HUMAN UMBILICAL CORD BLOOD CELLS AMELIORATE HUNTINGTON'S DISEASE IN TRANSGENIC MICE
A BRIEF REPORT

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Key Words: Amyotrophic lateral sclerosis, chorea, congenic bone marrow, human umbilical cord blood, human mononuclear cells, Huntington disease, life span, radiation, transgenic mice, weight loss.

Subjects: Transgenic mice having the Huntington disease transgene.

Abbreviations: HUCB = Human Umbilical Cord Blood, MNC = mononuclear cells.

Abstract

Human umbilical cord blood mononuclear cells given in megadose quantity (7.1-7.4 x 10^6 and 100-105 x 10^6) were able to increase the life span of B6CBA-TgN(Hd exon1)62Gpb mice (Huntington disease) from an average of 88 days to 97.8 and 103.4 days respectively. The rate of weight loss, which begins in these mice before the onset of symptoms of chorea, was far less in the animals receiving human cord blood mononuclear cells (p<0.01) than the weight loss in untreated control mice. With the full understanding that additional histological and

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behavioral studies are indicated, but because of the potential clinical significance, a brief report is being submitted.

**Introduction**

It has been postulated that there may be an association between the genetic defect of amyotrophic lateral sclerosis and Huntington disease (Beal, 1998). Our previous studies have shown that large doses of human umbilical cord mononuclear cells can produce a statistically significant delay in the onset of symptoms in SOD1 mice that carry the mutant transgene for amyotrophic lateral sclerosis, with some of the animals almost doubling their life span (Ende, Weinstein et al., 2000; Chen and Ende, 2000). Therefore, we postulated that a similar effect might be produced in Huntington disease mice (Ende and Chen, 2000). The transgenic mice B6CB-TgN(HDexon1)62 Gpb have the Huntington disease transgene expressed in all tissues of the mouse (Jackson Laboratory). This study was undertaken to determine if a significant clinical effect, similar to that which had occurred in SOD1 mice, would occur in the animals with the Hdexon1 transgene with megadoses of human umbilical cord blood mononuclear cells. The resulting fascinating and important data are reported with the full understanding that follow-up studies will be required to analyze the histological and behavioral consequences of this therapy.

**Material and Methods**

**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-I approved animal laboratory. The project was approved by the institutional animal review committee.

Fifty-one human umbilical cord blood samples (HUCB) 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 samples were then transferred into a special polyolefin blood collection bag (Cryocyte Freezing Container, Baxter Healthcare, Deerfield, IL) that allows gaseous transfer and stored at 4°C in a blood bank refrigerator for 10 to 13 days (Lemol, Tafuri et
al., 1992). Specimens that came from another institution were not transferred to Cryocyte containers for 48-72 hours. The 51 donor specimens, when available were combined 3-4 days before administration according to their blood type (ABO) and stored at 4°C. Frequently, however, only one donor was available at a time. After storage for 10 to 13 days at 4°C in a blood bank refrigerator, HUCB units were placed in 15 mL disposable centrifuge tubes and the 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). Portions or all of the cord blood mononuclear cells were removed to provide the desired number of cells (MNC) per mouse. 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.

Animals

The transgenic mice B6CBA-TgN (HDexon1)62Gpb have the Huntington disease Transgene (Mangiarini, Sathasivam et al., 1996). The Huntington disease transgene is expressed in all the tissues of the mouse. The animals exhibit weight loss, develop chorea and exhibit unusual vocalization. The animals were six weeks old when received. The age of onset of symptoms occurs between 9 to 11 weeks (Jackson Laboratory Bar Harbor, ME).

Antisera

Rabbit anti-Asialo GM1 antisera was purchased from Wako chemical (Richmond VA). Injection of 100 μL of this antibody depletes N.K. effector cell for a period of 2 weeks (Ende; Giuliani, et al., 1990).

Radiation

The animals received 800 cGy of irradiation from Mark-I irradiator (Cs-137).

Experimental Design

The research was divided into two parts, a preliminary and a confirmatory study. The preliminary study consisted of 24 B6CBA-TgN
(Hdexon1)62GPb mice. The mice were divided into four groups: (a) a control group of 7 untreated mice, (b) 5 mice that received congenic bone marrow from wild type mice B6CBAF1/J, (c) 5 mice that received 71-74 x 10^6 mononuclear cells, 800 cGy of irradiation, 0.1 mL of Asialo GM1 antisera before the onset of chorea and (d) 7 mice that received 800 cGy of irradiation and 71-74 x 10^6 human cord blood mononuclear cells after the onset of symptoms of chorea, 2 died within 24 hours of injection and were not evaluated.

The confirmatory studies consisted of 30 mice (a) 10 mice that received no treatment, (b) 10 mice that received congenic bone marrow (c) 10 mice that were given 100-105 x 10^6 human mononuclear cells, 0.1 mL of Asialo GM1 antisera and 800 cGy of irradiation before the onset of symptoms of chorea. Since these animals begin to lose weight early in their disease process, in the confirmatory studies, all animals were weighed weekly after injection of cord blood and the weight loss recorded.

Results

The observations on the onset of symptoms were made by the same observer. Any decision to euthanize an animal was made by a caregiver unfamiliar with the protocol.

In the preliminary study (Table I), the control group's average life span was 86 days with the animals living an average of 1.2 days after the onset of chorea. The average length of life, when the animals received 71-74 x 10^6 mononuclear cells and radiation prior to the onset of chorea, was 96.8 days. The average life span after the onset of chorea was 8 days with one animal surviving 15 days. The group that received similar treatment, after the onset of symptoms of chorea, the average increase in the length of life was 9.8 days with the average life span 93.3 days with one animal living 115 days not counting two mice that died within 24 hours of injection of cells.

