JOURNAL OF MEDICINE

Research Communications
Westbury, NY 11590-0966 USA

HUMAN UMBILICAL CORD BLOOD CELLS AMELIORATE ALZHEIMER'S DISEASE IN TRANSGENIC MICE

A BRIEF REPORT

Norman Ende, Ruifeng Chen and David Ende-Harris

Department of Pathology and Laboratory Medicine,
University of Medicine and Dentistry of New Jersey, New Jersey
Medical School, 185 South Orange Avenue, Newark, NJ 07103

Key Words: Alzheimer's disease, human umbilical cord blood; transgenic mice.
Subjects: Transgenic mice.
Abbreviations: APP = Amyloid precursor protein, SOD1 = amyotropic lateral sclerosis mice, HUCB = human umbilical cord blood, Hdexon1 = Huntington's disease mice, MNC = mononuclear cells.

Abstract

Having had success in extending the life of mice with a transgene for amyotropic lateral sclerosis (SOD1) mice and Huntington's disease (Hdexon1), we administered megadoses of human umbilical cord blood mononuclear cells to mice with Alzheimer's disease. These mice have an over-expression of human Alzheimer amyloid precursor protein (APP), die early and develop a CNS disorder that includes neophobia. When given $110x10^6$ human umbilical cord blood mononuclear cells, these mice (HuAPP 695.SWE) had considerable extension of life with a p value of 0.001 when compared to control animals.

Send reprint requests to: Norman Ende, MD, UMD-New Jersey Medical School, Department of Pathology and Laboratory Medicine, 185 South Orange Avenue, Room C501, Newark, NJ 07103-2714. Tel./Fax: 973-972-7493.

Introduction

In 1995, we described very immature stem cells in the umbilical cord blood of humans which we believed may have similar properties to fetal or embryonic stem cells (Ende, 1995, 2000). Under the assumption that these cells exist in small numbers in human umbilical cord blood, we administered large doses (megadoses) of human cord blood mononuclear cells to mice with the human transgene for amyotrophic lateral sclerosis (SOD1) (Chen and Ende, 2000; Ende, Weinstein *et al.,* 2000) and Huntington's disease (Hdexon 1) (Ende and Chen, 2000). By the use of megadoses of umbilical cord blood mononuclear cells, with and without immune suppression, we have obtained significant modulation in the animal's clinical manifestations and delayed death in these mice with neurodegenerative diseases. Since it has been suggested that a mitochondrial dysfunctional relationship exists in these neuro-degenerative diseases and Alzheimer's disease (Beal, 1998), we have carried out similar experiments on mice with Alzheimer's disease (Ende, Chen *et al.,* 2001).

These unusual data on Alzheimer's disease in mice are reported here with the full understanding that pathological and additional behavioral studies are indicated. Since, however, the survival studies were so striking and with its potential for therapeutic use, we believe a brief report should be made.

Materials and Methods

Animals

Breeding pairs of mice were obtained from Charles River Laboratories and according to information received were developed by Karen Hsiao, M.D., Ph.D. at the University of Minnesota as a model of Alzheimer's Disease [Tg(HuAPP 695.SWE)2576]. These mice were bred in the animal Facility Laboratory of the New Jersey School of Medicine, University of Medicine and Dentistry. The animals, as recommended, were genotyped before the experiments. These mice are relatively hyperactive and frequently with minimal stimulation would run in circles. Prior to their demise the animals would become hyperactive or develop a positive "cornering test" (neophobia) and became unable to eat or drink.

Cord Blood Collection and Storage

The study was approved by the Institutional Review Board of the New Jersey Medical School, Newark, NJ. The mice were housed in

AAALAC-1 approved animal laboratory. The project was approved by the Institutional Animal Review Committee.

Human umbilical cord blood (HUCB) samples were obtained from the placentas of healthy full-term neonates. Each cord blood sample was collected into a 50 mL sterile polypropylene test tube containing 5 mL of citrate phosphate dextrose as an anticoagulant. The volume of the HUCB collected varied from 20 mL to 40 mL. Samples were kept at room temperature and within 24 hours they were sent to the blood bank for storage. The HUCB sample was then transferred into a special polyolefin blood collection bag (Cryocyte Freezing Container, Baxter Healthcare, Deerfield, IL, USA) that allows gaseous transfer and stored at 4°C in a blood bank refrigerator for 10 to 13 days. The donor specimens, when available, were combined 3-4 days before administration according to their blood type (ABO). Frequently, however, only one donor was available. After storage for 10 to 13 days at 4°C, portions or all of each stored bag of cord blood were removed to provide the desired number of mononuclear cells (MNC) per mouse. With the use of 15 mL disposable centrifuge tubes, mononuclear cells (MNC) were separated from the whole cord blood by centrifugation for 30 minutes at 1700 RPMs with ficol histopaque (Sigma, St. Louis, MO, USA). The MNC cells were then washed twice with phosphate buffered saline (PBS) and centrifuged for 10 minutes at 1000 RPMs. One mL of PBS was added to the pellet for counting. After the viability and counting was determined, the mononuclear cells were centrifuge for 10 minutes at 1000 RPMs. 0.2 mL of PBS solution was added for final dilution and injection intravenously into the mouse (retro-ocular). This process was repeated the next day to bring the total count of HUCB mononuclear cells up to desired number.

