Intravenous Administration of Human Umbilical Cord Blood Reduces Behavioral Deficits After Stroke in Rats

Jieli Chen, MD; Paul R. Sanberg, PhD; Yi Li, MD; Lei Wang, MD; Mei Lu, PhD; Allison E. Willing, PhD; Juan Sanchez-Ramos, PhD; Michael Chopp, PhD

Background and Purpose—Human umbilical cord blood cells (HUCBC) are rich in stem and progenitor cells. In this study we tested whether intravenously infused HUCBC enter brain, survive, differentiate, and improve neurological functional recovery after stroke in rats. In addition, we tested whether ischemic brain tissue extract selectively induces chemotaxis of HUCBC in vitro.

Methods—Adult male Wistar rats were subjected to transient (2-hour) middle cerebral artery occlusion (MCAO). Experimental groups were as follows: group 1, MCAO alone (n=5); group 2, 3×10^6 HUCBC injected into tail vein at 24 hours after MCAO (n=6) (animals of groups 1 and 2 were killed at 14 days after MCAO); group 3, MCAO alone (n=5); group 4, MCAO injected with PBS at 1 day after stroke (n=8); and group 5, 3×10^6 HUCBC injected into tail vein at 7 days after MCAO (n=5). Rats of groups 3, 4, and 5 were killed at 35 days after MCAO. Behavioral tests (rotarod and Modified Neurological Severity Score [mNSS]) were performed. Immunohistochemical staining was used to identify cells derived from HUCBC. Chemotactic activity of ischemia brain tissue extracts toward HUCBC at different time points was evaluated in vitro.

Results—Treatment at 24 hours after MCAO with HUCBC significantly improved functional recovery, as evidenced by the rotarod test and mNSS ($P<0.05$). Treatment at 7 days after MCAO with HUCBC significantly improved function only on the mNSS ($P<0.05$). Some HUCBC were reactive for the astrocyte marker glial fibrillary acidic protein and the neuronal markers NeuN and microtubule-associated protein 2. In vitro, significant HUCBC migration activity was present at 24 hours after MCAO ($P<0.01$) compared with normal brain tissue.

Conclusions—Intravenously administered HUCBC enter brain, survive, migrate, and improve functional recovery after stroke. HUCBC transplantation may provide a cell source to treat stroke. **(*Stroke*. 2001;32:2682-2688.)**

Key Words: behavior ■ cerebral ischemia ■ fetal blood ■ neuronal plasticity ■ neuroprotection ■ transplantation ■ rats

Neural transplantation has been used to study and promote the regenerative potential of the brain after an ischemic insult. Fetal neural stem cells can reduce behavioral deficits in damaged and compromised brain in animals and in humans.[1,2] However, transplantation of embryonic grafts is plagued with logistical and ethical considerations. Thus, it is reasonable to seek alternative sources or an equivalent of stem cells. Stem cells have been isolated from various tissues in animals and humans, including adult bone marrow,[3–5] cord blood,[6–8] and even adult brain.[9]

Human umbilical cord blood cells (HUCBC) are rich in mesenchymal progenitor cells[8] and contain a large number of endothelial cell precursors.[10] Cord blood cells contain many immature stem/progenitor cells with extensive proliferation capacity in vitro. HUCBC have been used as a source of transplantable stem and progenitor cells[11] and as a source of marrow-repopulating cells for the treatment of pediatric disease.[12,13] HUCB stem cells have been used to reconstitute marrow in a child with severe Fanconi anemia and to treat amyotrophic lateral sclerosis in SOD1 mice.[14,15] However, little is known about survival and development of HUCBC transplantation in the central nervous system.

In the present study we tested whether intravenously infused HUCBC enter brain, survive, differentiate in the ischemic brain microenvironment, and improve neurological functional recovery after stroke in rats. In addition, we used an in vitro system to test whether ischemic brain tissue extract selectively induces chemotaxis of HUCBC.

Materials and Methods

HUCBC Sources and Preparation

HUCBC were provided and analyzed by Cryo-cell International, Inc. The cells contain 77.2% to 95% CD34+ cells. After centrifugation at 1000 rpm/min for 5 minutes at 4°C, the cells were washed with 0.1

Received April 11, 2001; final revision received July 10, 2001; accepted August 7, 2001.

From the Departments of Neurology (J.C., Y.L., L.W., M.C.) and Biostatistics and Research Epidemiology (M.L.), Henry Ford Health Sciences Center, Detroit, Mich; Department of Physics, Oakland University, Rochester, Mich (M.C.); and Departments of Neurosurgery and Neurology, Center for Aging and Brain Repair, University of South Florida, Tampa (P.R.S., A.E.W., J.S-R.).

