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Bone Marrow Transplantation for Niemann–Pick Type IA Disease

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Summary: Bone marrow transplantation has been undertaken with encouraging results as therapy for a wide variety of lysosomal storage diseases. We report a case of Niemann–Pick disease Type IA in which, despite the presence of only mild hypotonia with depressed reflexes, the clinical course of the disease appeared to be only slightly modified by this procedure, which was performed at the earliest practical opportunity. The patient was diagnosed early when asymptomatic, because of a family history of an affected sibling who died at 14 months. He received a bone marrow transplant from an HLA-identical, MLC non-reactive sibling donor, whose leukocyte sphingomyelinase activity was in the homozygote normal range. There was adequate engraftment as evidenced by persistently normal leukocyte sphingomyelinase activities, and there was no evidence of graft-versus-host disease. Visceral storage and neurological impairment were less rapidly progressive than in his untreated sibling but he eventually died at 30 months. Autopsy confirmed that this was essentially due to the effects of the underlying Niemann–Pick disease. We conclude that despite some success in other neurovisceral lysosomal storage disorders, bone marrow transplantation is not likely to be an adequate treatment for Niemann–Pick disease Type IA.

Bone marrow transplantation has been undertaken with encouraging results as a therapy for a wide variety of neurovisceral lysosomal storage disorders, such as the mucopolysaccharidoses and Gaucher disease (Krivit 1989). The rationale for this

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procedure is that circulating blood cells derived from transplanted, normal bone marrow become a continuing source of the missing enzyme to affected tissues. Donor macrophages, presumably originating from circulating monocytes, have been shown to repopulate the reticuloendothelial system of the liver and lungs following allogeneic bone marrow transplantation (Thomas et al 1976; Gale et al 1978). There is also *in vivo* evidence in murine models that following bone marrow transplantation donor bone marrow-derived cells are present as microglial cells in the central nervous system and are metabolically active (Hoogerbrugge et al 1988). These donor cells are believed to be responsible for removing the abnormal stored lipid *in vivo* by two possible methods. Normal macrophages from transplanted marrow may phagocytose and metabolize the stored material. Alternatively, circulating bone marrow-derived cells may supply the missing enzyme to affected cells by cell-to-cell transfer, an event which has been documented *in vitro* (Olsen et al 1981).

Niemann–Pick disease (McKusick 257200, 247220, 257250) is a clinically heterogeneous group of disorders involving lysosomal accumulation of sphingomyelin and cholesterol. The disease has recently been classified into Type I, associated with sphingomyelinase (EC 3.1.4.12) deficiency in various tissues, usually with activities less than 10% of control values, and Type II, with activities greater than 10% of control (Spence and Callahan 1989). Each type can present with acute (A), subacute (S) or chronic (C) varieties. Type IA (formerly Type A), presents with visceral storage and neurological problems early in infancy. There is a single previous report of liver transplantation undertaken for treatment of Niemann–Pick Type IA, at age 2 years, with minimal benefit (Daloze et al 1977). Marked benefit 9 months after bone marrow transplantation has been described in Niemann–Pick Type IS (formerly Type B) (Vellodi et al 1987), in contrast with another patient with Niemann–Pick Type IA, briefly reported, who showed no improvement despite normal sphingomyelinase levels following bone marrow transplantation (Krivit et al 1990a). Another 2-year-old girl with probable Type IA has been mentioned as having had a fetal liver cell transplant, but there were no signs of engraftment and she died of her disease 2 months later (Ringden et al 1990).

We describe the results of an allogeneic bone marrow transplant from an unaffected, HLA-identical sibling donor, to study prospectively the efficacy of this procedure in preventing the onset of the severe manifestations of Niemann–Pick Type IA disease.

METHODS

Sphingomyelinase assay: White blood cells were sonicated in ice-cold deionized water for 5 s and protein was determined by standard methods (Lowry et al 1951). Sphingomyelinase was measured using 2-*N*-(hexadenoyl)-amino-4-nitrophenyl-phosphoryl (HNP) by a modification of the method of Gal et al (1975). Two other lysosomal enzymes, β-galactosidase and β-glucosidase, were assayed as controls (Wenger et al 1976).

