Enzyme Replacement Therapy for Gaucher Disease

By E. Beutler, A. Kay, A. Seven, P. Garver, D. Thurston, A. Dawson, and B. Rosenbloom

Four patients with moderately severe type I Gaucher disease were treated with commercially available mannose terminated glucocerebrosidase (Ceredase; Genzyme, Boston, MA) for up to 13 months. The enzyme was administered at the rate of three to four times weekly at one fourth the total recommended dosage, greatly decreasing the cost. Marked regression of hepatomegaly and improvement in liver function tests, peripheral blood counts, and serum angiotensin-converting enzyme levels were documented. The two patients with pulmonary involvement manifested improvement in pulmonary function tests. Skeletal disease remained unchanged.

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LYSOSOMAL STORAGE diseases are the result of deficiencies of enzymes required to degrade complex molecules in cells. The most common of these disorders is Gaucher disease, in which glucocerebroside accumulates in macrophages because of a deficiency of the lysosomal β-glucosidase (glucocerebrosidase). Until recently, treatment of Gaucher disease has been almost entirely symptomatic. An exception is the use of allogeneic bone marrow transplantation, which cures the reticulo-endothelial manifestations of the disease, but at a high risk, which makes it an unsuitable modality in most cases, even when a matching donor is available.

In 1964 De Duve suggested that the lysosomal disorders might yield to enzyme replacement. Among the lysosomal storage diseases, Gaucher disease has been considered a prime candidate for such therapeutic intervention because the nervous system is usually spared and because cells of the macrophage system seem ideally suited to take up exogenously administered substances. Although a number of attempts to treat Gaucher disease by enzyme replacement were made in the 1970s, these were unsuccessful. They were probably doomed to failure partly because of an inadequate supply of enzyme. The enzyme supply problem has been overcome by production of glucocerebrosidase on a commercial scale. Moreover, modification of the native enzyme to expose mannose residues presumably improves its targeting to tissue macrophages.

Although only scant clinical data have previously been reported, the dose of mannose terminated glucocerebrosidase (Ceredase; Genzyme, Boston, MA) recommended by the manufacturer and most commonly used is 60 U/kg/2 weeks or approximately 1,560 U/kg body weight annually. This amount of enzyme administered to a 70-kg patient costs $382,000. Because it seems likely that a greater amount of enzyme will be delivered to the monocyte-macrophage system if it is administered more frequently, we have treated four patients with less than one fourth of this dose. Two units per kilogram was administered on alternate days or 2.3 U/kg was given three times weekly. In this communication we report a very satisfactory response to enzyme replacement therapy in all patients.

PATIENTS

All patients treated had moderately severe or severe type I Gaucher disease, confirmed by leukocyte β-glucosidase estimation. All manifested the 1,226? genotype as described previously. The treatment protocol was approved by the Institutional Review Board of the Scripps Clinic and Research Foundation, and all patients gave informed consent for enzyme administration and for the follow-up studies.

Clinical histories. S.W. is a 30-year-old woman of Ashkenazi Jewish ancestry. The diagnosis of Gaucher disease was made in 1965 at age 5 as a result of evaluation of a bleeding tendency and splenomegaly. Splenectomy was performed at age 7, and pain in the hips and knees began at age 8. In succeeding years, she suffered a fracture of the right hip and compression fracture of the 10th thoracic vertebra. At age 20, the patient developed progressive shortness of breath. At age 25, the dyspnea had become so severe as to limit performance of simple daily activities. Pulmonary function tests at that time showed decreased steady-state diffusion capacity of her lungs and a low ventilation/perfusion ratio that was believed to be due to right-to-left shunting. At bronchoscopy possible Gaucher-like cells were identified in bronchial washings, but transbronchial lung biopsy was normal. Since 1988, the patient had been on continuous nasal oxygen because of severe dyspnea, limiting even eating and speaking.

On starting enzyme therapy in February 1990 the patient had massive hepatomegaly, markedly abnormal liver function studies, and severe dyspnea, even at rest. There was mild reduction in the static lung volumes that was probably explained by her small chest size relative to her height because her lung elastic recoil pressure and status lung compliance were normal. She had very severe hypoxemia that may have been largely due to right-to-left shunting, although a reduced pulmonary capillary volume was indicated by a severe and proportional reduction of both the single breath and the steady-state diffusing capacity. Pertinent findings and clinical laboratory findings at the beginning of therapy are summarized in Tables 1 and 2.

