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Enzyme replacement therapy in Gaucher's disease: Preliminary clinical trial of a new enzyme preparation

(glucocerebrosidase)

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ABSTRACT **A patient with far-advanced adult type Gaucher's disease was treated with solubilized, highly purified placental glucocerebrosidase administered after entrapment in human erythrocytes or by direct intravenous injection. In some instances the enzyme-containing erythrocytes were coated with gamma globulin. No toxic side effects were observed after enzyme infusion. There were suggestive, but not conclusive, findings that enzyme infusion may have been beneficial. After therapy, there was a decrease in transfusion requirement, some improvement of liver function, possible decrease in liver size, and relief of subjective symptoms. Erythrocyte and plasma glucocerebroside levels were unchanged during therapy, but there was a possibly significant decrease in leukocyte and platelet levels of the glycolipid. No changes occurred in serum acid phosphatase or angiotensin-converting enzyme activity.**

Gaucher's disease is a glycolipid storage disorder in which glucocerebroside accumulates in phagocytic cells throughout the body. A rare disease in most population groups, this autosomal recessive disorder has a frequency of between 1 in 5000 and 1 in 10,000 among Jews of Eastern European origin. Clinical features include massive hepatosplenomegaly, bone marrow failure, and bone pain and fractures. The central nervous system is spared in the adult form of the disease. Because storage occurs primarily in the reticuloendothelial system, patients with Gaucher's disease have been considered among the most suitable candidates for enzyme replacement therapy (1, 2). Pentchev *et al.* (3) achieved about 4100-fold purification of detergent-solubilized glucocerebrosidase from human placenta with a yield of 5%. They infused the partially purified enzyme into three patients and detected a transient decline in erythrocyte glucocerebroside levels (4, 5). Liver biopsies taken before and after enzyme infusion seemed to show a modest decline in liver glucocerebroside content.

We have recently succeeded in solubilizing detergent-free glucocerebrosidase and in purifying the enzyme 6000- to 8000-fold with a yield of 40–60% (6, 7), and we now report clinical and laboratory observations in a patient with far-advanced Gaucher's disease whom we have treated with this enzyme.

MATERIALS AND METHODS

Glucocerebrosidase was purified from human placenta by previously described methods or minor modifications thereof (6). Enzyme activity was measured with 4-methylumbelliferyl β-D-glucoside as substrate. One unit of enzyme hydrolyzes 1 μmol of this substrate (or 0.4 μmol of glucocerebroside) per min at 37°. Comparison with units as described by Pentchev *et al.* (3) is difficult. The technique they used for the measurement of glucocerebrosidase activity is not applicable to the highly purified enzyme used in these studies: detergents that stimulate insoluble preparations inhibit the highly purified enzyme. However, 1 unit of our preparation probably corresponds to between 2×10^4 and 2×10^5 units of theirs. In some studies the enzyme was incorporated into normal human erythrocytes by adding enzyme to erythrocytes packed at a hematocrit value of 70%, dialyzing against 5 mM phosphate buffer, pH 7.4, for 2 hr at 4°, and then dialyzing against buffered saline at 25° (8).

For the estimation of glucocerebroside levels, erythrocyte preparations were freed of leukocytes by filtration through microcrystalline cellulose-α-cellulose (9). Leukocytes and platelets were separated into platelet-rich, granulocyte-rich, monocyte-rich, and lymphocyte-rich fractions (10). Glucocerebroside assays were carried out by high-pressure liquid chromatography with a modification of the method described by Evans and McCluer (11). A chloroform/methanol, 2:1 (vol/vol), extract of plasma or blood cells was dried under nitrogen, benzoylated with 0.6 ml of 50% benzoyl chloride in dry pyridine, and dried under nitrogen. After redissolving in hexane, it was washed six times with alkaline, acidic, and unmodified methanol, dried under nitrogen, redissolved in hexane, and chromatographed on a Corasil I column with a linear gradient of 0–0.75% or 0–1.00% methanol in hexane.