Correspondence to Michael Chopp, PhD, Department of Neurology, Henry Ford Hospital, 2799 W Grand Blvd, Detroit, MI 48202. E-mail chopp@neuro.hfh.edu

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mol/L PBS. Nucleated HUCBC were counted with a cytometer to ensure adequate cell number for transplantation. The final dilution was approximately 3×10^6 HUCBC in 500 μL saline for injection into the tail vein in each rat.

Animal Transient Middle Cerebral Artery Occlusion Model

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

Adult male Wistar rats weighing 270 to 300 g were used in all our experiments. Middle cerebral artery occlusion (MCAO) was induced by a method of intraluminal vascular occlusion modified in our laboratory.[16] Briefly, rats were initially anesthetized with 3.5% halothane and maintained with 1.0% to 2.0% halothane in 70% N_2O and 30% O_2 with the use of a face mask. The right common carotid artery, external carotid artery (ECA), and internal carotid artery (ICA) were exposed. A length of 4-0 monofilament nylon suture (18.5 to 19.5 mm), determined by the weight of the animal, with its tip rounded by heating near a flame, was advanced from the ECA into the lumen of the ICA until it blocked the origin of the middle cerebral artery (MCA). Two hours after MCAO, animals were reanesthetized with halothane, and reperfusion was performed by withdrawal of the suture until the tip cleared the lumen of the ECA.

In Vitro Chemotaxis Assay

Ischemic Brain Tissue Extracts

Animals were killed at 6 hours, 24 hours, and 1 week (n=3 per time point) after the onset of MCAO; a normal control group (n=3) was used in which the animals were not subjected to surgical procedures. Tissue extracts were obtained from the experimental rats and control rats.[17] Forebrain tissues were immediately obtained from interaural 12 mm to interaural 2 mm. Each specimen was dissected on a bed of ice into hemispheres ipsilateral right side and contralateral to the MCAO. The tissue sections were homogenized by adding Iscove's modified Dulbecco's medium (IMDM) (150 mg tissue per milliliter IMDM) and incubated on ice for 10 minutes. The homogenate was centrifuged, and the supernatant was extracted.

Ischemic Brain Tissue Extract Assay on HUCBC Migration

Chemotactic activity of ischemic brain tissue extracts toward HUCBC at different time points was evaluated with the use of a 48-well micro chemotaxis chamber technique, as described with some modification.[18] HUCBC were resuspended in IMDM (serum free) at 10^6 cells per milliliter. Twenty-five microliters of tissue extracts prepared from normal and ischemic brain at 6 hours, 24 hours, and 1 week after MCAO was placed in the lower chamber of the 48-well micro chemotaxis chamber. A polycarbonate membrane (8-μm pore size) strip was place over the lower wells, and 50 μL of HUCBC suspension (1×10^6 cells per milliliter) was placed in each of the upper wells. Migration of HUCBC was allowed for 5 hours at 37°C incubation, and the number of migrated cells into the lower wells was then measured.

In Vivo Treatment With HUCBC

Experimental Groups

Group 1 (control) involved MCAO alone without donor cell administration (n=5). In group 2, 3×10^6 HUCBC were injected into the tail vein at 24 hours after MCAO (n=6). The animals of groups 1 and 2 were killed at 14 days after MCAO. To test the effects of delayed (7-day) HUCBC treatment, we included additional groups, as follows: group 3 (control), MCAO alone without donor cell administration (n=5); group 4, MCAO with PBS injection at 1 day after stroke (sham control) (n=8); and group 5, 3×10^6 HUCBC injected intravenously at 7 days after MCAO (n=5). Rats of groups 3, 4, and 5 were killed at 35 days after MCAO. The selection of cell number was guided by our previous cell therapy studies.[19]

Implantation Procedures

At 1 or 7 days after ischemia, randomly selected animals received HUCBC. Animals were anesthetized with 3.5% halothane and were then maintained with 1.0% to 2.0% halothane in 70% N_2O and 30% O_2 with the use of a face mask mounted in a Kopf stereotaxic frame. Approximately 3×10^6 HUCBC in 0.5 mL total fluid volume were injected into a tail vein.

Functional Tests

In all animals, a battery of behavioral tests was performed before MCAO and at 1, 7, 14, 21, 28, and 35 days after MCAO by an investigator who was blinded to the experimental groups. The battery of tests consisted of the rotarod motor test and the Modified Neurological Severity Score (mNSS).