G.S. is a 45-year-old woman of Ashkenazi Jewish ancestry. She was first noted to have splenomegaly and easy bruising at age 7. She was diagnosed with Gaucher disease at age 5 as the result of evaluation of a bleeding tendency and splenomegaly. Splenectomy was performed at age 7, and pains in the hips and knees began at age 8. In succeeding years, she suffered a fracture of the right hip and compression fracture of the 10th thoracic vertebra. At age 20, the patient developed progressive shortness of breath. At age 25, the dyspnea had become so severe as to limit performance of simple daily activities. Pulmonary function tests at that time showed decreased steady-state diffusion capacity of her lungs and a low ventilation/perfusion ratio that was believed to be due to right-to-left shunting. At bronchoscopy possible Gaucher-like cells were identified in bronchial washings, but transbronchial lung biopsy was normal. Since 1988, the patient had been on continuous nasal oxygen because of severe dyspnea, limiting even eating and speaking.

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Table 1. Hematologic, Liver Function, and Bone Findings in Four Patients With Gaucher Disease Before and After Treatment With Ceredase

<table>
<thead>
<tr>
<th>Months of Treatment</th>
<th>Normal Range</th>
<th>S.W.</th>
<th>G.S.</th>
<th>A.M.</th>
<th>B.F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>14-18</td>
<td>10.3</td>
<td>11.2</td>
<td>5.5</td>
<td>7.9</td>
</tr>
<tr>
<td>Female</td>
<td>12-16</td>
<td>14.7</td>
<td>14.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td>20</td>
<td>32</td>
<td>16.5</td>
<td>25</td>
</tr>
<tr>
<td>Male</td>
<td>39-55</td>
<td>31</td>
<td>40</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>36-48</td>
<td>164</td>
<td>246</td>
<td>286</td>
<td>313</td>
</tr>
<tr>
<td>Platelets ($\times 10^9$/µL)</td>
<td>130-400</td>
<td>141</td>
<td>71</td>
<td>56</td>
<td>31</td>
</tr>
<tr>
<td>PT (s)</td>
<td>10.4-13.2</td>
<td>14.2</td>
<td>13.5</td>
<td>14.5</td>
<td>14.6</td>
</tr>
<tr>
<td>PTT (s)</td>
<td>22-36</td>
<td>38.8</td>
<td>40.4</td>
<td>37.5</td>
<td>41.2</td>
</tr>
<tr>
<td>SGOT U/L</td>
<td>2-40</td>
<td>41</td>
<td>56</td>
<td>58</td>
<td>54</td>
</tr>
<tr>
<td>SGPT U/L</td>
<td>2-40</td>
<td>22</td>
<td>41</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>30-120</td>
<td>194</td>
<td>84</td>
<td>145</td>
<td>137</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.0-1.5</td>
<td>0.7</td>
<td>1.1</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dL)</td>
<td>0.0-0.4</td>
<td>0.3</td>
<td>0.4</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>ACE (mU/mL serum)</td>
<td>16.4-52.4</td>
<td>324</td>
<td>220*</td>
<td>247</td>
<td>212T</td>
</tr>
<tr>
<td>Bone lesions</td>
<td></td>
<td></td>
<td>(H,F) Stable</td>
<td>(F) Stable</td>
<td>(F) NR</td>
</tr>
<tr>
<td>CompFx§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CompFx</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Abbreviations: NR, not repeated; I, infactions; H, humeri; F, femurs; CompFx, compression fractures.

*10 months.
†4 months.
§1 month.

‡Extensive involving thoracic and lumbar spine.

Involving thoracic spine only.

A.M. is a 10-year-old boy of Ashkenazi Jewish ancestry first noted to have splenomegaly at age 2. Bone marrow examination revealed the diagnosis of Gaucher disease. He did well until age 8, at which time increased bleeding at tonsillectomy required transfusion of platelets, plasma, and packed red blood cells. Because of continued episodes of epistaxis, modest thrombocytopenia, and massive splenomegaly, a partial splenectomy was performed. Since this time the patient has had repeated episodes of fever and bone pain. His height has been below the 10th percentile for his age. Modest hepatomegaly and enlargement of the residual spleen was evident on physical examination and confirmed by imaging. Pertinent clinical and laboratory findings at the time enzyme therapy was started in September 1990 are summarized in Table 1.

B.F. is a 39-year-old Jewish man known to have Gaucher disease since he was 4 or 5 years of age. The diagnosis was originally established because two older siblings also had Gaucher disease, and he had only minimal symptoms until he was about 30 years old.
His main symptoms in recent years had been related to massive hepatosplenomegaly, marked pancytopenia, and compression fractures of the thoracic spine. Although splenectomy had been repeatedly recommended to him over the past several years he had declined. In recent months he had several splenic infarcts and his blood hemoglobin concentration had decreased to as low as 5 g%. Detailed findings at the time that treatment was initiated are presented in Table 1.