Small samples of liver and brain were thawed and homogenized in 0.1% Triton X-100 and 0.05 mol/L NaCl. Enzyme assays were performed on the total homogenate after determination of the protein concentration (Lowry et al 1951). The liver

and brain homogenates were assayed for acid β-galactosidase activity using 4-methylumbelliferyl-β-D-galactopyranoside as described (Wenger et al 1976). Sphingomyelinase activity was measured with [^{14}C]methyl-labelled sphingomyelin at pH 5.0 in liver and brain. To rule out a contribution by Mg^{2+}-dependent sphingomyelinase in brain, additional samples were assayed in the presence of 0.5 μmol/L EDTA (Wenger et al 1981).

Lipid analysis: Liver biopsy and post-mortem samples were frozen immediately in a −70°C freezer and transported on dry ice (interval between death and post-mortem was 3 hours). Lipids in the samples were extracted by homogenizing a small sample in 20 volumes of chloroform–methanol (25:1 v/v). After filtering through a glass-wool filter, the solution was mixed with 1/5 volume of distilled water and centrifuged. The lower phases were evaporated with nitrogen and aliquots equal to 10 mg wet weight of tissue were spotted on a silica-gel thin-layer chromatography plate and run in chloroform – methanol – water (70:30:5, by vol.). Lipids were visualized by spraying with orcinol in sulphuric acid and heating at 110°C for 15 min. With this spray, glycolipids stained purple, cholesterol stained red, and phospholipids stained yellow. It is an excellent spray for detecting lipid storage in biopsy samples. The pre-transplant and post-mortem samples looked identical, with a large increase in the concentrations of sphingomyelin and cholesterol.

CASE HISTORY

The patient was the youngest of a family of four. He was born by spontaneous vaginal delivery after a normal 41-week gestation, and had an uneventful neonatal period. Diagnosis of Niemann–Pick Type IA disease was confirmed at 6 weeks of age by analysis of fibroblast sphingomyelinase activity of 0.25 nmol/h per mg protein (courtesy Dr Derek Applegarth, Vancouver, BC; normals 7.9–21 nmol/h per mg protein), and by finding ultrastructural lysosomal inclusions in peripheral blood leukocytes.

At diagnosis, his liver was palpable 2 cm below the right costal margin. There was no palpable spleen, there were no skin lesions, and he was neurologically normal. By 3 months of age his liver had enlarged to 5 cm below the costal margin, and the spleen to 4 cm below the costal margin. Head circumferences at 6, 12 and 16 weeks were 38.2 cm, 40 cm and 41.3 cm, all about the 50th centile. Neurologically he had slightly delayed performance on the Denver screen, with persistent head lag. In addition, he fed poorly and vomited excessively, resulting in failure to thrive. During this time he also had two respiratory infections treated with antibiotics.

Parents were unrelated, but both of Dutch extraction. Two siblings were normal homozygotes, one of whom was the male 22-month-old HLA-identical, mixed-lymphocyte-culture (MLC) non-reactive, and ABO-compatible bone marrow donor. An older sibling had been diagnosed with Niemann–Pick disease at age 4 months when he was noted to have reddish-brown skin lesions on his forehead that later spread to his trunk. Biopsy of the skin lesions revealed replacement of normal tissue with fibrohistiocytic cells containing lipid which showed lysosomal storage on electron microscopy. Leukocyte sphingomyelinase was deficient, confirming Niemann–Pick

disease (Wood et al 1987). He had progressive visceral storage: at 1 year his liver and spleen were enlarged, 11 cm and 14 cm respectively below the costal margin, and he had constant elevation of aspartate transaminase (AST) at 400–500 IU/L (normal < 40 IU/L). Developmental milestones were normal until 3 months, but there were no further gains. He became increasingly irritable and failed to thrive until his death at the age of 14 months from pneumonia.

Pre-transplant evaluation at age 4 months showed the patient to be just below the 5th centile in weight, but at the 25th centile for length. There were no skin lesions. His liver was 6 cm below the right costal margin, and spleen was 4.5 cm below the left costal margin. Neurological examination was normal except for mild generalized hypotonia with depressed reflexes (Table 2). The retina was normal, blood counts were normal, and the bone marrow contained numerous macrophages filled with lipid material. The peripheral leukocyte sphingomyelinase was 0.079 nmol/h per mg protein (normal 2.98 ± 0.25) (Table 1). A liver biopsy showed evidence of some early features of Niemann–Pick disease with complex lipid material in hepatocytes, reticuloendothelial cells, ductal epithelium and sinusoidal fibrosis, confirmed by electron microscopy.

