Cell Transplantation, Vol. 11, pp. 275–281, 2002
Printed in the USA.

0963-6897/02 $20.00 + .00

www.cognizantcommunication.com

Intravenous Administration of Human Umbilical Cord Blood Reduces Neurological Deficit in the Rat After Traumatic Brain Injury

Dunyue Lu,* Paul R. Sanberg,§ Asim Mahmood,* Yi Li,† Lei Wang,† Juan Sanchez-Ramos,¶ and Michael Chopp†‡

Departments of *Neurosurgery and †Neurology, Henry Ford Health Sciences Center, Detroit, MI
‡Department of Physics, Oakland University, Rochester, MI
Center for Aging and Repair, Departments of §Neurosurgery and ¶Neurology, University of South Florida, Tampa, FL

We measured the effect of treatment of traumatic brain injury (TBI) in the rat with human umbilical cord blood (HUCB) administered IV. HUCB cells were injected into the tail vein 24 h after TBI and the rats were sacrificed at day 28 after the treatment. The Rotarod test and the neurological severity score (NSS) were used to evaluate neurological function. The distribution of the donor cells in the brain, heart, lung, kidney, liver, spleen, bone marrow, and muscle were analyzed in recipient rats using immunohistochemical staining and laser confocal microscopy. HUCB cells injected IV significantly reduced motor and neurological deficits compared with control groups by day 28 after the treatment. The cells preferentially entered the brain and migrated into the parenchyma of the injured brain and expressed the neuronal markers, NeuN and MAP-2, and the astrocytic marker, GFAP. Some HUCB cells integrated into the vascular walls within the boundary zone of the injured area. Our data suggest that IV administration of HUCB may be useful in the treatment of TBI.

Key words: Traumatic brain injury (TBI); Rat; Human umbilical cord blood (HUCB); Intravenous administration

INTRODUCTION

Traumatic brain injury (TBI) continues to be an important cause of human morbidity with nearly 500,000 new cases in the US annually. As many as 50,000 people are killed and an equal number disabled by TBI each year (1). Despite much research and multiple studies, no therapeutic intervention has been found to be effective in promoting restitution of functional deficits after TBI. Neural transplantation has been used to treat the central nervous system diseases, including TBI, stroke, and neurodegenerative disorders (3). A major limitation in carrying out neural transplantation has been the limited availability of donor tissues and ethical problems associated with the use of embryonic cells.

"Cord blood" is the blood that remains in the umbilical cord and placenta after birth. Umbilical cord blood (UCB) is a plentiful and rich source of stem cells that can be used to treat a variety of life-threatening diseases including leukemia, other cancers, and blood and immune disorders (13). Because the number of pluripotent stem cells contained in one sample of cord blood is sufficient for engrafting into children and adults, cord blood banking has developed worldwide (15). Cord blood banking has several advantages, including availability of this source of stem cells and low viral infection rate at birth. Another advantage is a low risk of acute graft-versus-host disease, even with some degree of HLA mismatch (9). It has been well known that cord blood contains hematopoietic progenitor cells (24), endothelial progenitor cells (19), mesenchymal progenitor cells (11), autoimmune T cells (12), mononuclear cells (5), mast cells (25), and megakaryocyte progenitor cells (18). Thus, cord blood administration may be used to treat life-threatening diseases ranging from the hematopoietic system to the nonhematopoietic system (e.g., brain trauma). Recently, human umbilical cord blood (HUCB) has been used to treat a mouse model (SOD-1 Knock out) of amyotrophic lateral sclerosis (10). Large doses of HUCB cells ($3.4–3.5 \times 10^7$) produced considerable delay in onset of symptoms and death of the SOD-1 mice. More than 1500 cord blood transplants in humans have been reported worldwide but, to our knowledge, there is no report that IV administration of HUCB was attempted to treat TBI in humans and animals.

Therefore, using a controlled cortical impact (CCI)

Address correspondence to Michael Chopp, Ph.D., Henry Ford Hospital, Neurology Department, 2799 West Grand Boulevard, Detroit, MI 48202. Tel: (313) 916-3936; Fax: (313) 916-1318; E-mail: chopp@neuro.hfh.edu

model of TBI in the rat, we tested the hypothesis that HUCB administration reduces injury and improves functional outcome after TBI.

