Vol. 113, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
June 15, 1983 Pages 605-610

# BONE MARROW TRANSPLANTATION FOR NIEMANN-PICK MICE

T. Sakiyama, M. Tsuda, M. Owada, K. Joh*,
S. Miyawaki** and T. Kitagawa

Department of Pediatrics,
Nihon University Hospital,
Tokyo

Department of Pediatrics,
Jikei University School of Medicine*,
Tokyo

Research Laboratories,
Nippon Shinyaku Co., Ltd.,**
Kyoto, Japan

Received May 5, 1983

The Niemann-Pick mice which recieved bone marrow transplants showed a decreased accumulation of sphingomyelin and cholesterol quantitatively in their spleen. The sphingomyelin deposit in the bone marrow was also reduced histochemically. However, the neurological manifestations were not improved by the bone marrow graft.

Niemann-Pick disease like type A and C in humans are fatal lysosomal storage disorders with massive accumulations of sphingomyelin in the tissues. However, no therapeutic trials have been tested yet to try to prevent the further deposition of sphingomyelin and to arrest the clinical progression of this disorder. These necessary investigations should be first examined with appropriate animal models, because of the ethical and scientific limitation of human experimentation. Newly established Niemann-Pick mice(spm/spm) (1,2), mutated from a strain of C57BL/KsJ were an excellent model to assess the efficacy of some therapeutic trials. In this study, we tested the possiblity that a bone marrow transplant could provide an adequate amount of sphingomyelinase to degrade accumulated sphingomyelin deposits and prevent further deposition, which would result in the clinical improvement of the disease.

0006-291X/83 $1.50

## MATERIALS AND METHODS

### Animals

The Niemann-Pick affected mice(spm/spm) were found of the C57BL/KsJ strain and were maintained by transplanting affected female ovarium into normal mice(1). The detection of affected homozygous spm/spm mice was carried out by splenomegaly through a laparotomy in order to diagnose spm/spm mice at 4 weeks of age. This was done because the activity of sphingomyelinase in clipped tail homogenates failed to determine the genetic status of this disease(3). The clinical observation and life span study after receiving the bone marrow transplant were carried out under conventional room and feeding conditions at the laboratory of Nihon University Hospital. The span of life of three of the affected male mice was between 9 and 13 weeks under conventional room and feeding conditions. Those mice were fed as a parallel standard without any transplant when the other mice were evaluated for the efficiency of a bone marrow graft. The efficacy of the bone marrow grafts was determined by monitoring any changes in the clinical signs, such as the onset of ataxia, the body weight growth curve and the length of the life span. At the time of death a lipid analysis of the tissues was done in order to check the amount of accumulated lipid. A reduction in this amount would have been a positive sign even if death had been premature after the transplantation.

### Bone marrow graft

The collection, filtration and infusion of bone marrow cells was done in the following manner; bone marrow cells were collected from upper and lower leg bones of inbred mice and these were suspended in RPMI 1640(Gibco) containning 2% fetal calf serum and were sieved through sterilized tefron mesh. The collected cells were washed and precipitated at 1000 r.p.m for 10 min. and resuspended in the same medium. The cell count was adjusted to $2 \times 10^7$ in 0.2 ml medium. All procedures were performed on ice under sterile conditions. A 2% concentration of Trypan Blue staining solution was used to count the cells. 0.2 ml of this solution was injected into the affected spm/spm mice through their tail vain at the age of approximately 5 weeks. The mice were irradiated just before the transplantation as follows; two mice with 400 rad, 3 with 750 rad, 3 with 825 rad and 2 with 900 rad respectively.

### Lipid analysis and histochemical study

Tissues were removed as quick as posible when the grafted mice were dead, frozen and stored at -20°C for several weeks prior to being analyses for lipid. A chloroform-methanol (2:1) extract of tissues was prepared according to the method described by Folch et al(4). Total lipid contents in tissues were measured by the dried up weight of extracted lipid. Lipid analysis was carried out by thin-layer chromatography on HPTLC 60 silica-gel plates. Plates were one-dimensionally chromatographed using chloroform-methanol-water(60:35:8) as a solvent, and sphingomyelin was visualized by vaporing with Dittmer reagent and cholesterol was visualized with Anthrone reagent. Quantitative determination of sphingomyelin were estimated from the phospholipid contents which were scrapped off the area corresponding to sphingomyelin. Those of cholesterol were carried out according to Zak's method(5). The sphingomyelin in the bone marrow was stained by silver-diphenyl carbazone method(6).