Laboratory studies. The angiotensin converting enzyme (ACE) activity of serum was measured on samples that had been stored at −20°C. All samples from each patient were assayed simultaneously using hippuryl-L-histidyl-L-leucine as substrate. Plasma β-glucosidase activity was measured at pH 4.0 using 4-methyl umbelliferyl-β-glucoside as substrate. Other clinical laboratory studies were performed by standard methods.

Measurement of liver and spleen volume. Axial 1-cm contiguous slices through the liver were acquired using T1 weighted imaging parameters on a 1.5 Tesla GE MRI Scanner (General Electric, Milwaukee, WI). The liver and spleen contours were outlined on each slice with the area calculated by computer. The areas for each slice were then scanned to yield the total volume in cubic centimeters. The ultrasound measurements were obtained using a B-mode scanner (Picker International, Highland Heights, OH) selecting the longest dimensions in the transverse, anterior-posterior (AP), and longitudinal directions. An ultrasound size index was computed as the product of these dimensions.

Pulmonary function. The arterial oxygen saturations ($S_\text{O}_2$) were measured using a Hewlett-Packard ear oximeter (model 47201A). S.W. was continuously on nasal oxygen at 2 to 4 L/min. The reported value for $S_\text{O}_2$ was that at which she stabilized after 5 minutes at rest or 2 minutes of exercise walking on the treadmill at 0.7 mph. The pretreatment values represented the range on four studies, one before treatment and the other three during the first month of treatment. The posttreatment values are the range of two tests done during the 9th month and one test done during the 11th month.

The exercise $S_\text{O}_2$ of G.S. was recorded during a staged exercise test with the work rate increasing each minute. The $S_\text{O}_2$ was averaged each breath for the last 30 seconds of the stage. The values given are for walking at 4 mph at 6% grade, the highest stage completed on both pretreatment and posttreatment tests. Posttreatment studies were performed in the fifth month of therapy.

Enzyme administration. Mannose terminated glucocerebrosidase (Ceredase) was purchased from Genzyme. The preparation was infused intravenously over a 2-hour period. All patients were given the same total dose of enzyme, viz, 30 U of enzyme per month per kilogram of body weight. The dosage was fractionated so that the enzyme was administered every other day or three times a week, except that patient S.W. was given enzyme only twice a week for the first 14 doses because more frequent administration was not
allowed under the then Food and Drug Administration-approved protocol.

All patients except S.W. received enzyme therapy without interruption. Therapy of this patient was interrupted after 6 months. She was observed closely for 2 months to determine whether disease manifestations would recur rapidly. Through a misunderstanding S.W. was given a single dose of 30 U/kg body weight on day 243 of the study by another physician not connected with this study.

RESULTS

Clearance of enzyme from plasma. Blood anticoagulated in EDTA was drawn from B.F. before enzyme infusion, at the end of a 2-hour infusion of 2.3 U Ceredase/kg body weight, and at 10- or 11-minute intervals immediately following the infusion. The levels are shown in Fig 1. The calculated one-half time was 11 minutes.

Adverse effects. No adverse effects of enzyme infusion were noted in any patient.

Hematologic effects. The effect of treatment on the hemoglobin levels and the platelet counts of the patients is shown in Fig 2. In the case of patients S.W., A.M., and B.F., an initial decrease in the hemoglobin level was observed. This decrease may have been of iatrogenic origin, at least in part, because of the frequent blood tests required by the protocol during the first week of treatment. Subsequently all patients showed an increase in the hemoglobin, with normalization of counts in the case of S.W. and A.M., who were anemic at the beginning of the study.

The platelet counts of all of the patients also increased. At the beginning of treatment the platelet count of S.W. was near the lower limit of normal, and it progressively increased during treatment. G.S. was mildly thrombocytopenic, and her platelet counts rapidly returned to the normal range.

Liver function and liver and spleen size. Patient S.W. had markedly abnormal liver function tests at the outset of the study and these showed rapid deterioration during the first month of treatment. Subsequently, as shown in Fig 3, her liver function tests all improved except for a transient elevation of transaminase values during the fourth month, possibly as a result of a hepatic infarction. The other three patients had no major liver function abnormalities, and the results of liver function tests remained normal during the course of the study.

S.W. and G.S. manifested marked hepatomegaly and this regressed during treatment, as shown in Figs 4 and 5. The liver and spleen size of A.M. had each decreased 10% after 6 months of treatment. Quite remarkably the child had gained 2.9 kg or 10% in body weight during this time. He had grown 7 cm. The ultrasound size index of the liver of B.F. had decreased a remarkable 22% after only 2 months of treatment. The maximum length of his spleen had decreased from 34.7 to 23.4 cm and the maximum anterior-posterior diameter from 23.5 to 20.4 cm.