MATERIALS AND METHODS

All experimental procedures were approved by the Care of Experimental Animals Committee of Henry Ford Hospital.

Preparation of HUCB for Injection

The HUCB used was a gift from Cryocell International, Inc. (3165 McMullen Dr., Blvd. 3, Clearwater, FL 33761). The specimen ID number is 19 and the specimen was analyzed on June 27, 2000. The specimen was stored in liquid nitrogen and the cells were restored at 37°C. After centrifugation at 1000 rpm/min for 10 min at 4°C, the supernatant was removed and the cells were washed twice with 0.1 M PBS. Cell suspension (30 μl) was mixed with 30 μl of 0.4 % trypan blue stain and the number of the viable cells was counted with a hemacytometer and a counter under a phase contrast microscope. The total number of the harvested cells was calculated and the final dilution was 2×10^6 cells in 300 μl saline.

Controlled Cortical Injury Animal Model and the Injection of HUCB

Wistar rats were anesthetized with 350 mg/kg body weight chloral hydrate, IP. Rectal temperature was controlled at 37°C with a feedback-regulated water-heating pad. A controlled cortical impact device was used to induce the injury. Rats were placed in a stereotactic frame. Two 10-mm-diameter craniotomies were performed adjacent to the central suture, midway between lamda and bregma. The second craniotomy allowed for movement of cortical tissue laterally. The dura was kept intact over the cortex. Injury was induced by impacting the left cortex (ipsilateral cortex) with a pneumatic piston containing a 6-mm-diameter tip at a rate of 4 m/s and 2.5 mm of compression. Velocity was measured with a linear velocity displacement transducer (7,20).

Twenty-four rats subjected to TBI were divided into three groups. Experimental group ($n = 8$): 24 h after TBI, rats were slowly injected over a 10-min duration with 2×10^6 HUCB cells in 300 μl saline via a tail vein. Placebo control group ($n = 8$): 24 h after TBI, rats were slowly injected over a 10-min duration with 300 μl saline via a tail vein. TBI-only group ($n = 8$): the rats only were subjected to TBI and no treatment. All rats were killed 28 days after the treatment.

Tissue Preparation

Paraffin Sections. Four animals from each group were euthanized with an overdose of ketamine and xylazine administered IP and perfused with intracardiac heparinized saline followed by 10% buffered formalin. The brains, hearts, lungs, livers, kidneys, spleens, muscle, and bone marrow were removed and stored in 10% buffered formalin for 24 h. Seven 2-mm-thick blocks were cut on a rodent brain matrix and then embedded with paraffin. Blocks (2 mm thick) of the other organs were also cut and embedded with paraffin. A series of adjacent 6-μm-thick sections was cut and a section of each block of the brain and other organs was stained with H&E for morphological analysis under light microscopy.

Vibratome Sections. An additional four rats from each group received the IV administration of 1 ml of saline containing fluorescein isothiocyanate (FITC)-dextran (50 mg/ml, 2×10^6 molecular weight; Sigma, St. Louis, MO). This dye circulated for 1–2 min, after which the anesthetized rats were killed by decapitation. The brains were rapidly removed from severed heads and placed in 4% paraformaldehyde at 4°C for 48 h. Coronal sections (100 μm) were cut on a vibratome.

Immunohistochemistry

Single staining was performed for identification of HUCB cells using a primary mouse anti-human nuclei monoclonal antibody (MAB 1281), specific for all human cell types, and secondary Cy5-conjugated F(ab′)2 fragment rabbit anti-mouse IgG in the coronal sections of all organs. Double staining was also performed on coronal cerebral sections. Brains sections were initially stained for neuronal markers, NeuN and MAP-2, or an astrocytic marker, glial fibrillary acidic protein (GFAP), with the corresponding primary antibodies and the secondary FITC-conjugated F(ab′)2 fragment, and subsequently double stained with primary MAB 1281 antibody and second antibodies of Cy5-conjugated-F(ab′)2 fragment for identification of HUCB cells. Briefly, 6-cm-thick sections from TBI, TBI + saline, and TBI + HUCB groups were deparaffinized and the sections were put in boiling citrate buffer (pH 6) in a microwave oven for 10 min for identification of neurons. After cooling at room temperature, the sections were incubated in 0.1% saponin-PBS at 4°C overnight for mAb NeuN (dilution 1:400, Chemicon) and MAP-2 (dilution 1:400, Chemicon). Antimouse FITC-conjugated F(ab′)2 fragment (dilution 1:20, Calbiochem, CA) was then added and incubated for 1 week. To identify astrocytes, the sections were treated with 0.1% pepsin 37°C for 15 min and then pAb GFAP (dilution 1:400, Dakopatts) was added. The sections were incubated with anti-rabbit FITC-conjugated F(ab′)2 fragment (dilution 1:20, Calbiochem, CA) for 1 week. The above sections stained with FITC-conjugated F(ab′)2 fragment were subsequently processed for identification of a human cellular nuclei antigen with a primary mouse anti-human nuclei monoclonal antibody,