In the rotarod motor test,[19–21] the rats were placed on the rotarod cylinder, and the time the animals remained on the rotarod was measured. The speed was slowly increased from 4 to 40 rpm within 5 minutes. A trial ended if the animal fell off the rungs or gripped the device and spun around for 2 consecutive revolutions without attempting to walk on the rungs. The animals were trained 3 days before MCAO. The mean duration (in seconds) on the device was recorded with 3 rotarod measurements 1 day before surgery. Motor test data are presented as percentage of mean duration (3 trials) on the rotarod compared with the internal baseline control (before surgery).

Table 1 shows a set of the mNSS.[19,22–24] Neurological function was graded on a scale of 0 to 18 (normal score, 0; maximal deficit score, 18). The mNSS is a composite of motor, sensory, reflex, and balance tests. In the severity scores of injury, 1 score point is awarded for the inability to perform the test or for the lack of a tested reflex; thus, the higher the score, the more severe is the injury.

Histological and Immunohistochemical Assessment

Animals were allowed to survive for 14 or 35 days after MCAO, and at that time animals were reanesthetized with ketamine (44 mg/kg) and xylazine (13 mg/kg). Rat brains were fixed by transcardial perfusion with saline, followed by perfusion and immersion in 4% paraformaldehyde, and the brain, heart, liver, spleen, lung, kidney, and muscle were embedded in paraffin. The cerebral tissues were cut into 7 equally spaced (2 mm) coronal blocks. A series of adjacent 6-μm-thick sections was cut from each block in the coronal plane and stained with hematoxylin and eosin. The 7 brain sections were traced with the use of the Global Laboratory image analysis system (Data Translation).[19,25] The indirect lesion area, in which the intact area of the ipsilateral hemisphere was subtracted from the area of the contralateral hemisphere, was calculated.[19,25] Lesion volume is presented as a volume percentage of the lesion compared with the contralateral hemisphere.

Single and double immunohistochemical staining[19,26] was used to identify cells derived from HUCBC. Briefly, standard paraffin blocks from HUCBC-treated groups (n=11) and control groups (n=18) were obtained from the center of the lesion, corresponding to coronal coordinates for bregma −1 to 1 mm. A series of 6-μm-thick sections at various levels (100-μm interval) was cut from this block and analyzed by light and fluorescent microscopy (Olympus, BH-2). To detect the distribution of transplanted HUCBC in other organs (ie, heart, liver, lung, spleen, kidney, muscle, bone marrow), 3 sections (6-μm thickness, 100-μm interval) from each organ were obtained, and numbers of MAB1281-reactive cells were measured. MAB1281 (mouse anti-human nuclei monoclonal antibody, Chemicon International, Inc) is a marker for human cells.[27] Sections were treated with the monoclonal antibody (mAb) against MAB1281 diluted at 1:300 in PBS with fluorescein isothiocyanate (FITC)–conjugated antibody staining for identification of HUCBC. Analysis of MAB1281-positive cells is based on the evaluation of an average of 5 slides from each brain and 3 slides from each organ per experimental animal. No MAB1281 cells were detected in rats not receiving HUCBC infusion.

To visualize the cellular colocalization of MAB1281 and cell type–specific markers in the same cells, FITC (Calbiochem) and cyanine-5.18 (CY5, Jackson Immunoresearch) were used for double-

TABLE 1. Modified Neurological Severity Score Points

Motor tests	
Raising rat by tail	3
Flexion of forelimb	1
Flexion of hindlimb	1
Head moved >10° to vertical axis within 30 s	1
Placing rat on floor (normal=0; maximum=3)	3
Normal walk	0
Inability to walk straight	1
Circling toward paretic side	2
Falls down to paretic side	3
Sensory tests	2
Placing test (visual and tactile test)	1
Proprioceptive test (deep sensation, pushing paw against table edge to stimulate limb muscles)	1
Beam balance tests (normal=0; maximum=6)	6
Balances with steady posture	0
Grasps side of beam	1
Hugs beam and 1 limb falls down from beam	2
Hugs beam and 2 limbs fall down from beam, or spins on beam (>60 s)	3
Attempts to balance on beam but falls off (>40 s)	4
Attempts to balance on beam but falls off (>20 s)	5
Falls off; no attempt to balance or hang on to beam (<20 s)	6
Reflex absence and abnormal movements	4
Pinna reflex (head shake when auditory meatus is touched)	1
Corneal reflex (eye blink when cornea is lightly touched with cotton)	1
Startle reflex (motor response to a brief noise from snapping a clipboard paper)	1
Seizures, myoclonus, myodystony	1
Maximum points	18

