MATERIALS AND METHODS

Basic design

The study was designed as a phase I/II, open-label, single-dose (5 mg/kg), safety and efficacy study of rhGAA infused intravenously twice weekly in three patients with infantile Pompe disease. Given the limited life expectancy of the disease following diagnosis, no placebo control was used. Based on the historical control data that virtually all patients died before 1 year of age (Table 1), we defined the primary end point as heart failure-free survival at that age. The study was approved by the institutional review board, and parental written informed consent was obtained.

Study subjects

Inclusion criteria were infants affected with infantile GSD-II having virtually absent GAA activity (<1% of normal in skin fibroblasts and/or muscle biopsy) and less than 1 year of age. Exclusion criteria included severe cardiorespiratory failure at baseline and/or other medical conditions likely to decrease survival. Three infants fulfilling the criteria were enrolled in the study. Their baseline clinical data are shown in Table 2. At 2 months of age patient 1 presented with cardiac arrest during surgical repair of an inguinal hernia. By 4 months of age he demonstrated severe hypotonia, delayed motor development, and cardiomyopathy. Patients 2 and 3 were prenatally diag-

nosed with Pompe disease and were enrolled in the study when the diagnosis was confirmed postnatally in skin fibroblasts and/or muscle biopsy. Both patients 2 and 3 had evidence of motor delays and cardiomyopathy when enzyme therapy began; in addition, patient 2 had feeding difficulties. Each of the latter two patients had a sibling with infantile GSD-II who had died of cardiac failure before 1 year of age.

Clinical assessments for safety and efficacy

Clinical assessments included routine physical examinations, chest x-ray, and complete urine, hematologic, and clinical chemistry analyses (electrolytes, glucose, creatinine, blood urea nitrogen, CO₂, protein, albumin, alanine aminotransferase, aspartate aminotransferase, bilirubin, alkaline phosphatase, creatine kinase and isozymes, uric acid). Neurologic and motor function evaluations included manual muscle strength testing, Denver developmental testing, and the Alberta Infant Motor Scale (AIMS).¹³ Two-dimensional, M-mode, and Doppler echocardiography were used to assess left ventricular (LV) mass, wall thickness, and systolic and diastolic functions. Pulmonary functions included crying vital capacity, trend pulse-oximetry, end tidal carbon dioxide measurements, as well as negative inspiratory force maneuvers. Three months after the therapy, biopsies were obtained from the quadriceps muscles (contralateral thigh of pretreatment biopsies) 3 days after an rhGAA infusion.

Enzyme source

rhGAA purified from rhGAA secreting CHO cells constructed as previously described⁹ was provided as a GMP-grade, sterile, and colorless solution by Synpac (North Carolina) Inc. rhGAA was purified primarily as the 110-kD precursor protein with specific enzyme activity of 2.77 to 3.02 $\mu\text{mol}/\text{min}/\text{mg}$ protein.

ELISA for anti-rhGAA antibodies

The enzyme-linked immunosorbent assay (ELISA) for anti-rhGAA antibodies was a standard sandwich assay performed by Phoenix International Life Sciences, Inc. (Saint-Laurent, Quebec, Canada).

GAA activity, glycogen content, and Western blot analysis

GAA activity was assessed by measurement of 4-methylumbelliferyl- α -D-glucoside cleavage at pH 4.3.¹⁴ Glycogen content was determined by treatment of tissue extracts with *Aspergillus niger* amyloglucosidase and measurement of glucose released.⁹ Western blot analysis was performed with antibody raised in rabbits against purified placenta GAA.⁹

Histology

To avoid loss of glycogen, tissues were processed without en bloc staining with uranyl acetate. Semithin sections were stained with toluidine blue and thin section with uranyl acetate/lead citrate and mounted for electron microscopy.

Table 1

Historical control data of infantile glycogen storage disease, type II

	Onset (months)	Death (months)	Length of disease course (months)
Duke University Medical Center (<i>n</i> = 30) ^a			
Mean $\pm$ SD	5.1 $\pm$ 1.8	8.6 $\pm$ 2.4	3.5 $\pm$ 2.3
Range	2.4–10.3	3.3–12.4	0.0–9.0
Slonim et al. (<i>n</i> = 10) ^b			
Mean $\pm$ SD	2.5 $\pm$ 1.0	7.2 $\pm$ 2.8	4.7 $\pm$ 2.4
Range	1.0–4.0	3.0–12.0	2.0–9.0

^aData from Duke University Pompe Disease Registry.