MAB 1281 (dilution 1:200) and a Cy5-conjugated F(ab′)2 fragment rabbit anti-mouse IgG (dilution 1:20). The slides were analyzed using a fluorescent microscope (Olympus, BH-2). Because the primary antibodies used against MAB 1281, NeuN, and MAP-2 were monoclonal antibodies, nonspecific positive reaction could occur in double labeling staining. Therefore, a series of negative controls was used to assess and evaluate the immunohistochemical staining results. Negative control sections from each animal received identical staining preparation, except that the primary antibodies or the secondary antibodies were omitted.

Three-Dimensional Image Acquisition

In order to observe the relation of the donor's cells with the cerebral vessels, the vibratome sections were analyzed with a Bio-Rad (Cambridge, MA) MRC 1024 (argon and krypton) laser-scanning confocal imaging system mounted onto a Zeiss microscope (Bio-Rad). With the FITC-perfused tissue samples from each group, 10 vibratome sections from interaural 6.38 mm to interaural 1.0 mm (Paxinos and Watson, 1986) at 2-mm interval were screened at 488 nm under a 10× objective lens. Sections stained with the MAB 1281 antibody (Cy5) were excited by a laser beam at 647 nm (27).

Estimates of Cell Number

For measurement of MAB 1281-reactive cells, an average number of five equally spaced slides (approximately 100-μm interval) was obtained from each brain block and MAB 1281-reactive cells were counted within the seven 2-mm-thick blocks encompassing the forebrain. Nine slides from each of these blocks were first stained with FITC staining for identification of NeuN (3 slides), MAP-2 (3 slides), and GFAP (3 slides), and were followed by Cy5 staining for identification of HUCB cells. The number of the MAB 1281-reactive cells expressing NeuN, MAP-2, and GFAP was counted, respectively, using fluorescent microscopy within all seven blocks. In order to reduce biases introduced by sampling parameters, all sections for MAB 1281 identification from rats were stained simultaneously. The criteria for MAB 1281-positive cells were defined before the cells were counted by observers blinded to the individual treatment. All MAB 1281-reactive cells were counted throughout the coronal sections.

Neurological Functional Evaluation

Neurological motor measurement was performed using an accelerating Rotarod motor test. The rats were placed on the accelerating Rotarod treadmill (Lab-line Instruments, Inc.) and the rat's task was to walk and maintain its equilibrium on the rotating rod, which rotated at a gradually increasing speed. When the rat fell off the rod, a plate tripped and a liquid crystal recorded the endurance time in seconds. All rats were pretrained with five trials (warm-up trials) performed daily for 3 days prior to TBI to ensure stable baselines (14). After TBI and TBI following administration of HUCB or saline, the rats were tested on days 1, 4, 7, 14, and 28 until sacrifice. The motor test data are shown as a percentage of an average of five trials on the Rotarod test compared with the internal baseline values.

Twenty-four hours after TBI or administration of HUCB or saline, all rats were evaluated using the neurological severity scores (NSS) (23). NSS is a composite of the motor (muscle status, abnormal movement), sensory (visual, tactile, and proprioceptive), and reflex tests. One point was given for failure to perform a task. Thus, the higher score, the more severe the injury, with a maximum of 14 points. Rats were reevaluated on days 1, 4, 7, 14, and 28 after the treatment. All measurements were performed by observers blinded to individual treatment.