Bone marrow transplantation was undertaken after permission from the Committee for Protection of Human Subjects of The Children's Hospital of Philadelphia was obtained, and informed-consent forms were signed by both parents. Pre-conditioning consisted of busulfan 1 mg/kg every 6 hours for 4 days (total dose 16 mg/kg), followed by cyclophosphamide 50 mg per day intravenously for 4 days (total dose 200 mg/kg) (Krivit et al 1990a,b). For graft-versus-host disease prophylaxis, he was started on daily cyclosporin 2.5 mg/kg intravenously on day −3, and received methotrexate 15 mg/m^2 on day +1, and 10 mg/m^2 on days +3, +6 and +11 (Ramsay et al 1982).

Table 1 Peripheral blood leukocyte sphingomyelinase activities pre- and post-bone marrow transplant expressed as nanomoles substrate hydrolysed/h per mg protein

Day	*Sphingomyelinase*	*β-Galactosidase*	*β-Glucosidase*
−20	0.079	—	19.1
+28	2.71	128	—
+39	1.38	131	13.5
+69	1.77	151	11.8
+98	2.61	164	19.4
+140	2.36	191	20.6
+161	2.36	142	16.9
+190	1.68	108	11.4
+288	2.20	138	27.9
+371	1.90	104	20.8
+542	2.90	131	26.8
+573	3.80	118	18.0
Normals mean ± SD (number)	2.98 ± 0.25 (14)	94 ± 18 (41)	7.6 ± 0.25 (32)
Family study			
Father	1.60		
Mother	0.81		
Donor	2.57		

Table 2 Neurodevelopmental studies

	Pre-transplant, 4 months old	*6 months post-transplant, 11 months old*	*12 months post-transplant, 17 months old*
Developmental assessment	Language, cognitive, adaptive, motor skills age-appropriate (Gessell Schedules)	Cognitive skills 7- to 8-month level (MDI = 58; mild range developmental delay) Motor skills at 6-month level (PDI > 50; moderate range developmental delay) (Bayley Scales of Infant Development; Bayley 1969)	Cognitive skils at 12-month level (MDI = 52; moderate to mild range developmental delay) Motor skills still at around 6-month level (PDI < 50; moderate to severe range developmental delay (Bayley Scales of Infant Development)
MRI/CT	Both normal	MRI normal	MRI: delayed myelination of deep white matter
EEG	Normal	Not done	Abnormal with diffuse slowing and disorganization of background activity

Whole donor marrow was administered at a dose of 1×10^8 nucleated cells/kg recipient weight. Approximately 7 days after marrow infusion, his liver and spleen enlarged, and he became markedly jaundiced (total bilirubin 200–240 μmol/L, indirect 32 μmol/L), but with no other derangement of liver function. At the same time he was mildly hypertensive.

Cyclosporin was thought to be contributing to the jaundice and hypertension, and was discontinued. His jaundice and hypertension then subsided, and the liver and spleen decreased slightly in size. Another episode of jaundice of unknown aetiology occurred from day 20 to day 28. Bone marrow aspirate at 1 month showed return to normal cellularity, but multiple large vacuolated macrophages were still present. Allogeneic engraftment was documented by peripheral leukocyte sphingomyelinase activities in the normal range (Table 1), and his haemoglobin, white count and platelets remained normal throughout his course following bone marrow recovery. No other probes were used to demonstrate complete haematopoietic engraftment.

Forty-two days after bone marrow transplant, he was discharged clinically well, but with persistent hepatosplenomegaly and elevation of serum transaminases. By 60 days post-transplant, AST decreased, but alkaline phosphatase rose to 1447 IU/L (normal < 120 IU/L); bilirubin was 43 μmol/L. Liver biopsy at this time showed unchanged lipid storage, with no evidence of hepatitis or graft-versus-host disease. Over the next two months, clinically his hepatosplenomegaly remained constant, each 7 cm below the costal margin, with normalization of bilirubin and alkaline phosphatase but persistent mild elevation of AST (200–500 IU/L).

Six months post-transplant, peripheral leukocyte sphingomyelinase was stable at the donor level (Table 1). Height remained at the 25th centile, and head circumference on the 50th centile (45.2 cm), but weight had dropped to −2.5 SD from the mean.