One point is awarded for inability to perform the tasks or for lack of a tested reflex: 13–18, severe injury; 7–12, moderate injury; 1–6, mild injury.

label immunoreactivity. Each coronal section was first treated with the primary MAB1281 mAb with FITC staining for identification of HUCBC. This was followed by treatment with cell type–specific antibodies: a neuronal nuclear antigen (NeuN for neurons; dilution 1:200; Chemicon); microtubule-associated protein 2 (MAP-2 for neurons; dilution 1:200; Boehringer Mannheim); an astrocytic marker, glial fibrillary acidic protein (GFAP; dilution 1:1000; Dako); and an endothelial marker, FVIII (von Willebrand factor; dilution 1:400; Dako) with CY5 staining. Negative control sections from each animal received identical preparations for immunohistochemical staining, except that primary antibodies were omitted. A total of 500 MAB1281-positive cells per animal were counted to obtain the percentage of MAB1281 cells colocalized with cell type–specific markers (MAP-2, NeuN, GFAP, FVIII) by double staining.

Laser Scanning Confocal Microscopy

Colocalization of MAB1281 with neuronal markers (NeuN, MAP-2, GFAP) was conducted by laser scanning confocal microscopy (LSCM) with the use of a Bio-Rad MRC 1024 (argon and krypton) laser scanning confocal imaging system mounted onto a Zeiss microscope (Bio-Rad).[28] For immunofluorescence, double-labeled green (FITC for HUCBC) and red (Cy5 for MAP-2, NeuN, GFAP, or FVIII) fluorochromes on the sections were excited by a laser beam at 488 and 647 nm; emissions were sequentially acquired with 2 separate photomultiplier tubes through 522- and 680-nm emission filters, respectively. Areas of interest were scanned with a ×40 oil immersion objective lens in 260.6×260.6-μm format in the *x-y* direction and 0.5-μm format in the *z* direction.

Statistical Analysis

The behavioral scores (rotarod and mNSS) were evaluated for normality and equal variances. Data transformation or nonparametric analysis approach (eg, analysis on the ranked data) would be considered if data were ill behaved. We also tested the balance of the baseline variables between the treated and the control groups. Any imbalance of variables would be included in the analysis for the treatment effect. Two control groups were involved in the study: MCAO alone and MCAO with PBS (sham-operated) controls. Repeated-measures ANOVA was conducted to test the treatment by time interactions and the effect of treatment over time on each behavioral score. If an interaction of treatment by time or overall treatment effect was significant at the 0.05 level, then subgroup analysis would be conducted for the effect of treatment at each time point at the 0.05 level. Otherwise, the subgroup analysis would be considered exploratory. The means (SE) and P-values for testing the difference between treated and control groups are presented.

For the 2 MCAO alone control groups, one control group had complete behavioral scores up to 14 days after stroke before they were killed for histological analysis, and the other control group had complete behavioral scores up to 35 days after stroke. The controls were shared for testing the treatment effect with HUCBC at different times after stroke.

To evaluate the chemotactic activity of HUCBC migration, counts of intact cells were performed on the normal brain tissue extracts and ischemic brain tissue extracts at 6 hours, 24 hours, and 1 week after ischemic onset. We tested the normality and equal variances of each outcome measure. Data transformation or permutation tests would be

