Enzyme replacement therapy: conception, chaos and culmination

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Soon after the enzymatic defects in Gaucher disease and in Niemann–Pick disease were discovered, enzyme replacement or enzyme supplementation was proposed as specific treatment for patients with these and related metabolic storage disorders. While relatively straightforward in concept, successful implementation of this approach required many years of intensive effort to bring it to fruition. Procedures were eventually developed to produce sufficient quantities of the requisite enzymes for clinical trials and to target therapeutic enzymes to lipid-storing cells. These achievements led to the development of effective enzyme replacement therapy for patients with Gaucher disease and for Fabry disease. These demonstrations provide strong incentive for the application of this strategy for the treatment of many human disorders of metabolism.

Keywords: lipid storage disorders; enzyme replacement; benefits to patients

1. BACKGROUND

Reports of some of the features of patients with conditions that turned out to be sphingolipid storage disorders began to appear in the latter part of the nineteenth century. The earliest of these was a description in 1881 of a cherry-red spot in the fundus of the eye in an infant with the condition that became known as Tay–Sachs disease. It was quickly followed by a report of splenomegaly in a patient with Gaucher disease in 1882, and, in 1887, skin abnormalities in two patients with Fabry disease. With time, additional clinical manifestations of these conditions were documented. Descriptions of patients with Niemann–Pick disease, Krabbe disease, generalized GM1 gangliosidosis and fucosidosis followed (Brady 1978). Patients with Gaucher disease have been subdivided into type 1 (non-neuronopathic), type 2 (acute neuronopathic) and type 3 (chronic neuronopathic) clinical presentations. Similarly, Niemann–Pick patients were classified as type A with organomegaly and severe brain involvement and type B with organomegaly, but without neurological signs. In addition, it was gradually realized that the extent and severity of the clinical problems, the time of their clinical onset and rate of progression varied greatly within the patient groups.

Aside from additional sporadic case reports, little insight into the nature of these conditions was available until 1934 when the material that accumulated in patients with Gaucher disease was correctly identified by Aghion (1934) as glucocerebroside. It was subsequently shown by Klenk (1934) that sphingomyelin accumulated in the organs and tissues of patients with Niemann–Pick disease. Later ganglioside GM2 was reported by Svennerholm (1962) to

accumulate in Tay–Sachs disease, and ceramidetrihexoside was shown by Sweeley & Klionsky (1963) in 1963 to accumulate in Fabry disease.

Even in the middle of the twentieth century, nothing was known about the metabolism of the accumulating sphingolipids. Because galactocerebroside was known to be a major component of the central and peripheral nervous systems, it could be conceived that the glucocerebroside that accumulated in patients with Gaucher disease might be due to an abnormality of galactose metabolism. Therefore in 1956, I performed a galactose tolerance test in a patient with type 1 Gaucher disease and learned that there was no apparent abnormality in galactose metabolism. I then decided to explore glucocerebroside formation in surviving slices of spleen tissue obtained at splenectomy that was frequently performed because of splenomegaly and severe haematological problems associated with Gaucher disease. These studies revealed that there was no abnormality in the biosynthesis of either glucocerebroside or galactocerebroside, nor was there an augmented rate of formation of glucocerebroside over that in control human spleen specimens. These results prompted the speculation that Gaucher disease might be caused by an abnormality of glucocerebroside catabolism (Trams & Brady 1960). Studies with unlabelled glucocerebroside did not provide an answer because enzyme assays with this material were too insensitive. Biosynthetic attempts to label glucocerebroside did not yield products with sufficient radioactivity to carry out tracer studies. Labelling glucocerebroside throughout the molecule with ^3H by the Wilzbach technique was unsatisfactory because background radioactivity in non-incubated control reaction vessels was too high. Finally, David Shapiro, Julian Kanfer and I chemically synthesized glucocerebroside with ^{14}C in the fatty acid moiety in one preparation and ^{14}C in the glucose moiety in another. We found no conclusive evidence for an enzyme that catalysed the hydrolytic cleavage

