

Bone Marrow Transplantation and Mesenchymal Stem Cells in Niemann Pick A Disease

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Abstract

A patient suffering from Niemann Pick A Disease, underwent an haploidentical bone marrow transplantation, which was then followed by intrathecal and I.V injections of mesenchymal cells.

Results: While the outcome of the bone marrow transplantation was a complete failure, after one month after from the treatment with the mesenchymal cells the patient improved from the psychomotor and from the parenchymal storage perspective. Hypersplenism was solved and platelets rose quickly from 20,000 to 120,000/microliter. After the discontinuation of the therapy the disease slowly relapsed; however the patient is alive at the age of 8 years, while the survival of this disease does not exceed 3 years.

Conclusions: A bone marrow transplantation resulted ineffective while cellular therapy should be considered as a possible choice for of treatment of for Niemann Pick disease.

Keywords: Bone marrow transplantation; Parenchymal; Sphingomyelin

Introduction

Niemann Pick disease encompasses a group of genetic autosomal disorders; they are caused by a series of mutations of the sphingomyelin phosphodiesterase-1 gene (SMPD1) which encodes the acid sphingomyelinase (ASM), involved in the degradation of sphingomyelin [1]. The acid sphingomyelinase defect leads to the accumulation of sphingomyelin in the cells of the liver, spleen, bone marrow, lungs, and in some patients, brain [2-5].

Niemann Pick type A is a severe neurodegenerative disease with little or no enzyme activity; it develops in infancy with abdominal enlargement due to hepatosplenomegaly, feeding difficulties, cherry red macula. In addition, it involves with progressive loss of acquired motor skills. The increased accumulation of sphingomyelin in the central nervous system (CNS) leads to neurologic disturbances and mental retardation generally resulting in death by 3 years of age [6,7].

Niemann Pick type B is a slow paced disease with the same gene defect but it has more residual enzyme activity. The disease develops in pre-teen years with the enlargement of the liver and spleen; while in adulthood pulmonary difficulties and ataxia are the major complications although the CNS is not involved [8].

So far there are no specific pharmacological treatments that assure a cure for these diseases. Clinical and preclinical experiments such as enzyme replacement therapy (ERT), miglustat, hystone deacetylase, cyclodextrine, and bone marrow transplantation gave rather unsatisfactory results [9-13]. The best results were achieved with mesenchymal, neural and amniotic stem cells [14-16].

Since 1986 we have cured a series of patients with Niemann Pick type B by means of a subcutaneous injection of amniotic membrane cells [17]. Amniotic membranes cells share many features with mesenchymal cells in the CD phenotype and in their biological activity [18-20].

Seven years ago we put forward a child with Niemann Pick type A for a bone marrow transplant, who had failed to improve lysosomal storage in his organs and to slow down the worsening of the CNS symptoms. Later we infused I.V. and intrathecally the mesenchymal cells isolated from the marrow of the same donor.

Patient's History

At the beginning of 2008, a 10-month-old child was admitted to the Bone Marrow Transplantation Department of Trieste. He suffered from a rapid worsening of psychomotor skills; he was not able to sit down, or even to keep his head in an upright position unaided. The liver and spleen were enlarged. In the bone marrow smear and in the liver biopsy there were large foamy cells typical of Niemann Pick disease. A defect of sphingomyelinase was demonstrated in the leukocytes. The parents consented to a Bone Marrow Transplantation (BMT) even though they were aware of the slim chance of success.

Bone marrow transplantation

Since there was no HLA compatible sibling, and the search of a matched unrelated donor (MUD) would have taken too much time, we chose his haploidentical father as a donor, given the patient's rapidly deteriorating condition.

The conditioning regimen was: fludarabine, busulphan, thiotepa, cyclophosphamide and antithymocyte globulin (ATG). The donor's marrow was treated with vincristine and methylprednisolone as has

been performed in Trieste since 1986, for mismatched bone marrow transplantations [21].

The engraftment of the marrow was rather quick, with minor problems of Graft versus Host Disease (grade 1). The engraftment of the donor's cells was confirmed by means of HLA control and DNA polymorphism. Unfortunately after three months the size of the liver and spleen was even larger. In the liver biopsy and in the marrow smear there were still foam cells. The number of platelets after a first growth not sustained by transfusions fell and never exceeded 20,000/microl.