Statistical Analysis

NSS and Rotarod test scores were measured before injury and at 1, 4, 7, 14, and 28 days after TBI. The numbers of MAB 1281-reactive cells were counted at 28 days after treatment. The analysis of covariance for ANOVA (repeated measures) was conducted to test the treatment by time interactions, and the effect of treatment over time. The same analysis approach was used to analyze the outcome of Rotarod test score. Paired *t*-test was used to test the difference in means of cell counts between the injured hemisphere and the control hemisphere.

RESULTS

Histological Analysis of Organs

Sections from the blocks of brain and organs were stained with H&E staining for the general histopathological evaluation. The architectural integrity of all organs analyzed under light microscopy was not disrupted except for the initial mechanical injury of the brain. Bleeding, invasion of white cells, inflammatory response, and neoplasm were not observed on any slides aside from the brain.

Distribution of MAB 1281-Positive Cells

No MAB 1281-positive cells were observed in the slides from only TBI and TBI + saline groups, which did not receive the injection of HUCB cells. Large numbers of MAB 1281-positive cells were found in the vessels of the brain, heart, lung, liver, kidney, spleen, muscle, and even bone marrow of the rats receiving the injection of HUCB. A few scattered MAB 1281-positive cells were found in the parenchyma of these organs. In the brain, MAB 1281-labeled cells were observed in the

boundary zone of the injured area, cortex, striatum, and corpus callosum of the ipsilateral hemisphere. The MAB 1281-positive signals were detected in the nuclei of the capillary endothelial cells surrounding the injured area. Using laser confocal microscopy, the implanted cells were confirmed to be integrated into sprouting vessels in the boundary zone of the injured area (Fig. 1g–i). The total number of MAB 1281-positive cells migrating into the parenchyma of both the ipsilateral and contralateral hemispheres of the brain was counted and analyzed in the TBI + HUCB group. The numbers of MAB 1281-positive cells in the ipsilateral hemisphere (43,597 ± 4265) were significantly greater than those in the contralateral hemisphere (13,742 ± 6471, $p < 0.05$). The data indicate that HUCB cells delivered to the brain via an IV route preferably migrate into the injured brain tissue.

Phenotypical Identification of MAB 1281-Reactive Cells

Double fluorescent staining showed that some MAB 1281-positive cells expressed neuronal markers, NeuN and MAP-2, and an astrocytic marker, GFAP (Fig. 1a–f). These double-labeled cells were observed only in the ipsilateral hemispheres of the rats in the HUCB-treated group. Most of these positive cells were located in the boundary zone of the injured area: 6.9 ± 1.3% of MAB 1281-labeled cells in the ipsilateral hemispheres in the HUCB-treated group expressed NeuN, 5.8 ± 2.4% expressed MAP-2, and 9.7 ± 2.8% expressed GFAP. These data demonstrate that some implanted cells express neuronal and astrocytic phenotypes.

Neurological and Motor Function Evaluation

Two days after TBI, significantly lower scores of the Rotarod test and significantly higher scores of NSS in three groups compared with preinjury were found. Rotarod test scores were significantly improved in the TBI + HUCB group (138.0 ± 11.3% and 155.2 ± 16.2%) when compared with the TBI group (118.5 ± 17.0% and 129.2 ± 12.2%) and TBI + saline group (117.2 ± 13.6% and 133.2 ± 10.7%) ($p < 0.05$) at days 14 and 28 after administration of HUCB. The neurological severity scores were also significantly improved in the TBI + HUCB group (4.2 ± 1.3 and 3 ± 0.8) when compared with the TBI group (7.5 ±