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of the fatty acid from glucocerebroside, but all of the tissues in the human body contain an enzyme called glucocerebrosidase that catalyses the cleavage of glucose from glucocerebroside (Brady *et al.* 1965*a*). We then turned to more slice experiments and found that glucocerebrosidase activity was significantly reduced in tissue specimens obtained from patients with Gaucher disease (Brady *et al.* 1965*b*, 1966*a*). I had anticipated that there would be no glucocerebrosidase activity in tissues from patients with Gaucher disease. I was therefore quite surprised to learn that all patients who survive the immediate post-partum period have demonstrable residual glucocerebrosidase activity. This situation later turned out to be quite fortuitous when enzyme replacement therapy trials were undertaken.

The following year, the enzymatic defect in Niemann–Pick disease was shown with chemically synthesized ^{14}C -sphingomyelin (Brady *et al.* 1966*b*). The next year the metabolic defect in Fabry disease was demonstrated with generally labelled ^3H -ceramidetrihexoside that had been purified sufficiently to reduce background radioactivity to acceptable levels (Brady *et al.* 1967). Two years later, the specific biochemical abnormality in Tay–Sachs disease was identified using biosynthetically labelled ganglioside GM2 (Kolodny *et al.* 1969; Tallman *et al.* 1972). The metabolic defects in all of the sphingolipid storage disorders were eventually shown to be due to insufficient activity of specific hydrolytic enzymes.

2. CLINICAL APPLICATIONS

(a) *Diagnostic tests*

The first clinical use of this information was the development of easily performed tests to diagnose patients with these disorders (Kampine *et al.* 1967), identify the majority of the carriers of these conditions (Brady *et al.* 1971*a*) and for prenatal diagnosis (Brady *et al.* 1971*b*). These tests are in worldwide use at the present time. Genotyping is employed when there is uncertainty concerning the identification of heterozygotes.

(b) *Nascence of enzyme therapy*

Quite soon after learning the nature of the enzyme defects in Gaucher disease and Niemann–Pick disease, I postulated that enzyme replacement or supplementation might be beneficial for patients with sphingolipid storage disorders (Brady 1966). At the time, I was not aware that glucocerebrosidase and sphingomyelinase were lysosomal enzymes. When performing subcellular fractionation of tissue homogenates using isotonic sucrose, most of the glucocerebrosidase activity was found in the high-speed supernatant fraction (Brady *et al.* 1965*a*). Moreover, the pH optimum for the activity of this enzyme was between 5.9 and 6.0, which is considerably higher than the usual lysosomal enzymes. Further investigation revealed that the highest specific activity of glucocerebrosidase and sphingomyelinase is present in purified lysosomes (Weinreb *et al.* 1968), but the bulk of the activity is in other subcellular fractions. This statement was particularly true for sphingomyelinase. Subsequently, convincing evidence for at least one non-lysosomal sphingomyelinase was found and another form of glucocerebrosidase was forthcoming. Nevertheless, the concept of enzyme

replacement for lysosomal enzyme deficiencies had been enunciated prior to my preparing the review article (Brady 1966), and full credit is due to Christian de Duve (1964) for his articulation of this possibility.