Objectively, liver size had increased (by CT scan to 437.6 cm^3 from 298 cm^3 pre-transplant), but the spleen was constant (by CT scan to 63.4 cm^3 from 62 cm^3 pre-transplant) (Heymsfield et al 1977; Moss et al 1981). He was still hypotonic with depressed reflexes and could not sit for more than a moment, but had demonstrated some neurological progress by managing to hold his head when prone, and having no head lag when pulled to sitting by 4 months post-transplant. Fundoscopy remained normal. He had made some developmental gains since the evaluation at 4 months, but had not maintained a normal developmental rate (Table 2). There was no evidence of graft-versus-host disease. Bone marrow was normocellular with marked reduction in numbers of Niemann–Pick cells. Immunological reconstitution was complete.

At 1 year post-transplant, neurological examination suggested slight motor gains despite a recent, temporary loss of skills after a short 'flu-like illness. He had better head control, although he was still not able to sit unassisted. However, developmental testing indicated that there had been essentially no gains in his physical skills (Table 2), but though the patient remained delayed in his cognitive development, he continued to learn new skills. In contrast, motor nerve conduction studies showed marked slowing in the demyelinating range with severe dispersion of proximally evoked CMAPs (compound motor action potentials); sensory nerve action potentials were unelicitable. The MRI, which was normal at 6 months post-transplant, now showed delayed myelination of deep white matter.

Other findings at 1 year post-transplant were hepatosplenomegaly persisting at the same level as 6 months post-transplant with slightly elevated liver enzymes and normal bilirubin. Bone marrow studies showed fewer Niemann–Pick cells than at 6 months post-transplant, and his peripheral leukocyte sphingomyelinase levels were still in the donor range (Table 1).

By 2 years post-transplant, there was progressive clinical deterioration. The liver and spleen slowly enlarged (9 cm and 8 cm, respectively, below the costal margin), with an increase in AST to 744 IU/L. There was radiological evidence of chronic diffuse interstitial lung disease. He became more hypotonic, and had several reported seizures. An EEG showed generalized grade III dysrhythmia consistent with a severe encephalopathic process, but no frank epileptic activity. His head circumference had been static for the previous 18 months and now was on the 5th centile. A cherry-red spot was now clearly visible at the macula. He became irritable and feeding was increasingly difficult; a gastrostomy tube was used. Two weeks later, at age 30 months, he suddenly developed respiratory difficulty, and died within hours.

A complete post-mortem examination was performed, revealing selective organomegaly including hepatomegaly (1100 g; normal for age 390 g), splenomegaly (200 g; normal for age 30 g), cardiomegaly (80 g; normal for age 56 g), and renomegaly (120 g; normal for age 93 g) (Copolletta and Wolbach 1933). Diffuse lymphadenopathy was also present.

Histopathological examination revealed extensive involvement of liver, spleen, lymph nodes and adrenal glands with storage-type macrophages and patchy involvement of myocardium and bone marrow. The lungs contained large numbers of vacuolated histiocytes, as expected for this disease, with no direct evidence of aspiration. The central nervous system showed storage in neurons throughout, with

no particular zones showing more severe involvement than others. Additional findings included a lymphocytic infiltrate of the myocardium, possibly representing viral myocarditis, but all cultures were negative. There was no evidence of opportunistic infection or histopathological evidence of graft-versus-host disease.

The lipid pattern in post-mortem liver samples was identical to pre-bone marrow transplant liver biopsy samples. Sphingomyelinase levels in liver and brain were practically undetectable (Table 3).

DISCUSSION

We describe the clinical course of a patient with Niemann–Pick Type IA disease following allogeneic bone marrow transplantation. The bone marrow transplant was performed in infancy soon after diagnosis, at a time when the risk for graft-versus-host disease is low and prior to onset of major neurological damage. The donor was an HLA-A, B and D identical sibling with normal sphingomyelinase activity. There were relatively few transplant-related problems (except for the poorly understood hepatic dysfunction during the first few months post-transplant and the precipitating cause of death, which could possibly have been transplant-related in that subtle post-transplant immune incompetence may have led to infectious myocarditis).