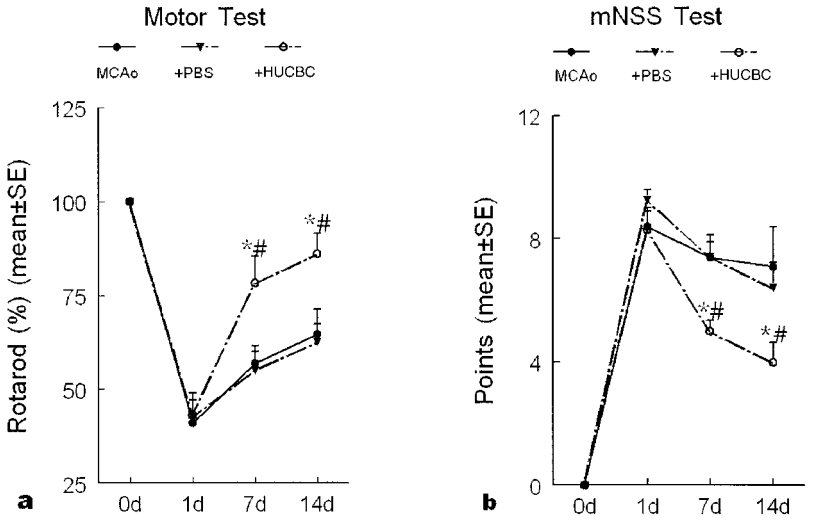

Figure 1. Behavioral functional tests before and after MCAO. Groups were as follows: group 1, MCAO alone (n=10); group 2, MCAO with PBS (sham control; n=8); group 3, intravenous infusion of HUCBC (3×10^6; n=6) at 24 hours after MCAO. Rats of group 1 and 3 were killed at 14 days after MCAO. a, Motor test; b, mNSS. *$P<0.05$ vs MCAO alone; #$P<0.05$ vs MCAO+PBS (sham control).

considered if data were ill behaved. The HUCBC migration activity was evaluated between normal tissue and ischemic tissue. If the main effect was significant at the 0.05 level, then subgroup analysis would be considered, with a significant effect at the 0.05 level. The means (SE) are reported.

Results

HUCBC Treatment Improves Functional Recovery After MCAO

The overall effects of HUCBC on functional recovery were significant for rotarod and mNSS tests compared with MCAO alone ($P<0.05$). Similar HUCBC effects were shown compared with MCAO with PBS controls. Treatment at 1 day after MCAO with HUCBC significantly improved functional recovery, as evidenced by improved rotarod test and mNSS scores at 7 and 14 days ($P<0.05$) compared with MCAO alone and MCAO with PBS, respectively (Figure 1).

Slight differences in regard to MCAO abnormality were observed between the treatment and control groups before the treatment. However, the differences are not significant ($P>0.38$ compared with MCAO alone; $P>0.18$ compared with MCAO with PBS). Rats treated with HUCBC at 7 days after stroke showed a significant reduction of neurological deficit only on mNSS ($P<0.05$ from 14 days after the treatment compared with the PBS control group; $P<0.05$ at days 14, 28, and 35; $P=0.12$ at 21 days after treatment compared with MCAO alone group). No treatment effect on the rotarod test ($P=0.55$) was detected (Figure 2).

Intravenously Infused HUCBC Enter Brain, Survive, and Differentiate

Dark and red neurons were observed in the ischemic core of all rats subjected to MCAO with and without donor transplantation at 14 and 35 days after MCAO. No significant reduction of volume of ischemic damage was detected in rats with donor treatment at 24 hours and 7 days after ischemia, compared with control rats subjected to MCAO alone and MCAO with PBS (Table 2). Within the brain tissue, HUCBC survived and were distributed throughout the damaged brain (including cortex, subcortex, and striatum) of recipient rats, with the vast majority of cells localized to the ischemic boundary zone. Few cells were observed in the contralateral hemisphere. Significantly more HUCBC were found in the ipsilateral hemisphere than in the contralateral hemisphere (Table 2; $P<0.05$). Thus, HUCBC delivered to brain via an

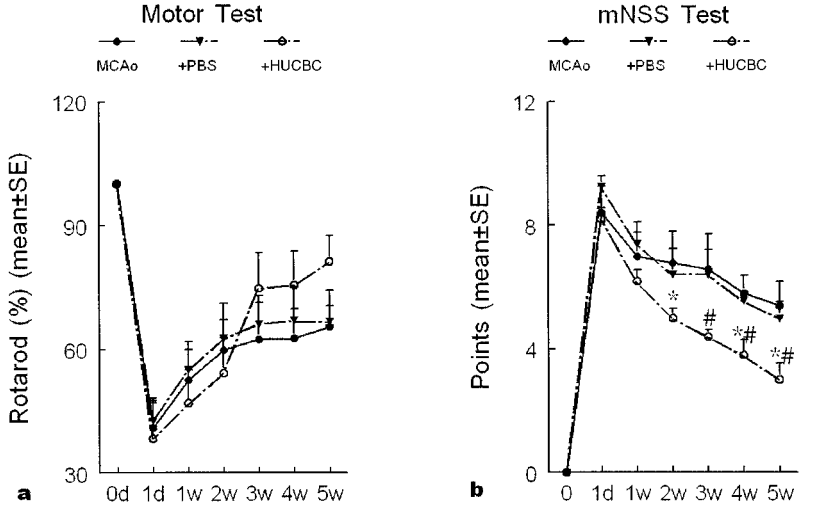

Figure 2. Behavioral functional tests before and after MCAO. Groups were as follows: group 1, MCAO alone (n=5); group 2, MCAO with PBS (sham control; n=8); group 3, HUCBC treatment group (n=5) at 7 days after MCAO. Rats were killed at 35 days after MCAO. a, Motor test; b, mNSS. *$P<0.05$ vs MCAO; #$P<0.05$ vs MCAO+PBS (sham control).