Patient. The patient was a Jewish woman, born in 1944, who was known since age 5 to have Gaucher's disease. Details of her clinical history were published in 1973 (1). During the past few years she had had a gradual downhill course, with chronic arterial hypoxemia secondary to pulmonary shunting and repeated episodes of fever, infection, and life-threatening bleeding from her nose and rectum. Her outlook was considered to be grave in early 1976 when these studies were initiated. The therapeutic trials were undertaken only after detailed explanation of the procedure and possible risks to patient and family, and after approval by the institutional review committee. The patient was given five courses of enzyme as summarized in Table 1. Resealed erythrocytes that had not been coated with gamma globulin were cleared quite slowly from the circulation (12). After coating with gamma globulin the half-life (^{51}Cr) of the cells was only 4.8 hr. Enzyme infused directly into the circulation was cleared from plasma with a half-life of approximately 19 min.

Two months after conclusion of these trials, the patient had another episode of profuse nasopharyngeal hemorrhage. Bleeding could not be controlled surgically or by infusion of fresh-frozen plasma and the patient died.

RESULTS

Side Effects. Enzyme infusion had no effect on pulse, blood pressure, respiratory rate, or body temperature. No subjective symptoms were associated with enzyme infusion. Measurement

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Table 1. Courses of glucocerebrosidase treatment

Course	Date	Glucocerebrosidase dose, units	Carrier*
1	4-14-76	0.006	RBC
	4-15-76	0.096	RBC
	4-19-76	0.435	RBC
2	7-26-76	0.79	0
	7-27-76	2.91	0
3	9-21-76	2.20	C-RBC
4	10-28-76	1.41	C-RBC
5	2-1-77	1.5	0
	2-2-77	1.5	0
	2-3-77	1.5	0
	2-4-77	1.5	0
	2-7-77	1.5	0
	2-9-77	1.5	0
	2-12-77	1.5	0
	2-15-77	1.5	0
	2-21-77	1.5	0

* RBC, resealed erythrocytes; C-RBC, resealed erythrocytes coated with anti-Rh serum; 0, infused without carrier.

of plasma clotting Factors I, II, V, VII, VIII, IX, X, XI, and XII before and after enzyme infusion showed no change.

Peripheral Blood Cell Counts. The patient's transfusion requirements had averaged 3.5 units of packed erythrocytes per month in the 3 months preceding the first course of therapy. The transfusion requirement was 2 units of packed cells per month during the last 5 months of life. No significant change was observed in the leukocyte or platelet count.

Serum Acid Phosphatase and Angiotensin-Converting Enzyme. There was no consistent change in the levels of serum acid phosphatase or angiotensin-converting enzyme.

Liver Function. The mean (±SEM) serum glutamate-oxaloacetate transaminase value gradually fell from a pretreatment level of 124.8 ± 8.6 units/ml (n = 4) to 97.6 ± 2.8 units/ml (n = 7) (normal = 10–40 units/ml). The mean (±SEM) serum glutamate-pyruvate transaminase value fell from 30.0 ± 2.9 units/ml (n = 4) before treatment to 21.6 ± 1.2 units/ml (n = 7) after treatment (normal = 5–35 units/ml). Results of clotting factor studies remained essentially unaltered.

Liver Size. Liver size as determined by external palpation appeared to regress after the second course of therapy (Fig. 1). However, no further decrease in liver size could be established after additional courses of therapy, and the reliability of external measurements of liver size is low when the liver is massively enlarged. Measurement of liver size by technetium-99m sulfur colloid scintigram and by ultrasound suggested that a decrease of about 1 cm in each dimension occurred after the conclusion of the last course of therapy. Moreover, serial technetium scintigrams of the liver (Fig. 2) appeared to show improvement in an area of decreased uptake that had been present for at least 8 years. Spider hemangiomata showed a distinct decrease during the period of observation.