At that moment we, along with the parents, decided to proceed with a further attempt with mesenchymal cells.

Cellular Therapy

A large core ("carrot") of bone marrow stroma cells was harvested from the iliac crest of the father. The spongy bone sample was fragmented with surgical forceps in phosphate buffered saline (PBS). The cell suspension was then centrifuged and the pellet resuspended in culture medium (D-MEM, 10% fetal bovine serum (FBS), glutamine and antibiotic/antimycotic (Gibco®). The mixture was distributed in four 75 cm² Falcon flasks and incubated in a CO₂ incubator for 24 hours. Then the non-adherent cells and bone fragments were poured out and the adherent cells resuspended in culture medium which were cultivated for three weeks. The medium was changed twice a week. At the end the cells were detached from the plastic wall with trypsin, washed and cryopreserved in aliquots in a 10% DMSO solution.

This system of harvest and the culture allowed elimination of all the haemopoietic stem cells, and at the end of the culture the phenotype of the cells was CD90+, CD73+, CD 105-, CD44+, CD31-, CD34-, CD45-. We underline that CD105 is usually weak/negative in the first three weeks of culture, while it becomes positive in the fourth week.

On the day of the treatment one aliquot was washed twice with physiological saline and used for IV injection; two million cells were diluted in 50 ml of PBS and infused IV within 10 minutes.

Another aliquot was washed, resuspended in PBS and 10% patient's serum, and incubated for 40 mins with 6 microl/ml of a solution of retinoic acid in ethanol (10 mg in 10 ml). This incubation started the initial differentiation of the stem cells into the neurological line, as demonstrated by means of the expression of neurological markers (assay at 2 hours) as III tubulin and neurofilaments M (NF-M) in real time experiments with reverse transcriptase. After incubation, the cells were centrifuged and resuspended in 2 ml of physiological solution, and immediately injected into the lumbar spine of the patient. The same treatment was repeated after 2 months.

Outcome

The patient had no untoward effects due to these injections. After 25 days the number of platelets rose to 100,000/microl. The size of the liver and spleen was reduced to one half. One week later the child was able to keep his head in an upright position unaided. His relationship with his parents improved and the achievements brought him up to a psychomotor level compatible with a 7-8 months old child. We did not consider it ethically acceptable to perform a further liver biopsy. After a second infusion we were not able to continue the treatment for 2 years. The psychomotor skills maintained a plateau. After 2 years the patient showed a clear worsening mainly from the neurological perspective. He became unable to swallow and was then fed by naso-

gastric tube. An anesthesiologist refused to put him under anesthesia to insert a PEG, due to the high anesthesiological risk (Figure 1).

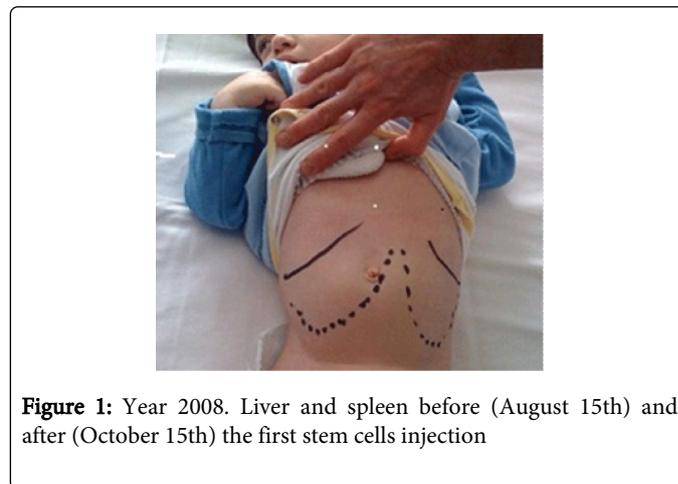


Figure 1: Year 2008. Liver and spleen before (August 15th) and after (October 15th) the first stem cells injection

In 2012, in the Hospital of Brescia, he restarted another cycle with intratecal and I.V. mesenchymal cells. This time the mother was the donor. After one month from the first injection he was able to swallow normally, and was fed with a spoon. The liver and spleen remained within normal size. His psychomotor skills (movements, relationship with the parents) improved, but he remained a severely handicapped child.