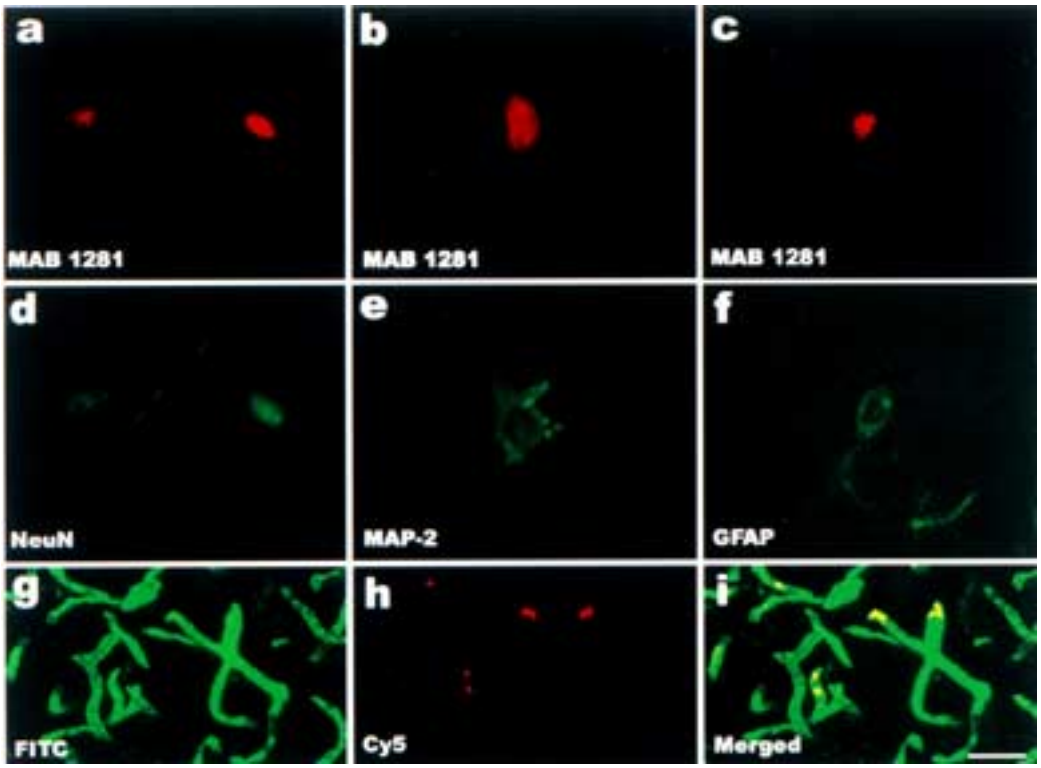

Figure 1. Localization of MAB 1281-positive cells by fluorescent immunohistochemical stain in the nucleus of human cord blood cells migrating into the brain (a, b, c) and vessel (h) when stained by Cy5-conjugated secondary antibody (red). Double staining of the same section shows expression of neuronal phenotype markers, NeuN (d) and MAP-2 (e), and astrocytic marker, GFAP (f), by these cells. (g) FITC-dextran perfused vessels photographed under the laser confocal microscopy. (i) Merged photograph of (g) and (h), indicating adherence or integration of the HUCB into the microvessels. Scale bar (showed in i) = 25 μm in (a, b, c, d, f) and 50 μm in (g, h, i).

1.73 and 6.3 ± 1.3) and the TBI + saline group (7.3 ± 0.9 and 5.75 ± 0.9) ($p < 0.05$) at days 14 and 28 after injection. The results indicate that IV administration of HUCB 24 h after TBI reduces the motor neurological functional deficits caused by TBI (Figs. 2 and 3).

DISCUSSION

Our major findings in this study were: 1) HUCB cells injected IV enter the brain by day 28 after HUCB cell administration; 2) IV injection of HUCB reduces motor and neurological deficits by days 14 and 28 after administration; 3) the cells migrating into the parenchyma of the brain express the neuronal markers, NeuN and MAP-2, and the astrocytic marker, GFAP; 4) HUCB cells integrated into the vascular wall within target organs; 5) these cells are also present in other organs and primarily localize to the vessels, without any obvious adverse effects. Our data suggest that IV administration of HUCB cells may be useful in the treatment of TBI.