## RESULTS

The 400, 750 and 825 rad spm/spm mice survived after the transplantation but their lifespans were not as long as expected. The onset of neurological signs such as ataxia and body weight loss started at around 8 weeks of age with death at 10-12 weeks, almost the same time as non-grafted spm/spm mice (Fig.1). However, the accumulation of sphingomyelin in the bone marrow of the affected mice was apparently reduced by the bone marrow graft. This reduction was proven by histochemical staining (Fig. 2). In addition, an accumulation of sphingomyelin and cholesterol in the spleens of the mice was remarkably decreased. The

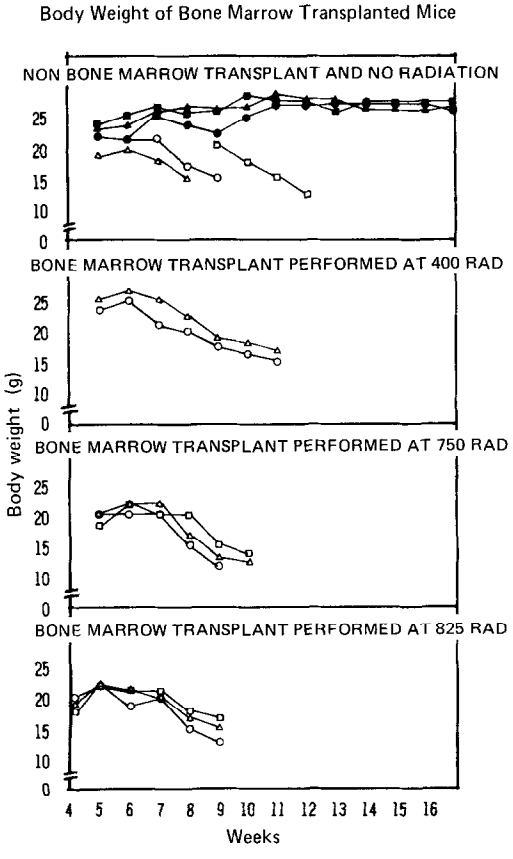


Figure 1. The life spans and the body weight curves of spm/spm mice. In the top graph, the closed marks indicate the control mice while the open marks denote the affected mice. The other graphs represent mice which had bone marrow transplants.