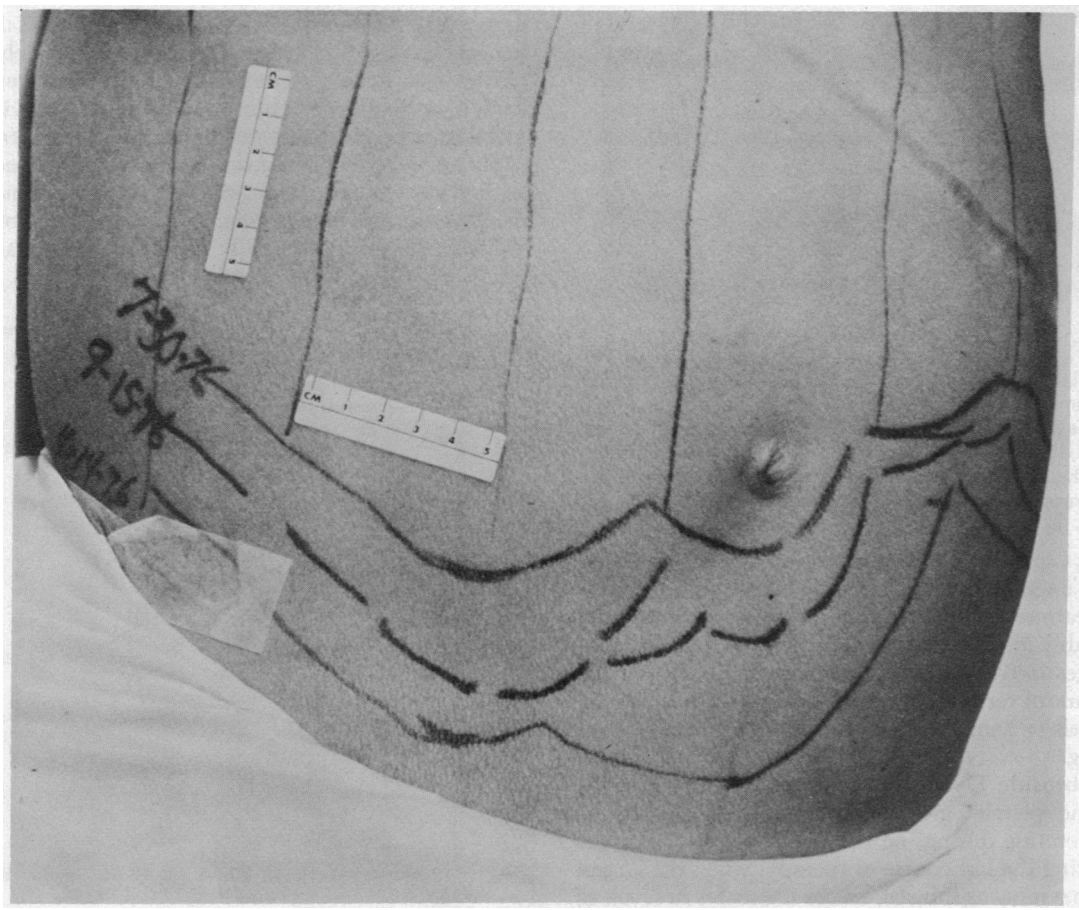

FIG. 1. The position of the inferior liver margin, as determined by external palpation on 4-14-76, 7-30-76, and 9-15-76. Marked regression of liver size was observed between the first two measurements, but some subsequent enlargement appeared to occur in spite of further therapy. Precise measurement of liver size is not possible by external palpation.