These data demonstrate that a few injected cells migrate into the parenchyma of the brain, heart, lung, kidney, liver, spleen, muscle, and bone marrow. Because our study was designed to measure the effect of HUCB administered IV on TBI, the numbers of HUCB cells (MAB 1281-positive cells) present in the cerebral parenchyma were counted and analyzed in the TBI + HUCB group. Significantly more MAB 1281-positive cells were found in the ipsilateral hemisphere than in the contralateral hemisphere. This indicates that the injected cells preferably migrate into the injured hemisphere, especially to the boundary zone of the injured area after TBI. Nearly all of the MAB 1281-positive cells expressing NeuN, MAP-2, and GFAP were located in the ipsilateral hemisphere, demonstrating that the microenvironment of the brain after injury may drive HUCB cells into a neural cell phenotype. The mechanisms responsible for MSC engrafting into the brain remain unclear. HUCB cells may enter the brain from blood–brain barrier disruption or in response to signals from cytokines and cell surface receptors and antigens (11,16,25). HUCB cells significantly increase in the presence of 24-h-old ischemia brain tissue extracts (4). These data demonstrate that the injured brain tissues secrete factors that induce the migration of HUCB cells into the brain. Brain injury also activates an enzymatic cascade of matrix metalloproteinases (MMPs) that are responsible for degradation of extracellular matrix proteins such as collagen, proteoglycan, and laminin (22). In the central nervous system, endothelial cells, microglia, and astrocytes express MMPs in response to injury that facilitate cell migration (22).

The observation that the injected cells appear to integrate into the vascular structures surrounding the injured area suggests that the IV-injected HUCB cells may be involved in the angiogenesis of the cerebral vessels after TBI. Endothelial progenitor cells of HUCB may participate in the rebuilding of the injured vessels. This hypothesis is supported by data in which locally trans-

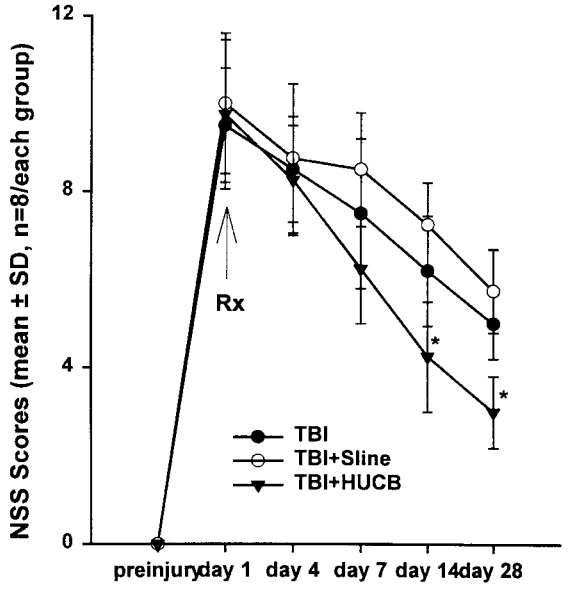

Figure 2. Neurological severe scores preinjury and at different time points after traumatic brain injury. *$p < 0.05$ compared with TBI only and TBI + saline groups at the same time point. Rx, treatment time point.

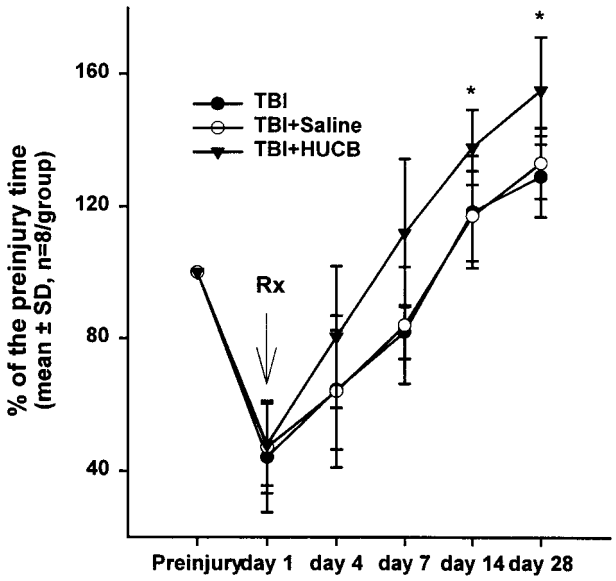

Figure 3. Rotarod test results preinjury and at different time points after traumatic brain injury. *$p < 0.05$ compared with TBI only and TBI + saline groups at the same time point. Rx, treatment time point.

planted endothelial precursor cells (EPCs) isolated from umbilical cord blood into the ischemic tissues of immunodeficient nude rats survived and participated in building capillary network (19). Our study in treatment of brain ischemia with HUCB showed that HUCB cells integrated with vascular structure and expressed the endothelial cell marker, Factor VIII (4).