Following 4 injections of the new cycle he had to stop the therapy due to burocratical reasons in the hospital of Brescia (spring 2013). The improvement lasted six months and then we witnessed a new worsening of the psychomotor symptoms, and the child was fed again by means a naso-gastric tube.

Noteworthy

Today even with severe health problems (recently the platelets fell to 50,000/microl) this child is 8 years old; this survival is definitely unusual in Nieman Pick A disease. No untoward effect was seen after this treatment.

Discussion

We are aware that this patient was submitted to the treatment with mesenchymal cells too late. Maybe if we had chosen this treatment much earlier the outcome would have been different. The effect of mesenchymal cells lasted less than 2 years, after which the symptoms reappeared. The treatment was given again with a different donor, with significant - but not complete and short lasting- results.

Since the first time we used the father as a donor (his "twin" from the immunological point of view), this could be a particular situation from the immunological aspect, maybe more favorable than in cases when there could be an HLA mismatch.

However, since these cells have no HLA-DR on their surface and are immunosuppressive of their own, the choice of the donor may have been irrelevant.

The crucial point of our method seems to be the short incubation with retinoic acid, which starts a differentiation along the neurological line, without achieving the complete maturation expected to occur in vivo. As long as the cells maintain the features of stem cells, they are

able to move across the blood brain barrier and to “feel” the chemical messages of cellular damage. Mature neurons are unable to move more than one mm from the site of injection [22] (Figure 2).

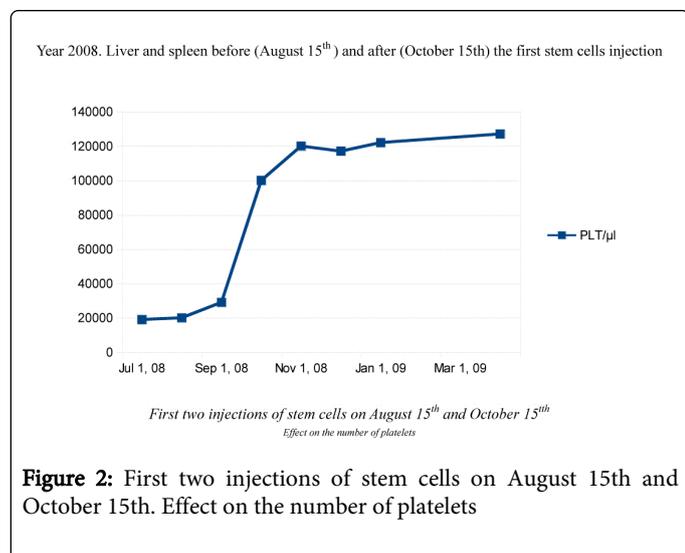


Figure 2: First two injections of stem cells on August 15th and October 15th. Effect on the number of platelets

Our method seems much more efficient than treatments that use undifferentiated mesenchymal cells in neurodegenerative diseases. Our previous experience with untreated mesenchymal cells in Spinal Muscular Atrophy (SMA1) resulted in a very short lived activity [23].

This report deals with only one case; therefore the result must be confirmed in a larger cohort of patients. We must be aware that mesenchymal cells do not cure this disease, even if they produce significant improvements and stop the progression of the disease.

However at the moment cellular therapy with mesenchymal cells could be the right answer for the treatment of Niemann Pick diseases.

Legal and Ethical Issues

The treatment in the hospital of Brescia achieved the positive opinion (“nothing against this treatment”) of the Italian Agency for Drugs (AIFA), according to a decree (DM 12/5/2006) that allowed even without authorization a cellular therapy in life-threatening cases.

The treatment was discontinued twice due to the different interpretation of the Italian laws that changed in the last years. Therefore the treatment was permitted, then prohibited, at last even imposed by a Civil Court.

The Hospital Ethical Committee granted permission for the procedure. The patient’s parents signed an informed consent.

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