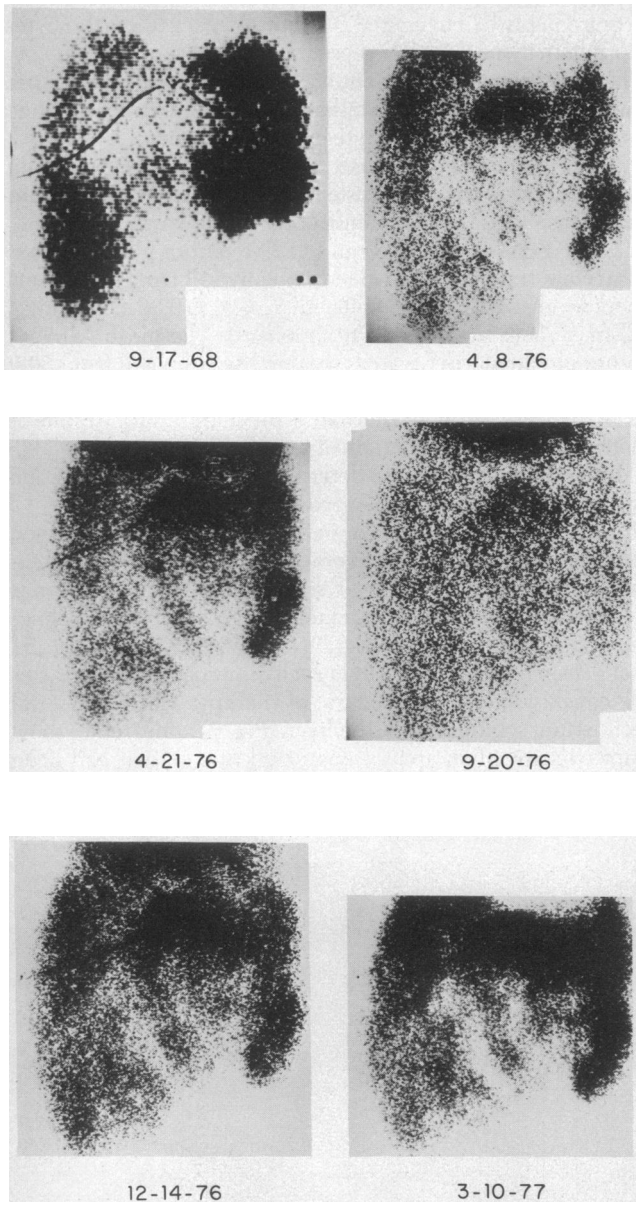

FIG. 2. Serial ^{99m}Tc sulfur colloid scintigrams of the liver carried out between September 1968 and March 1977. The photographic reduction in size of each scintigram is identical. Variations in overall density may be due to differences in technique. However, some improvement in the area of decreased uptake in the midportion of the liver and a decrease in overall liver size seemed to occur.

Subjective Changes. Gradual improvement was observed in the patient's activity status and in her feeling of well-being during her treatment. Her family was quite convinced that she was considerably improved. Always difficult to appraise, subjective changes in this patient were particularly difficult to evaluate because of various perturbing factors such as infections, changing levels of hemoglobin, and intermittent episodes of nasal bleeding.

Glucocerebroside Determinations. Plasma, erythrocyte, leukocyte, and platelet glucocerebroside levels showed no consistent alteration during the first four courses of enzyme therapy. During the last course of therapy, when the largest amount of enzyme was infused, minor decreases in levels of blood cell glucocerebroside were documented (Fig. 3); unfortunately, only two estimations of leukocyte glucocerebroside levels could be carried out because of the large quantity of blood required for isolation of sufficient numbers of cells. Liver glucocerebroside levels were determined at autopsy, 2 months after the last course of therapy. The glucocerebroside concentrations in six samples taken from different parts of the liver and weighing 770–1360 mg each ranged from 14 to 29 (19.7 ± 5.8) mg/g wet weight. Seven samples taken from a single portion of the liver with a Vim-Silverman biopsy needle and weighing 1 to 17 mg had glucocerebroside levels ranging from 9.8 to 33.8 (21.6 ± 7.7) mg/g.

DISCUSSION

Various model systems have been devised for the study of enzyme replacement therapy. Infusion of beef β-glucuronidase into β-glucuronidase-deficient mice has shown that exogenous enzyme may be incorporated into organs of the recipient animal and may remain active for several days (13). The administration of bacterial dextranase to dextran-loaded rats suggests that exogenously infused enzyme may hydrolyze an otherwise unmetabolizable storage material in the liver (14). The data gleaned from the studies of other enzymes are not necessarily applicable to β-glucosidase. In the case of some lysosomal enzymes, evidence derived from fibroblast uptake suggests that the enzyme may even exist in different forms in different organs and that some are readily taken up and others are not (15). One instance of glucocerebroside storage disease was found at autopsy in a dog (16); however, animal models suitable for study do not currently exist. Model systems for the study of uptake of β-glucosidase are difficult to implement; preliminary studies in our laboratory suggest that a small amount of partially purified glucocerebrosidase is incorporated into Gaucher's disease monocytes. Another possible model system relies on the well-known capacity of monocytes to ingest gamma globulin-coated particulates; in fact, we have found that resealed erythrocytes loaded with glucocerebrosidase and coated with gamma globulin are rapidly ingested by monocytes *in vitro*.