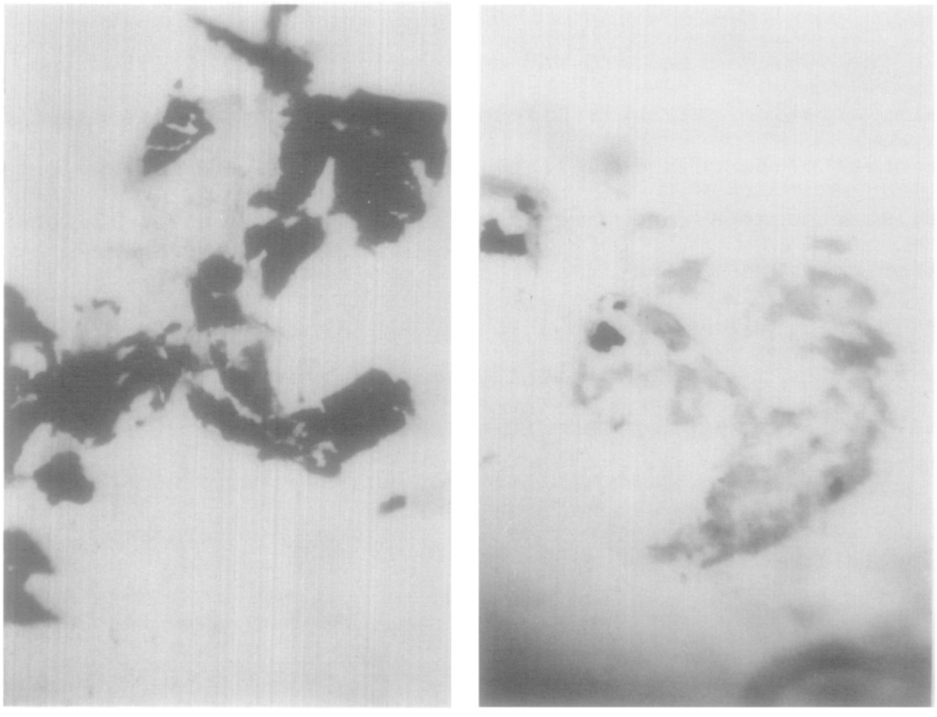

Figure 2. The left side illustrates the accumulation of sphingomyelin in the bone marrow of spm/spm mice. This was stained black by sliver-diphenylcarbazone. The Photo on the right illustrates the decrease of the sphingomyelin staining after the bone marrow graft.

content of sphingomyelin and choresterol in the liver was slightly reduced. Those reductions were confirmed by quantitative analysis (Table 1). The 400, 750 and 825 rad mice all reacted similarly. The 900 rad mice survived only 2 days after the graft.

## DISCUSSION

Niemann-Pick disease in humans has tentatively been classified into five different phenotypes. Type A and C are fatal and no effective treatment is available at present. A search has been looked for using a suitable animal model. Spm/spm mice showed overt hepatosplenomegaly with marked accumulation of sphingomyelin and cholesterol as well as fatal clinical manifestations with neurological involvement(1,2). Thus the Niemann-

Table I. Amounts of Total lipid, Total cholesterol and Sphingomyelin in Liver and Spleen of spm/spm mice with or without Bone Marrow Transplantation.

| | | Total lipid | Total cholesterol | Sphingomyelin |
|---|---|---|---|---|
| | | mg/g tissue | | |
| Liver: | | | | |
| no-grafted spm/spm | 1 | 292 | 45.0 | 14.7 |
| | 2 | 284 | 46.0 | 16.1 |
| 400 rad spm/spm | | 132 | 51.1 | 10.6 |
| 825 rad spm/spm | 1 | 132 | 52.1 | 11.1 |
| | 2 | 116 | 36.7 | 10.2 |
| Spleen: | | | | |
| no-grafted spm/spm | 1 | 108 | 29.3 | 7.5 |
| | 2 | 80 | 23.4 | 8.1 |
| 400 rad spm/spm | | 42 | 12.5 | 2.7 |
| 750 rad spm/spm | | 54 | 9.9 | 2.9 |
| 825 rad spm/spm | 1 | 38 | 7.2 | 1.7 |
| | 2 | 49 | 8.6 | 2.6 |

Pick mice would be an excellent model for the trials of treatment of lysosomal storage disorders.

Recently, bone marrow grafts have been considered as one form of treatment for certain inborn errors of metabolism. It has been proven by some animal models, such as the acatalasemic mouse (C57BL/6An1/Csb)(7) and the C3H/HeJ mouse(8) with an enzyme $\beta$-glucuronidase deficiency, that the replacement therapy for congenital enzyme deficiency could succeed in providing a continuous and permanent source of the missing enzyme by allogenic bone marrow transplant. Moreover, human application of bone marrow transplant for the patients with Hurler's disease(9) seems to be a successful treatment, providing a lasting adequate amount of the deficient enzyme $\alpha$-iduronidase. Therefore the bone marrow graft method was applied to the treatment of Niemann-Pick disease.

From our experiment with Niemann-Pick mice, the bone marrow graft did not improve the neurological manifestation. However,

the accumulation of sphingomyelin and cholesterol in the spleen was decreased remarkably. In addition, the sphingomyelin deposit in the bone marrow was also reduced histochemically. Since no neurological improvement was noticed, this could be ascribed to some possible causes, such as the existance of blood-brain-barriers which prohibit the leucocytes from entering the necessary areas of the brain. Also it might be possible that the sphingomyelinase in the brain reacts differently with the leucocytes and monocytes.

A human subject might react differently due to the genetic difference between humans and animals. Our result has encouraged us to apply bone marrow transplantation to the patients with Niemann-Pick disease except the neurological manifestation type. It also might warn of unqualified application of bone marrow transplants to humans with lysosomal storage disease showing neurological involvement.

ACKOWLEDGEMENT

This work was supported in part by a grant in aid of new drug development from the Ministry of Health and Welfare of Japan and by a grant in aid of a study of enzyme replacement therapy for lipid storage disorders from the Ministry of Education in 1982.

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