Experimental Design

The mice were divided into three groups. No immunosupression was used. One group of 9 were controls, untreated; a second group of 7 mice received $5.6x10^6$ congenic bone marrow cells and a third group of 8 mice received 110 x 10^6 human cord blood mononuclear cells. The "cornering test" utilized to identify neophobia was given twice a week.

Statistics

The Kaplan-Meier survival statistics were utilized in this study.

Results

At the end of 266 days all except 2 of 9 control mice were dead. Only one of the two control mice was still alive at 406 days, when the experiment was terminated.

Six of the seven animals that received congenic bone marrow were all dead at 168 days except for one that lived to 336 days.

Of the eight animals that received $110x10^6$ human cord blood mononuclear cells, none were dead at 266 days. One animal developed a positive "cornering" test at 350 days and was dead by day 357. Another mouse developed a positive "cornering" test and 15 days later it was dead by day 392. The remaining 6 out of 8 mice were alive at 406 days when the experiment was terminated.

The survival curves of the untreated animals and those receiving congenic bone marrow cells were similar. When compared to controls the survival curve of the Alzheimer's mice that received megadoses of human umbilical cord blood mononuclear cells (110×10^6) was significant, and the p value was 0.001 (Figure 1).

Discussion

There has been an increasing acceptance of a potential pivotal role for mitochondrial dysfunction in familial amyotrophic lateral sclerosis, Huntington's disease, sporadic Alzheimer's disease and other neurodegenerative diseases (Beal, 1998). These diseases of widely disparate genetic etiologies may show a final common pathway. Having found that megadose of human umbilical cord blood mononuclear cells could delay the onset of symptoms and death in amyotrophic lateral sclerosis mice (SOD1) (Chen and Ende, 2000) and Huntington mice (Hdexon1) (Ende and Chen, 2000), we attempted to determine if a similar effect could be produced in mice carrying the transgene associated with Alzheimer's disease (Ende, Chen et al., 2001). These transgenic mice, over-expressing human Alzheimer amyloid precursor protein (APP), die early and develop a CNS disorder that includes neophobia. (Hsiao, Borchelt *et al.*, 1995) The brain shows diminished glucose utilization and astrogliosis. Those animals that died relatively young did not show fibrillary tangles or significant amyloid deposits. The authors concluded that APP over-expression could accelerate or accentuate a natural occurring age-related central nervous system disorder in FVB/N mice without amyloid deposition in the brain. The age of onset of neophobia and age at death decreases with increasing levels

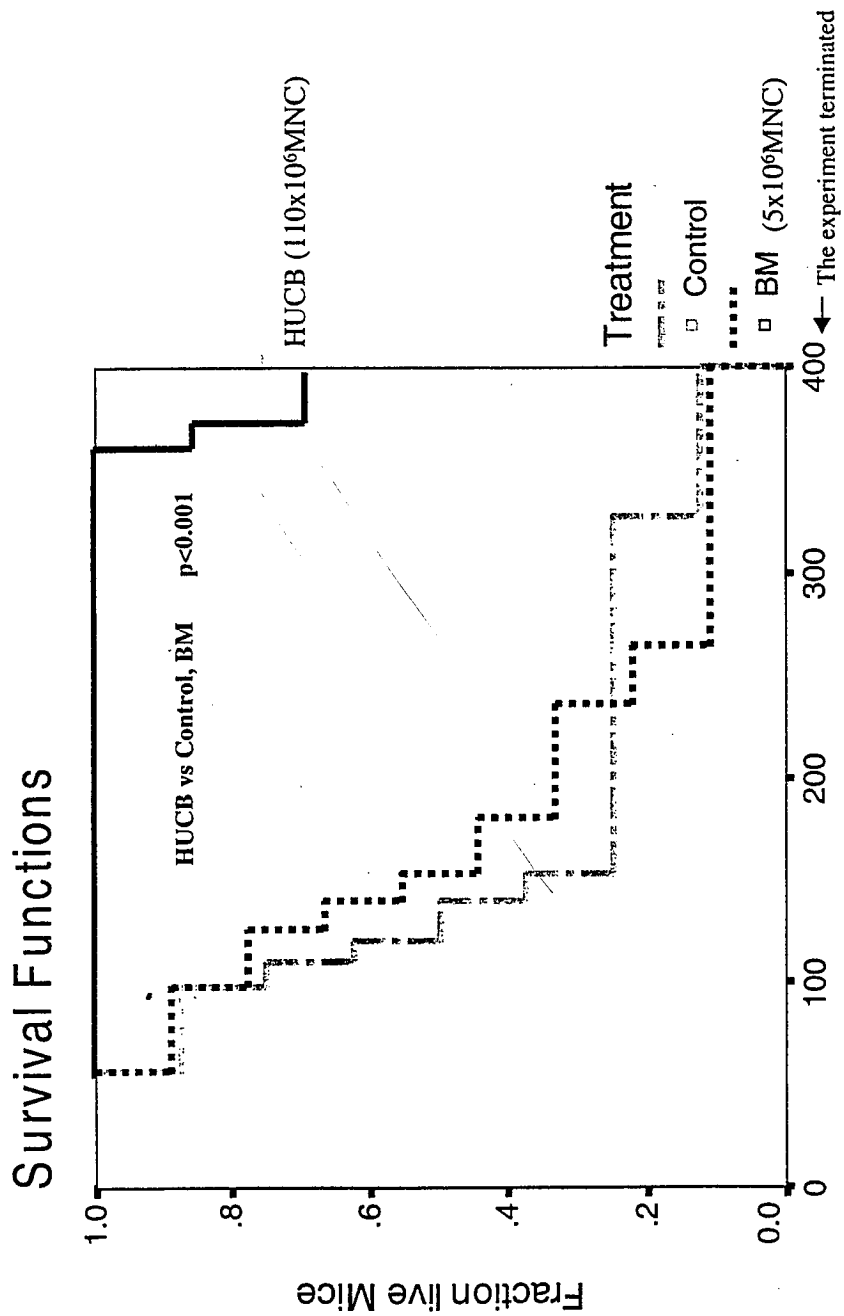
Survival Functions
HUCB vs Control, BM p<0.001
HUCB (110x10⁶MNC)
Treatment
Control
BM (5x10⁶MNC)
The experiment terminated
Fraction live Mice
1.0
.8
.6
.4
.2
0.0
0
100
200
300
400
Days after Birth