Angiotensin converting enzyme. The level of angiotensin converting enzyme was greatly increased in all patients, as is characteristic of Gaucher disease. It tended to decrease during treatment (Fig 6), except for patient B.F., whose follow-up was only 1 month.

Skeletal changes. The major skeletal manifestations of Gaucher disease (marrow infarction and endoskeletal expansion) did not change in radiographic appearance with treatment in any of the patients. Patient S.W. continued to have fleeting bone pains and A.M., who had been subject to sporadic episodes of severe bone pain, suffered one episode of moderate pain after about 4 months of treatment.

Pulmonary function. Modest improvement of pulmonary function was documented in patients S.W. and G.S., who had impaired lung function at the beginning of treatment. The results of these studies are summarized in Table 2.

Subjective symptoms. All four patients experienced subjective improvement during the course of treatment. S.W. was able to resume limited activities, driving an automobile, and engaging in handicraft hobbies and some social activities. She had been unable to engage in any of these activities for a year or so before beginning enzyme therapy.
The frequency and severity of episodes of bone pain diminished. G.S. no longer had appreciable shortness of breath and felt stronger. A.M. had an improved appetite, became more active, and began to gain weight rapidly. Bone pains occurred less frequently. B.F. felt a marked increase in well being as his hemoglobin increased from 5 g% to over 7 g%. He has resumed his teaching responsibilities as a chemistry professor and no longer requires afternoon naps. His appetite and meal portions have increased as his organomegaly has decreased.

**DISCUSSION**

In the 1970s several groups of investigators attempted to treat patients with Gaucher disease by administering...
purified placental glucocerebrosidase. We treated seven patients with enzyme purified in our own laboratory with total doses per patient of 2.2 U to 122 U, corresponding to catalytic activity of about 3 U to 200 U of Ceredase. Enzyme was either infused directly intravenously or was encapsulated in erythrocytes that were coated with Ig to deliver them more effectively to the macrophage-monocyte system. The patient receiving the largest dose was treated over the course of a year. She weighed about 45 kg, and therefore received only about 4 U/kg over the entire year, a little more than 1% of the dose used in the present study. Thus, it is not surprising that the clinical responses of our patients were disappointing, although there was a tendency for the enzyme activity to decrease during treatment.

Recent preliminary reports of the results of treatment of Gaucher disease with mannose terminated glucocerebrosidase have been much more encouraging. Not only is the amount of enzyme available for administration much greater, but the exposed mannose of Ceredase may serve to target it more efficiently to the macrophage-monocyte system. Barton et al have presented a detailed account of a child with moderately severe Gaucher disease treated with Ceredase over a 2-year period. The minimum amount of enzyme administered by Barton et al was 60 U/kg body weight each 2 weeks. At the price of $3.50 per unit the cost of treating a 70-kg patient is approximately $382,000 per year for enzyme alone.

Enzyme administered intravenously is very rapidly cleared. We had earlier documented a $T_{1/2}$ of placental enzyme of about 30 minutes, and the rate of clearance of the modified enzyme was even more rapid. The mannose receptors of macrophages have been studied extensively, but their density and the rate of which they turn over is not precisely known. Moreover, it is entirely possible that the sensitivity of cells of the macrophage-monocyte system to the therapeutic effect of enzyme may vary during maturation of the monocyte to the Gaucher cell. Thus, the injection of an enzyme bolus every 2 weeks could be a very inefficient way to administer this very expensive product. The present study was undertaken to determine whether a much smaller, and thus much less costly, amount of enzyme would exert a significant clinical effect if the enzyme was administered at more frequent intervals. Because there is marked heterogeneity in the clinical expression of Gaucher disease and only scant published data about the results of the much larger doses that have been used heretofore, quantitative comparison of results is not feasible. However, it seems apparent that patients with Gaucher disease respond very well to the much smaller dose of Ceredase that we have used, and the response observed seems, if anything, more rapid than that described with much larger total doses administered at infrequent intervals. We were able to document decrease in organomegaly in all patients and improvement in blood counts in those instances in which anemia or thrombocytopenia was present. Over the relatively short period encompassed by these studies no objective changes in skeletal manifestations were observed, although bone pain decreased. Longer-term administration of enzyme appears to improve the x-ray appearance of the skeletal lesions. The relatively slow response of skeletal lesions is probably a reflection of the rate at which bone is remodeled.

Future investigations will deal with the effect of the enzyme when given by continuous infusion by portable pump or frequent self administration. It is hoped the effectiveness of even smaller doses of the enzyme will place it within the reach of more patients with severe and moderately severe Gaucher disease.
REFERENCES


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