In view of the diagnosis at birth, bone marrow transplantation was performed at the earliest opportunity considered safe for a patient so young. Consequently, despite the presence of mild hypotonia with depressed reflexes at the time of transplantation, the patient was otherwise asymptomatic; this was an excellent opportunity to study the role of allogeneic bone marrow transplantation in the therapy of Niemann–Pick disease. The patient achieved normal peripheral leukocyte levels of sphingomyelinase soon after transplant, which were maintained during clinical deterioration 18 months to 2 years later. Despite this, we were unable to detect the enzyme in liver and brain at post-mortem. However, in comparison to his affected sibling there was some evidence of an ameliorated course. There was clearing of storage cells from the bone marrow, no skin lesions developed, and there was a less rapid progression of visceral

Table 3 Lysosomal enzyme activities in liver and brain

	Sphingomyelinase (nmol/h per mg protein)	*β-galactosidase* (nmol/h per mg protein)
Liver		
Patient (pre-BMT)	0.0	435
Patient (autopsy)	0.1	204
Controls ($n = 8$)		
Mean	22.9	163
Range	6.6–35.4	92–243
Brain		
Patient (autopsy)	0.15[a]	34.2
Controls ($n = 6$)		
Mean	19.8	14.7
Range	8.9–27.8	11.7–17.9

[a]Identical value with or without EDTA

storage. There was also somewhat slower neurological deterioration, with sparing of cognitive functions. Similar results have been reported on bone marrow transplantation in an animal model, the Niemann–Pick mouse; there was reduced visceral storage, but neurological deterioration and survival were unaffected (Sakiyama et al 1983; Yasumizu et al 1990).

The apparent lack of efficacy of bone marrow transplantation in the current case may be contrasted with its more beneficial effects in other lysosomal diseases such as Gaucher disease (Ringden et al 1988) and metachromatic leukodystrophy (Krivit et al 1990b). Gaucher disease is characterized by the accumulation of glucosylceramide in several tissues, most prominently in the spleen (Barranger and Ginns 1989).

There may be differences in the kinetics of the accumulation of sphingomyelin and glucocerebroside due to differences in rates of synthesis, as well as accessibility for catabolism, and the specific activities of the catabolic enzymes. Sphingomyelin, which is derived from the turnover of membrane lipids, is degraded *in situ* through the action of lysosomal sphingomyelinase (Maziere et al 1982; Spence et al 1983). In contrast, whole-body metabolism of glucosylceramide is characterized by a complex intercellular transfer of lipid involving their carriage through the circulation bound to lipoproteins (Dawson and Oh 1977), ultimately leading in Gaucher disease to the deposition of lipid in tissues of the reticuloendothelial system (Barranger and Ginns 1989). Hence, the better response to treatment in Gaucher disease may indicate a readier accessibility to the lipid by the transplanted macrophages, which can remove material that has left the intracellular compartment. In contrast, the therapeutic efficacy of transplantation in Niemann–Pick disease may be inhibited by the intracellular sequestration of sphingomyelin.

Furthermore, changes in amounts and cellular location of sphingomyelin and cholesterol may alter membrane function. Sphingomyelin has been postulated to be a membrane stabilizer (Barenholz and Thompson 1980). Altered membranes of affected cells may prevent uptake of enzyme which has been delivered by donor cells. Also, the polypeptide of sphingomyelinase is heterogenous in human tissues for unknown reasons. For example, in placenta and spleen the enzyme has been reported to range from 70 000 to 90 000 daltons in size, while in the brain, urine and fibroblasts, the range is from 70 000 to 120 000 (Spence and Callahan 1989). This apparent tissue specificity could decrease activity of the haematopoietic-derived enzyme in other tissues. The possibility also exists that antibody is raised to the new protein. Measurements were not undertaken to test this.

This case demonstrates that lysosomal storage disorders are not a homogeneous group of diseases equally amenable to bone marrow transplantation, and that providing a constant source of enzyme this way may not always effectively treat all the manifestations. Intravenous administration of enzyme, or transplantation of autologous bone marrow containing exogenous, normal genes following gene transfer, might be equally ineffective for the same reasons. Gene transfer directly to affected tissues may be a more promising strategy (Anderson 1992).

In summary, we have shown only selective improvement in a patient with a lysosomal storage disease, Niemann–Pick Type IA, following allogeneic bone marrow transplantation. There is much to be learned regarding the properties of

sphingomyelinase, and the metabolism of sphingomyelin and cholesterol before the final goal of curing this devastating disease can be realized.

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