Figure 1

of amyloid precursor protein. This raises intriguing possibilities when human cord blood mononuclear cells can substantially increase the length of life of these animals.

Although it is obvious that additional studies are needed, the current findings could have significant clinical potential.

Acknowledgments

We thank Dr. Milton Ende and Ms. Adaline Woods and the Obstetrics Staff of the Southside Regional Medical Center, Petersburg, VA; Dr. Leslie Iffy, Dept. of Obstetrics and Gynecology, Luz Bagtas-Ricafort, Blood Bank, New Jersey Medical School, Newark, N.J.; Judith M. Walker, R.N.; Veronica N. Anokute, BSN, RNC, MPA; and Brain A. McCarthy for their help and cooperation. The study was supported by the Abraham S. Ende Research Foundation, Petersburg, Virginia.

References

Beal, M.F. (1998).: Mitochondrial dysfunction in neurodegenerative diseases. Biochim. Biophys Acta. 1366: 211-223.

Chen, R. and Ende, N. (2000).: The potential for the use of mononuclear cells from human umbilical cord blood in the treatment of amyotrophic lateral sclerosis in SOD1 mice. J. Med. 31: 21-29.

Ende, N. (1995).: Use of human umbilical cord blood for stem-cell transplantation (HLA-matched, unmatched. Clinical, ethical, and legal aspects). In: *Hematopoietic Stem Cells*. Edited by: D. Levitt and R. Mertelsmann. Marcel Decker Inc. New York, NY. pp. 333-347.

Ende, N. (2000).: The Berashis Cell: A Review. Is it similar to the embryonic stem cell? J. Med. 31: 113-130.

Ende, N. and Chen, R. (2000).: The effect of a megadose of human umbilical cord blood mononuclear cells on Huntington's disease mice. Amer. J. Clin. Pathol. 114: 4, abst. 89.

Ende, N., Chen R. and Ende-Harris, D. (2001).: The Effect of Megadose of Human Umbilical Cord Blood Mononuclear Cells on Alzheimer's Disease. Mod. Pathol. 14: 207, abst. 1217.

Ende, N., Chen, R., Weinstein, F., Bagtas-Ricafort, L. and Ende, M. (2000).: Human umbilical cord blood (HUCB) and effect on SOD mice (amyotrophic lateral sclerosis). Mod. Pathol. 13: 1 abst. 1137.

Ende, N., Weinstein, F., Chen, R. and Ende, M. (2000).: Human umbilical cord blood effect on SOD mice (amyotrophic lateral sclerosis) Life Sci. 67: 53-59.

Hsiao, K.K., Borchelt, D.R., Olson, K., Johannsdottir, R., Kitt, C., Yunis, W., Xu, S., Eckman, C., Younkin, S., Price, D., Iadecola, C., Clark, H.B. and Carlson, G. (1995).: Age-related CNS disorder and early death in transgenic FVB/N mice over-expressing Alzheimer amyloid precursor proteins. Neuron, 15: 1203-1218.