^bData from Slonim et al.²

Table 2
Baseline clinical data on three infantile Pompe disease patients

Patient no./sex	Ethnic background	Age rhGAA started (months)	Cardiac status	Pulmonary function	Motor development (AIMS score) (percentile)	GAA activity in skin fibroblasts (% of normal)	Current age (months)
Patient 1/male	Caucasian	4	Severe cardiomyopathy; status post cardiac arrest	Borderline normal, left main bronchus compression due to markedly enlarged heart, O ₂ desaturation	<<5th	0.84	22
Patient 2/male	African-American	3	Severe cardiomyopathy	O ₂ desaturation during crying	<5th	0.57	18
Patient 3/male	Caucasian	2½	Cardiomyopathy noted	Normal	<<5th	0.69	16

RESULTS

Safety evaluation

Three patients with infantile Pompe disease have been receiving twice-weekly intravenous infusions of rhGAA for 14 to 17 months. Despite more than 250 intravenous administrations of rhGAA (5 mg/kg), only three episodes of skin rash, accompanied by a mild fever and increased irritability, occurred (patient 1 had two episodes, patient 2 a single episode). These symptoms resolved promptly after intravenous administration of diphenhydramine. Patients 1 and 2 have subsequently been routinely premedicated with oral diphenhydramine prior to each rhGAA infusion. All hematologic, liver, and renal function parameters have remained in the normal range throughout the therapy period in all the treated patients.

Cardiac status

Prior to the initiation of the enzyme therapy, patients 1 and 2 had severe hypertrophic cardiomyopathy associated with an increased LV mass, concentric thickening of the ventricular wall, and a decrease in size of the ventricular cavity, all classical findings noted in infantile GSD-II patients. With treatment, steady decreases of LV masses were seen; both patients after 1 year of therapy had LV masses reduced to 60–70% of the baseline pretreatment levels (Fig. 1). The decreases of LV masses suggest the overall decrease in heart sizes, a finding confirmed by chest x-ray images (Fig. 2). The initial small ventricular cavity as evidenced by reduced LV end-diastolic and end-systolic volumes was also normalized with the treatment (data not shown). Both patients had normal ventricular functions at the latest follow-up.

Patient 3 had cardiomyopathy at two standard deviations above the norm (LV mass of 64 g/m², normal 48.8 ± 8), but an otherwise normal baseline cardiac evaluation, and has continued to have normal cardiac assessments 14 months after the therapy.

Neurodevelopment and motor assessment

AIMS was used to evaluate the motor development in the treated infants. AIMS scores for all three patients started below

Fig. 1 Longitudinal two-dimensional echocardiographic measurements of LV mass in three infantile Pompe disease patients receiving enzyme replacement therapy. Week 0 depicts measurements at the time of enzyme therapy initiation. Straight lines represent linear regression analyses with $r = -0.71$ and $r = -0.72$, for patients 1 and 2, respectively; each $P < 0.01$.

the 5th percentile for age (Fig. 3). Patient 1 showed initial increases in the AIMS score, before beginning to decline at week 13 of the therapy. Patient 2's AIMS scores rose to the 25th percentile by week 5 but dropped back below the 5th percentile after week 7, with declines between weeks 13 and 17. The onset of clinical declines in these patients was concomitant with the rising titers of anti-rhGAA antibodies (Fig. 3). These antibodies were IgG subclass and were associated with complement consumption.

In contrast to patients 1 and 2, patient 3 has shown a steady increase of AIMS score for the entire treatment period, rising over the 10th percentile by week 11 of the therapy and rising above the 25th percentile by week 20, and has now reached 90th percentile at latest follow-up (Fig. 3). Remarkably, he has been walking independently since 12 months of age and has been able to move between squatting and standing without hand use since 14 months of age. Notably, anti-rhGAA antibodies have not been detected in patient 3.

Denver developmental evaluations demonstrated that both patients 1 and 2 have normal personal-social development for

Fig. 2 Chest x-ray findings of three infantile Pompe disease patients before and 9 months after the enzyme therapy.

age but delay in all other domains. Patient 3 is currently evaluated as age-normal for both neurologic and Denver developmental evaluations in all domains.

Pulmonary function

In the first 2 months of therapy, improvements in pulmonary functions were evident by increases in crying vital capacity (>28% and 70%, improvements in patients 1, and 2, respectively) over baseline capacities, and normalization of O₂ desaturation during crying. The initial improvements noted in the pulmonary functions of both patients, however, plateaued over the next 2 to 3 months and declined subsequently, concomitant with rising anti-rhGAA antibodies. Both patients have subsequently become ventilator-dependent after episodes of viral pneumonia precipitated respiratory insufficiency. In contrast, patient 3 had normal pulmonary functions at initiation of therapy and has continued to demonstrate normal pulmonary function testing at the latest follow-up.