(c) *Early investigations on enzyme replacement*

To explore enzyme replacement in patients with sphingolipid storage disorders, my associates and I began to explore the availability of the requisite enzymes. I wanted to use a human source if one were available. Our initial investigation was carried out with hexosaminidase A isolated from human urine and injected intravenously into an infant with the O-variant (Sandhoff) form of Tay–Sachs disease (Johnson *et al.* 1973). We learned that there was a quite rapid decrease of the elevated globoside in the circulation after infusion of the enzyme. It returned to the pre-infusion level rather rapidly. We also learned in this investigation that none of the intravenously administered enzyme reached the brain. The preparation was quite pyrogenic, and I therefore wanted to explore the possibility of other human sources of sphingolipid hydrolases and eventually thought of human placenta. I obtained some fresh placental tissue and found that extracts of it contained ceramidetrihexosidase and glucocerebrosidase activity. Dr William Johnson purified a small amount of ceramidetrihexosidase, now called α -gal A, from this tissue (Johnson & Brady 1972). Following injection of this enzyme into two patients with Fabry disease, there was a rapid reduction of ceramidetrihexoside, now called Gb3, in the blood (Brady *et al.* 1973). Gb3 returned to the pre-injection level within 3 days after infusing the enzyme. We were not permitted to obtain tissue specimens in this investigation. Shortly thereafter, Dr Peter Pentchev succeeded in purifying small amounts of glucocerebrosidase from placental tissue (Pentchev *et al.* 1973). We obtained permission to perform percutaneous needle biopsies of the liver before and after intravenous infusion of glucocerebrosidase in two patients with Gaucher disease. There was a 26% reduction in the quantity of hepatic glucocerebroside by 24 h following injection of the enzyme (Brady *et al.* 1974). Many investigators were sceptical of this result and postulated that the difference was due to the tissue sampling procedure. We ruled out this objection by multiple analyses of needle biopsy samples in a post-mortem liver from a patient with Gaucher disease. However, some persons were still unconvinced about the validity of the findings. Somewhat reassuring was the data concerning the blood level of glucocerebroside which was between three and fourfold higher than normal before infusing glucocerebrosidase. The quantity in the blood returned to normal over a period of 72 h. Most importantly, it remained in the normal range for a number of weeks and only gradually re-accumulated in the circulation over a period of many weeks (Pentchev *et al.* 1975). We were greatly encouraged by these observations that were performed on many samples from each of the recipients. They seemed to indicate that one might expect a clinical benefit if we could provide enough glucocerebrosidase to patients with Gaucher disease.

(d) *Large-scale purification of glucocerebrosidase from human placental tissue*

The optimism generated by the preceding findings was short-lived. We worked for 1 year and obtained 9 mg of

purified placental glucocerebrosidase that was injected into a splenectomized patient with type-1 Gaucher disease. She had 20 times more glucocerebroside in her liver than the first recipient and 10 times more than the second. We saw only an 8% reduction of hepatic glucocerebroside, which was actually within the sampling error of these determinations. Moreover, there was no reduction of glucocerebroside in the blood. The enzyme preparation was fully active catalytically, and we deduced that we had not administered enough of it to this patient. We were then at a major roadblock. Because we could not scale-up Pentchev's purification procedure, we had to stop investigations with patients for 2 years while we developed a satisfactory isolation procedure that took advantage of the extraordinary hydrophobicity of glucocerebrosidase. To make the enzyme adhere to hydrophobic affinity columns, we had to extract an early fraction of the placental glucocerebrosidase with butanol to remove lipids that were present in the preparation. With this modification, we were able to purify large amounts of placental glucocerebrosidase (Furbish *et al.* 1977).

(e) *Targeting glucocerebrosidase to lipid-storing cells*

Once again, we encountered an extraordinary difficulty. In three of seven patients who received this new enzyme preparation, we observed a reduction of hepatic glucocerebroside. In four other recipients, however, we could not detect an effect, and we were greatly puzzled by these findings. The enzyme was fully active with ^{14}C -labelled glucocerebroside. We eventually learned that most of this glucocerebrosidase preparation was taken up by hepatocytes in the livers of experimental animals. This was completely undesired because most of the glucocerebroside that accumulates in the liver is in macrophages (Kupffer cells). We eventually learned that extracting the enzyme with butanol had untoward effects. It removed phosphatidylserine that was discovered to be an activator of glucocerebrosidase (Dale *et al.* 1976) and can also direct materials such as proteins to macrophages (Kupffer cells) (Raz *et al.* 1981).