Although the basis of the neurological function improvements is not yet understood, several mechanisms of action that can contribute to these improvements should be considered, including HUCB cell integration into brain parenchyma and HUCB cells as a source of trophic factors. HUCB cells produce an array of tropic factors and cytokines (11,16,26). Our studies in stroke and spinal cord injury demonstrate rapid improvement of function after the transplantation of marrow stromal cells, strongly suggesting a trophic factor response is primarily responsible for the therapeutic benefit (6,17). HUCB cells integrating into the vascular structure may increase angiogenesis after injury and subsequently promote improvement of the neurological function. In addition, another important mechanism mediating the improvement of neurological function may be that the temporary HUCB graft is rich in superoxide dismutase and directly and indirectly provides adequate superoxide dismutase to scavenge free radicals released by the injured tissue after TBI (26).

Our data indicate that a small percentage of HUCB cells express proteins phenotypic of neurons and astrocytes. However, protein expression does not imply cellular replacement and integration into tissue. In addition, the relatively few cells that express parenchymal cell proteins are insufficient to provide adequate replacement of tissue and thus support the hypothesis that therapeutic benefit derives from HUCB cells as a source of trophic factors, angiogenesis, and free radical scavengers. Cell replacement improves functional deficits in the adult brain through different mechanisms except the replacement of the lost tissue (2). Transplanted cells may produce neurotrophic or neurotroprotective factors that can counteract degeneration or promote regeneration, and release neurotransmitters (such as acetylcholine). They may also modify the response to injury and assist in structural repair, stimulate axon outgrowth, and promote remyelination (8,21). Because HUCB contains many different progenitor cells (such as hematopoietic progenitors, mesenchymal progenitors, and endothelial progenitors), HUCB treatment can promote repair of the injured tissue via different mechanisms.

CONCLUSION

We show for the first time that the IV administration of human umbilical cord blood cells reduces functional deficits after severe traumatic brain injury in the rat. The injected cells enter the brain, migrate into the injured area, and a few express the neuronal and astrocytic phenotypes and integrate into the vascular structures. Cord blood administration may be a useful treatment for traumatic brain injury.

ACKNOWLEDGMENTS: This work was funded by Saneron CCEL Therapeutic, Inc. (Tampa), and the State of Florida High Tech Corridor Grant.

REFERENCES

1. Becker, D. P.; Gade, G. F.; Yound, H. F.; Feuerman, T. F. Diagnosis and treatment of head injury in adults. In: Youmans, Jr., ed. Neurological surgery. Philadelphia: W. B. Saunders; 1990:2017–2148.
2. Björklund, A.; Lindvall, O. Cell replacement therapies for central nervous system disorders. Nat. Neurosci. 3(6): 537–544; 2000.
3. Borlongan, C. V.; Stahl, C. E.; Cameron, D. F.; Saporta, T. B.; Freeman, D. W.; Cahill, P. R. CNS modulation of graft survival and rejection. Neurol. Res. 18:297–304; 1988.
4. Chen, J. L.; Sanberg, P. R.; Li, Y.; Chopp, M. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke 32(11):2682–2688; 2001.
5. Chen, R.; Ende, N. 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(1–2):21–30; 2000.
6. Chopp, M.; Zhang, X. H.; Li, Y.; Wang, L.; Chen, J.; Lu, D.; Lu, M.; Rosenblum, M. Spinal cord injury in rat: Treatment with bone marrow stromal cell transplant. Neuroreport 11(13):3001–3005; 2000.
7. Dixon, E.; Clifton, G.; Lighthall, J. W. A controlled cortical impact model of traumatic brain injury in rat. J. Neurosci. Methods 39:253–262; 1991.
8. Duncan, I. D.; Grever, W. E.; Zhang, S. C. Repair of myelin disease: Strategies and progress in animal models. Mol. Med. Today 3:554–561; 1997.
9. Ende, N.; Lu, S.; Ende, M.; Giuliani, D.; Ricafort, R. J.; Alcid, M. G.; Deladisma, M. D.; Bagtas-Ricafort, L. Potential effectiveness of stored cord blood (non-frozen) for emergency use. J. Emerg. Med. 14(6):673–677; 1996.
10. Ende, N.; Weinstein, F.; Chen, R.; Ende, M. Human umbilical cord blood effect on sod mice (amyotrophic lateral sclerosis). Life Sci. 67:53–59; 2000.
11. Erices, A.; Conget, P.; Minguell, J. J. Mesenchymal progenitor cells in human umbilical cord blood. Br. J. Haematol. 109(1):235–242; 2000.
12. Fredrikson, S.; Sun, J. B.; Huang, W. X.; Li, B. L.; Olsson, T.; Link, H. Cord blood contains high numbers of autoimmune T cells recognizing multiple myelin proteins and acetycholine receptor. J. Immunol. 151(4):2217–2224; 1993.
13. Gluckman, E.; Rocha, V.; Chastang, C. Cord blood stem cell transplantation. Best Pract. Res. Clin. Haematol. 12: 279–292; 1999.
14. Hamm, R. J.; Pike, B. R.; O'Dell, D. M. The rotarod test: An evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. J. Neurotrauma 11: 187–196; 1994.
15. Inoue S. Cord blood transplantation: Current and future issues. Indian J. Pediatr. 65:163–171; 1998.