Muscle GAA activity and glycogen content

After 4 months of rhGAA treatment, GAA activity increased 2- to 3-fold over baseline pretreatment levels in both patients 1 and 2, and 18-fold in patient 3 (Table 3). The absolute amounts

of GAA activity in the latter patient approached 8% of the GAA activity seen in normal muscles. There were no appreciable changes in the muscle glycogen content in patients 1 and 2 after treatment, but glycogen levels were reduced to within the normal range in patient 3.

Histology

The pretreatment biopsies of all patients showed marked vacuolization of the muscle fibers. Evaluation of the semithin sections demonstrated the fibers to be expanded by glycogen with the formation of glycogen lakes (Fig. 4A). In some fibers faint outlines of residual membranes could be discerned. Electron microscopy confirmed the presence of glycogen both in expanded lysosomes and lying free in the cytoplasm.

The 4-month posttreatment biopsies of patients 1 and 2 were similar to the pretreatment biopsies in terms of glycogen deposition and overall muscle fiber architecture. The post-treatment biopsy of patient 3, however, had a marked decrease in visible glycogen and essentially normal histology in most of the muscle fibers (Fig. 4B).

Western blot analysis

To investigate why anti-rhGAA antibodies developed in patients 1 and 2, but not 3, we performed a Western blot analysis specific for detection of expressed (but nonfunctional) GAA protein in fibroblasts derived from each of the patients. No GAA protein was detected in the fibroblasts of patients 1 and 2, whereas a readily detectable precursor form of GAA protein (110 kD) was found in patient 3. These patterns were previously seen in other patients with infantile GSD-II.¹⁴ Normal fibroblasts as expected have GAA protein predominantly of 95 kD and 76 kD (Fig. 5).

DISCUSSION

In this clinical trial, we not only evaluated the safety of rhGAA therapy in patients affected by infantile GSD-II, but we also evaluated the treated patients for preliminary evidence of efficacy. Infantile GSD-II is a rapidly fatal disorder. Because of limited life expectancy following diagnosis, no placebo control was used; instead we used heart failure-free survival at 1 year of age as the primary clinical end point to determine efficacy of the therapy. This was based on our evaluation of historical control data from a total of 40 infantile GSD-II patients (see Table 1: 30 from Duke Pompe registry, 10 from a recent report by Slonim et al.²); both results are comparable in terms of early lethality of the disease by 1 year of age. The three treated patients described in this report have well passed the critical age of 1 year (now 16, 18, and 22 months old); all still have normal cardiac functions, and all have heart sizes that have decreased.

Similar cardiac improvements in four infants treated with rhGAA from rabbit milk for 9 months have recently been reported¹⁵; however, the doses of rhGAA from rabbit milk necessary for the therapeutic effects were 4 times higher than the present study of rhGAA from CHO cells. Variable skeletal muscle improvements were mentioned, but no AIMS scores or

Fig. 3 Longitudinal data on motor development as assessed by AIMS (closed diamonds) and titer of anti-rhGAA antibody (open diamonds) in three patients with infantile Pompe disease receiving enzyme replacement therapy. The arrow indicates when the enzyme therapy was initiated. AIMS scores in normal subjects are plotted as dotted curves against age (5th, 10th, 25th, 50th, 75th, and 90th percentile, from bottom to top).

Table 3

Muscle acid α -glucosidase activity and glycogen content in infantile Pompe disease patients treated with rhGAA

	GAA activity (nmol/hr/mg protein)	Glycogen content (% wet weight)
Patient 1		
Pretherapy	0.41	5.90
Posttherapy	0.95	7.50
Patient 2		
Pretherapy	0.67	5.68
Posttherapy	1.97	4.42
Patient 3		
Pretherapy	0.1	5.13
Posttherapy	1.84	1.43
Control	23.92 $\pm$ 8.63	0.94 $\pm$ 0.55 ^a

^aUpper normal limit; 1.5%.

muscle histology was shown. The antibody status of their patients in relation to the varied clinical responses was also not presented.

Skeletal muscle functions improved in our patients; patient 3, who had severe motor delay and extensive muscle glycogen storage prior to the therapy, is now essentially a normal 16-month-old infant. Muscle biopsies derived from patient 3 confirmed the quantitative and qualitative correction of glycogen storage and improvements in histopathology.