We learned two important facts at this point. It was reported that lysosomal enzymes were glycoproteins (Goldstone & Koenig 1970) and that macrophages had a lectin on their surface with high avidity for mannose-terminal glycoconjugates (Stahl *et al.* 1978). It was found that glucocerebrosidase has four oligosaccharide side chains, three of which are called the complex type whose terminal molecules are *N*-acetylneuraminic acid or galactose. The occurrence of uncovered galactose is particularly deleterious as hepatocytes have a particularly strong lectin for this sugar (Ashwell & Morell 1974). To divert glucocerebrosidase from hepatocytes to macrophages, we carried out three investigations. The first was covalent addition of linear pentamannosyl chains to the enzyme. This strategy did not increase the targeting of glucocerebrosidase to macrophages. The second was covalent linking of dilysyltrimannosyl residues to the protein. This procedure increased the uptake of glucocerebrosidase by macrophages fourfold (Doebber *et al.* 1982). While encouraging, we did not consider this increase to be sufficient for clinical benefit. We eventually elected to remove the three terminal oligosaccharides from the complex oligosaccharide chains of glu-

cocerebrosidase with specific exoglycosidases to produce mannose-terminal glucocerebrosidase (Furbish *et al.* 1981). This strategy resulted in a 50-fold increase in the uptake of glucocerebrosidase by Kupffer cells over that of the unmodified enzyme (Brady & Furbish 1982).

(f) *Initial clinical investigations with mannose-terminal glucocerebrosidase*

Encouraged by the enhanced delivery of glucocerebrosidase to lipid-storing cells, my colleagues and I carried out a trial consisting of weekly administration of 190 U of mannose-terminal glucocerebrosidase to seven adults and one child with type 1 Gaucher disease over a period of six months. Of these, only the child, a 5-year-old male, had clearly observable responses. Several weeks after the initiation of enzyme infusions, his haemoglobin, which was in the anemic range, began to increase. In addition, his blood platelets, which were severely depressed, gradually began to rise (Barton *et al.* 1990). The patient was receiving *ca.* 13 U kg^{-1} of body weight each week. The adults were receiving *ca.* 2 U kg^{-1} of body weight. Because we wished to be certain that these effects were not due to a spontaneous recovery of his abnormal haematology, we discontinued the administration of enzyme. Both the haemoglobin and platelets gradually declined to levels that were present before enzyme injections had been given. We then treated him with weekly infusions of glucocerebrosidase at 32 U kg^{-1} of body weight. His haemoglobin and platelet counts rose rapidly to normal values. He has been maintained on bi-weekly infusions of glucocerebrosidase, and these values have been normal ever since. In addition to the haematological responses, the size of his greatly enlarged liver and spleen returned to normal, and the skeletal damage that was evident before enzyme therapy has been completely corrected. Based on the discrepant results between the child and the adults, we deduced that the latter patients had not received sufficient enzyme to achieve clinical benefit.

(g) *Dose-response and clinical efficacy trials*

Because we deduced that the inconsistency of the haematological responses that occurred in the preceding trial was due to inadequate dosing of the adult recipients, we performed a dose-response trial in adults resorting to liver biopsies before and after infusing a wide range of mannose-terminal glucocerebrosidase. We found that the most consistent reduction of hepatic glucocerebroside followed the administration of 60 U of enzyme per kg of body weight. Based on this observation, we devised a clinical efficacy trial with 12 non-splenectomized patients with type-1 Gaucher disease. Patients were from 9 to 42 years in age. All of them had significant improvement in their haemoglobin and platelet levels; the size of the spleen and liver was reduced, and the skeletal manifestations of Gaucher disease were improved (Barton *et al.* 1991). On the basis of these findings, the US Food and Drug Administration approved mannose-terminal glucocerebrosidase for the treatment of patients with Gaucher disease on 5 April 1991. The Genzyme Corporation subsequently produced glucocerebrosidase by recombinant technology in Chinese hamster ovary cells. This product was found to be as effective as the placental enzyme (Grabowski *et al.* 1995). It was approved for the treatment of patients with

Gaucher disease by the US Food and Drug Administration in 1994. This therapy has been approved by regulatory agencies in 54 additional nations. Currently, more than 3000 patients with Gaucher disease are receiving enzyme replacement therapy. Beneficial responses to enzyme replacement in patients with Gaucher disease have been reported in more than 100 publications. A summary of such benefits has recently appeared (Weinreb *et al.* 2002).