In the confirmatory study (Figure 1) 30 mice were used. The average length of life of the 10 control animals was 89.4 days with the longest living 97 days. The 10 animals that received a congenic bone marrow transplant, the life span was 89.5 days and the mice lived an average of 2.4 days after onset of symptoms. For the 10 animals that received 100-105 x 10^6 human cord blood mononuclear cells and irradiation prior to the onset of chorea the average length of life was 103.4 days (p<0.05) with one animal living for 135 days (Table I, Figure 1).
<table>
<thead>
<tr>
<th>Preliminary Study</th>
<th>Age when treated in days</th>
<th>Average age at onset of Chorea in days</th>
<th>Average age at death in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=7)</td>
<td></td>
<td>84.57 ± 3.59</td>
<td>86.14 ± 3.93</td>
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<tr>
<td>Congenic Marrow Cells (n=5)</td>
<td>56 ± 5</td>
<td>85 ± 4.79</td>
<td>86.4 ± 4.27</td>
</tr>
<tr>
<td>71-74 x 10^6 Mononuclear Cells prior to Chorea onset (n=5)</td>
<td>56 ± 5</td>
<td>89.6 ± 2.07</td>
<td>96.8 ± 8.67</td>
</tr>
<tr>
<td>Mononuclear Cells after Chorea onset (n=5)</td>
<td>89 ± 4</td>
<td>83.71 ± 4.15</td>
<td>93.28 ± 14.13</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Confirmatory Study</th>
<th>Age when treated in days</th>
<th>Average age at onset of Chorea in days</th>
<th>Average age at death in days</th>
<th>3-week Average Wt. Loss</th>
</tr>
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<tr>
<td>Control (n=10)</td>
<td></td>
<td>87.6 ± 5.08</td>
<td>89.4 ± 5.05</td>
<td>2.24g</td>
</tr>
<tr>
<td>Congenic Marrow (n=10)</td>
<td>55 ± 4</td>
<td>87.9 ± 5.19</td>
<td>89.5 ± 4.97</td>
<td>2.23g</td>
</tr>
<tr>
<td>100-105 x 10^6 MNC prior to Chorea onset (n=10)</td>
<td>56 ± 4</td>
<td>95.8 ± 13.19</td>
<td>103.4 ± 15.8</td>
<td>0.75g</td>
</tr>
</tbody>
</table>
Fig 1

Survival Functions

Kaplan-Meier

HUCB(100x10^6MNC) vs Control p<0.001
HUCB(70x10^6MNC) vs Control p<0.01

Fraction Live Mice

Days after birth

Treatment
- ▲ HUCB- high
- ▼ HUCB- low
- ▲ control
- ▼ BM (5x10^6MNC)
Huntington's mice weight loss

P<0.01

Average weight loss vs. Time (weeks)
The difference in weight loss between the controls and those mice receiving human umbilical cord blood mononuclear cells was striking (Figure 2). Only 4 animals out of 10, during the 3 weeks period after receiving human cord blood mononuclear cells, had a weight loss of over 1 g. While all of the control mice, including those that received bone marrow, had a weight loss greater than 1 g, many losing over 2 g during the three weeks period. Three of the four animals that had received cord blood but had a rapid weight loss, died during the same time period as the controls. The difference in weight loss between the controls (including untreated and bone marrow treated) and the mice receiving cord blood mononuclear cells had a p value of <0.01, (Figure 2).

Discussion

Having had significant improvement in the life span and clinical course of SOD1 mice which have a transgene associated with amyotrophic lateral sclerosis (Chen and Ende, 2000; Ende, Weinstein et al., 2000,) we attempted similar studies on B6CBA-TgN(HD exon1) 62 Gpb mice (Mangiarini, Sathasivam et al., 1996). These mice have a Huntington disease transgene expressed in all tissues of the mice similar to humans. The average life span of Huntington disease mice that received megadose of human cord blood mononuclear cells was significantly longer than the controls. Although the length of time of survival of the animals receiving cord blood was not as great as the SOD1 mice that had received similar treatment as the Huntington disease mice, the patterns were similar (Chen and Ende, 2000; Ende, Weinstein et al., 2000).

The results in the clinical findings and death of the individual animals treated with cord blood, as in all our previous experiments with human cord blood mononuclear cells, were quite variable. The logistics of collection and storage of cord blood at our institution even with pooling, makes it impossible to obtain uniform cord blood samples as to their most immature cells (Berashis cells) (Ende, 1995; Lu and Ende, 1997). Since 100 x 10^6 cord blood mononuclear cells obtained may represent all the cord blood mononuclear cells we obtain from a single donor, even with pooling according to blood type, the treated mouse frequently received cells only from a single donor. Both the number of cells per mL and volume of each donation during the experimental time period were quite variable.
As in all our previous experiments using human cord blood on irradiated animals and SOD1 mice, the clinical response was dose related (Chen and Ende, 2000; Ende, Lu et al., 1996). The greater the dose of mononuclear cells the greater the number of mice that survived lethal irradiation, the longer the delay in the onset of symptoms and death in both the SOD1 mice and the Hdxon1 mice. From additional studies we have undertaken (Ende, 2000) the irradiation dose given the animals can probably be reduced or eliminated.

Conclusion

Megadose of human umbilical cord blood mononuclear cells can significantly delay the onset of symptoms and increase the life span of B6CB-TgN(HD exon1)62Gpb mice. This may provide a readily available form of therapy for Huntington disease.

Acknowledgments

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References