A nontypical form of infantile GSD-II has been previously described.^{1,2} These patients have a lesser degree of cardiomyopathy and a better prognosis and can survive longer than 12 months. These patients have higher residual GAA activity than the typical infantile patients,² and in our assessments of their

GAA activity levels, the nontypical patients have GAA activity (measured with 4-methyl-umbelliferyl- α -D-glucoside as the substrate) in skin fibroblasts $>1\%$ of normal, while the typical infantile GSD-II patients have activity $<1\%$. Both patients 1 and 2 had severe cardiomyopathy at the initiation of the therapy; patient 3, who was treated earlier, had only mild cardiomyopathy at the time of the therapy. At first glance it might be argued that patient 3 had a “nontypical form or muscle variant” of infantile GSD-II^{1,2} because of the absence of marked cardiomyopathy. Several observations, however, argue against this possibility: (1) we confirmed that both muscle biopsies and skin fibroblasts obtained from patient 3 had virtually absent GAA activity; (2) patient 3 is clinically normal at the age of 16 months, whereas patient 3 had an affected brother (full sib) who had died of cardiac failure, profound hypotonia, motor delay, and respiratory insufficiency at 11 $\frac{3}{4}$ months of age; (3) patient 3’s affected deceased brother also had virtually absent GAA activity and had the same Western blot pattern as patient 3; and (4) we are not aware of significant phenotypic variations occurring among affected siblings diagnosed with the classical, infantile form of this uniformly lethal disease, and certainly not to the degree described here.

Patients 1 and 2 have had a variable course during rhGAA therapy. It is important to note, however, that their heart sizes have decreased significantly, and normal cardiac functions have been maintained throughout the study. In effect, patients 1 and 2 phenotypically now resemble the early-juvenile form of GSD-II, rather than the lethal infantile form of GSD-II. Preferential uptake of rhGAA by the heart could be one explanation as to why cardiac function seems to be well preserved. Cardiac tissue has greater numbers of mannose 6-phosphate receptors than skeletal muscle,^{16,17} possibly allowing for a more robust rhGAA uptake in the heart.

Fig. 4 Semithin sections (0.5 μ m) of muscles stained with toluidine blue, $\times 198$. A: Pretreatment biopsy of patient 3. The pink material is glycogen. B: Posttreatment biopsy of the same patient. Note the marked decrease in glycogen.

We presume that the declines in muscle function noted in patients 1 and 2 were primarily due to the presence of anti-rhGAA antibodies, since appearance of these antibodies correlated with the onset of clinical declines. In support of this, patient 3, who continues to progress normally, has not developed anti-rhGAA antibodies. It is noteworthy that patients 1 and 2 were cross-reacting immunologic material (CRIM) negative for endogenous GAA (Fig. 5), and therefore likely recognized rhGAA as a foreign protein; this condition is similar to that of patients with severe hemophilia who develop antibody against infused factor VIII resulting in reduced treatment efficacy.¹⁸ Patient 3, however, was CRIM positive, which likely renders this patient immunologically tolerant to the rhGAA, a phenomenon also noted in the enzyme replacement therapy for other diseases, such as Gaucher disease.¹⁹ Most Gaucher patients are CRIM positive and do not develop antibodies against the infused enzyme. Only 12.8% of the enzyme-treated Gaucher patients eventually develop antibodies; these antibodies, however, usually do not influence the clinical efficacy. It should be noted that about 50% of infantile patients, 100% of juvenile patients, and 100% of adult GSD-II patients are CRIM positive, suggesting that these patient populations may also ultimately respond to the rhGAA therapy as favorably as did patient 3.

Fig. 5 Western blot analysis of acid α -glucosidase in fibroblasts. A and B represent results from two separate flasks of fibroblasts. Normal fibroblasts contain predominantly 95 and 76 kD GAA, while patient 3 has only 110 kD precursor form of GAA.

Antibodies to rhGAA may reduce the effectiveness of rh-GAA therapy via several mechanisms, including neutralization of enzyme activity, alteration of enzyme targeting, tissue distribution, and/or clearance.²⁰ At this time we do not know the exact mechanism(s) by which anti-rhGAA antibodies might alter the efficacy of the therapy. In addition to the elicitation of anti-rhGAA antibody, the stage of the disease when treatment was initiated in each patient may have also impacted upon the observed responses. It appears that patients 1 and 2 had more advanced disease than patient 3 (Table 2). It is possible that some extensive muscle damage might be beyond repair, despite the initiation of rhGAA.

In conclusion, our data indicate that rhGAA is capable of improving cardiac and skeletal functions in infantile Pompe patients, although anti-rhGAA antibody and stage of the disease might influence the effectiveness of the therapy. Further studies in larger numbers of patients combined with longer-term follow-up will be required to assess the overall safety and potential of this rhGAA therapy for all forms of GSD-II.

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