(h) *Enzyme replacement in Fabry disease*

A year before placental glucocerebrosidase became available for the initial investigation of its effect in Gaucher patients, my colleague Dr William Johnson obtained a small amount of highly purified α -gal A from placental extracts (Johnson & Brady 1972). Infusion of this enzyme into two males with Fabry disease caused a rapid reduction of ceramidetrihexoside (now called Gb3) in the circulation (Brady *et al.* 1973). By 72 h, Gb3 in the blood had returned to the pre-injection level, a kinetic response quite distinct from that observed in Gaucher patients. Two additional infusions of placental α -gal A were carried out, but in each instance, a technical complication prevented the acquisition of useful data. Further exploration of enzyme therapy in patients with Fabry disease was delayed for many years until α -gal A was produced recombinantly in cultured human skin fibroblasts. When this enzyme preparation became available, a phase one safety and dose-response study was carried out in 10 males with the disorder (Schiffmann *et al.* 2000). In nine of the 10 patients, there was a significant reduction of Gb3 in the blood. Moreover, three weeks after infusing a single dose of α -gal A, there was a large reduction of Gb3 in the urine sediment in nine of the patients. Reduction of urinary Gb3 also occurred in the 10th patient but at two weeks post-infusion. These results were particularly encouraging as they probably represented an improvement in the function of damaged renal tubular cells, a primary aspect of the pathophysiology in Fabry disease.

Encouraged by these findings, we carried out a randomized placebo-controlled phase two clinical efficacy trial of enzyme therapy in Fabry patients (Schiffmann *et al.* 2001a). The α -gal A used in this investigation was produced by a gene-activation technique developed by Transkaryotic Therapies, Inc., Cambridge, MA, using a continuous human cell line. Because most patients with Fabry disease have a debilitating peripheral neuropathy accompanied by severe painful episodes, we looked at the effect of the enzyme on this manifestation of the disorder. There was a significant reduction in the severity of pain in patients who received the enzyme compared with those on placebo ($p = 0.02$). There was improvement of the quality of life of the recipients of α -gal A compared with those on placebo ($p = 0.05$). There was dramatic decrease in mesangial widening in kidney glomeruli of the recipients ($p = 0.01$) and improvement of creatinine clearance ($p = 0.02$). There was also reduction of plasma Gb3 along with improvement in cardiac conduction and an increase of body weight. After six months, patients in the placebo arm were also given the enzyme, and the original recipients were continued on enzyme for 2 years. In both groups, patients' renal disease became stabilized or improved (Schiffmann *et al.* 2001b). Significant clearance

of renal micro-vascular endothelial deposits of Gb3 have been reported following infusions of recombinant α -gal A produced in Chinese hamster ovary cells (Eng *et al.* 2001).

These salutary findings provided the basis for the approval of enzyme replacement therapy for patients with Fabry disease by member countries of the European Union and seven additional countries throughout the world. A decision concerning a biological licence from the US Food and Drug Administration is anticipated in late 2002 or early 2003. Moreover, taken together, the positive results of enzyme therapy for Gaucher disease, and now for Fabry disease, provide strong impetus for similar studies in other disorders. Chief among them are Pompe disease (Amalfitano *et al.* 2001) and the Hurler syndrome (Kakkis *et al.* 2001). Much additional effort is anticipated before enzyme therapy becomes effective for patients with metabolic storage disorders with central nervous system damage. A recent preclinical investigation was highly encouraging in this regard (Zirzow *et al.* 1999).

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GLOSSARY

α -gal A: α -galactosidase A
Gb3: globotriaosylceramide