16. Li, Y.; Douglas, S. D.; Ho, W. Human stem cells express substance P gene and its receptor. J. Hemtother. Stem Cell Res. 9(4):445–452; 2000.
17. Lu, D.; Mahmood, A.; Wang, L.; Li, Y.; Lu, M.; Chopp, M. Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. Neuroreport 12(3):559–563; 2001.
18. Miyazaki, R.; Ogata, H.; Iguchi, T.; Sogo, S.; Kushida, T.; Ito, T.; Inaba, M.; Ikehara, S.; Kobayashi, Y. Comparative analysis of megakaryocytes derived from cord blood and bone marrow. Br. J. Haematol. 108:602–609; 2000.
19. Murohara, T.; Ikeda, H.; Duan, J.; Shintani, S.; Sasaki, Ki.; Eguchi, H.; Onitsuka, I.; Matsui, K.; Imaizumi, T. Transplanted cord blood-derived endothelial precursor cells augment postnatal neovascularization. J. Clin. Invest. 105(11):1527–1536; 2000.
20. Postmantur, R.; Kampel, A.; Simon, R. A calpain inhibitor attenuates cortical cytoskeletal protein loss after experimental traumatic brain injury in the rat. Neuroscience 77: 765–787; 1997.
21. Raisman, G. Use of Schwann cells to induce repair of adult CNS tracts. Rev. Neurol. 153:521–525; 1997.
22. Romanic, A. M.; White, R. F.; Arleth, A. J.; Ohlstein, E. H.; Barone, F. C. Matrix metelloproteinase expression increses after cerebral focal ischemia in rats: Inhibition of matrix metalloproteinase-9 reduces infarction size. Stroke 29:1020–1030; 1998.
23. Shohami, E.; Novikov, M.; Bass, R. Long-term effect of HU-211, a novel non-competitive NMDA antagonist, on motor and memory functions after closed head injury in the rat. Brain Res. 674:55–62; 1995.
24. Todaro, A. M.; Pafumi, C.; Pernicone, G.; Munda, S.; Pilastro, M. R.; Russo, A.; Farina, M.; Messing, A. Haematopoietic progenitors from umbilical cord blood. Blood Purification 18:144–147; 2000.
25. Welker, P.; Grabble, J.; Gibbs, B.; Zuberbier, T.; Henz, B. M. Nerve growth factor-beta mast-cell marker expression during in vitro culture of human umbilical cord blood cells. Immunology 99(3):418–426: 2000.
26. Yunoki, M.; Kawauchi, M.; Ukita, N.; Noguchi, Y.; Nishio, S.; Ono, Y.; Asari, S.; Ohmoto, T.; Asanuma, M.; Ogawa, N. Effects of lecithinized SOD on sequential change in SOD activity after cerebral contusion in rats. Acta Neurochir. Suppl. (Wien) 71:142–145; 1998.
27. Zhang, Z. G.; Chopp, M.; Goussev, A.; Lu, D.; Morris, D.; Tsang, W.; Powers, C.; Ho, K. L. Cerebral microvascular obstruction by fibrin is associated with upregulation of PAI-1 acutely after onset of focal embolic ischemia in rats. J Neurosci. 19(24):10898–10907; 1999.