Such findings encouraged us to initiate replacement therapy with glucocerebrosidase. In the final analysis, clinical efficacy can be evaluated only in clinical trials. Appraisal of efficacy is difficult, however, especially in a patient with far-advanced

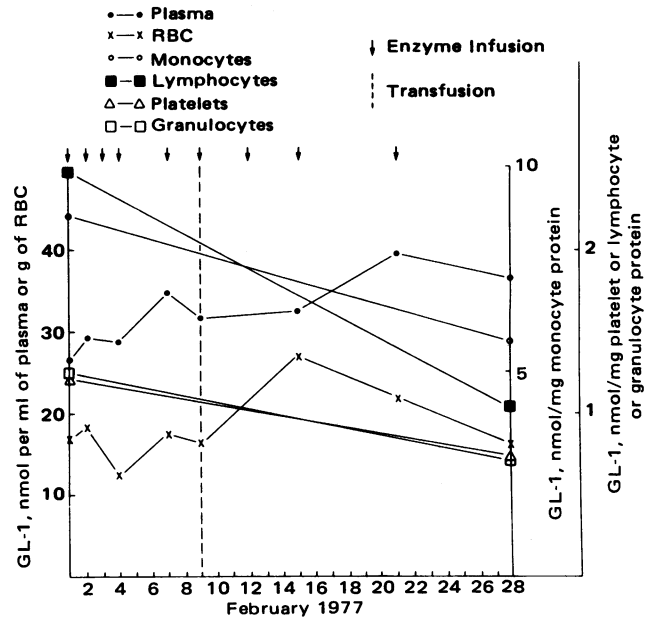

FIG. 3. Changes in plasma and blood cell glucocerebroside content during the patient's last course of therapy. Each arrow represents direct intravenous injection of 1.5 units of enzyme.

disease, and we are unable to draw clear-cut conclusions regarding the effect of treatment. The possibility that some benefit was obtained was suggested by the following findings: (*i*) a slight decrease in the size of the liver, both by external palpation and through technetium scanning and ultrasound measurements; (*ii*) improved homogeneity in liver technetium uptake; (*iii*) decrease in serum glutamate-oxaloacetate and glutamate-pyruvate levels; (*iv*) decreases in platelet, monocyte, granulocyte, and lymphocyte glucocerebroside levels; and (*v*) reduction in transfusion requirement.

On the other hand, many disease parameters were unaffected, including the following: (*i*) pulmonary failure; (*ii*) coagulation defects (which led to the patient's death); and (*iii*) serum acid phosphatase and angiotensin-converting enzyme activities. The massively enlarged liver weighed 7.7 kg at autopsy and contained amounts of glucocerebroside that are at the upper portion of the range found in patients with Gaucher's disease.

It is difficult to compare our observations with those reported by Brady *et al.* (5). Even if differences in enzyme properties are not taken into account, the amount of enzyme given by these investigators in a single injection may have been 2 or 3 times the total amount we infused. The liver of our patient was not biopsied because of her marked bleeding tendency. Furthermore, we believed that such biopsies would not be useful because the heterogeneous distribution of liver glycolipid would lead to too much random variability in glucocerebroside content in small samples. The fact that this was the case was verified when the liver was examined at autopsy. The relatively sharp fall in glucocerebroside level reported by Brady (4), but not observed in our studies, may be due at least in part to differences in quantity of enzyme infused. More effective management of Gaucher's disease may require infusion of larger amounts of enzyme or the development of a better delivery system, or both. In addition, it will be desirable to carry out investigations in patients who have not yet reached the end-stage of their disease. Our studies demonstrated the safety of repeated administration of partially purified glucocerebrosidase, both by direct intravenous injection and after encapsulation in erythrocytes. Further studies would seem clearly justified, and necessary if efficacy is to be evaluated.

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