TABLE 2. Lesion Volume and MAB1281-Positive Cells in Brain

Group	n	Lesion Volume, %	MAB1281-Positive Cells in Recipient Brain: Ipsilateral Hemisphere	MAB1281-Positive Cells in Recipient Brain: Contralateral Hemisphere
1	5	34.6±3.2		
2	6	29.9±4.1	32 600±1689*†	9050±772
3	5	35.2±3.7		
4	8	33.8±3.1		
5	5	33.1±3.6	27 250±1561†	11 050±902

*$P<0.05$, group 2 (injected HUCBC at 1 day after MCAO) MAB1281-positive cells in ipsilateral hemisphere compared with group 5 (injected HUCBC at 7 days after MCAO).

†$P<0.05$, ipsilateral hemisphere MAB1281-positive cells compared with contralateral hemisphere.

intravenous route preferably migrate into the injured tissue. HUCBC in the ipsilateral hemisphere were significantly higher when treatment was initiated at 24 hours than at 7 days after stroke (Table 2; $P<0.05$). Controls were performed, and the data demonstrate no MAB1281-positive cells without HUCBC infusion (MCAO alone and MCAO with PBS).

In organs other than brain, as an approximate percentage of endogenous organ-specific cells, some HUCBC were detected in bone marrow (≈3%) (Figure 3P) and spleen (≈1%), and scattered HUCBC were detected in muscle (Figure 3Q), heart, lung, and liver (0.01% to 0.5%). Most HUCBC encircle vessels of these organs, with few cells located in parenchyma.

HUCBC (Figure 3A, 3D, 3G, 3J) were reactive for the neuronal markers NeuN (Figure 3B) and MAP-2 (Figure 3H), for the astrocyte marker GFAP (Figure 3K), and for endothelial cell marker FVIII (Figure 3E). The percentage of HUCBC that expressed NeuN, MAP-2, GFAP, and FVIII proteins was ≈2%, ≈3%, ≈6%, and ≈8%, respectively. LSCM shows colocalization of HUCBC with immunofluorescent labels for MAP-2 and MAB1281 (Figure 3M), GFAP and MAB1281 (Figure 3N), and NeuN and MAB1281 (Figure 3O).

Ischemic Brain Tissue Extract Assay on HUCBC Migration

Ischemic tissue elicited an increase in HUCBC migration (Figure 4). A significant increase in HUCBC migration activity was detected in the presence of ischemic cerebral tissue harvested at 24 hours after the onset of stroke ($P<0.01$). A trend of increase in HUCBC migration activity was apparent on tissue harvested at 6 hours and 1 week after MCAO ($P>0.09$) compared with HUCBC migration activity measured in the presence of normal nonischemic brain tissue.

Discussion

Our data show that at 14 and 35 days after transplantation, intravenously injected HUCBC were found in the brain, and significantly more HUCBC were found in the ipsilateral hemisphere than in the contralateral hemisphere. Many cells migrated into the boundary zone of ischemic brain. HUCBC survive, and some express cell type–specific markers GFAP, NeuN, MAP-2, and FIII. Most importantly, a significant improvement in functional outcome on motor and mNSS tests was found in animals given HUCBC intravenously at 1 day after stroke. In vitro, our data showed that there was significant HUCBC migration activity in the presence of ischemic cerebral tissue harvested at 24 hours after MCAO ($P<0.01$) compared with normal nonischemic brain tissue.

The mechanisms by which transplanted HUCBC induce functional benefit after stroke are not clear. Growth factors are the molecular signals by which the body regulates cell survival, proliferation, and differentiation.[29] Exogenously administered neurotrophic growth factors may limit the extent of acute ischemic neural injury and enhance functional recovery after stroke.[30] The intravenous administration of HUCBC and the migration of these cells into the injured tissue may provide a trophic factor production source that bypasses the blood-brain barrier. HUCBC contain a large number of hematopoietic colony-forming cells,[31] thrombopoietin, and interleukin-11.[32] The hematopoietic cytokine

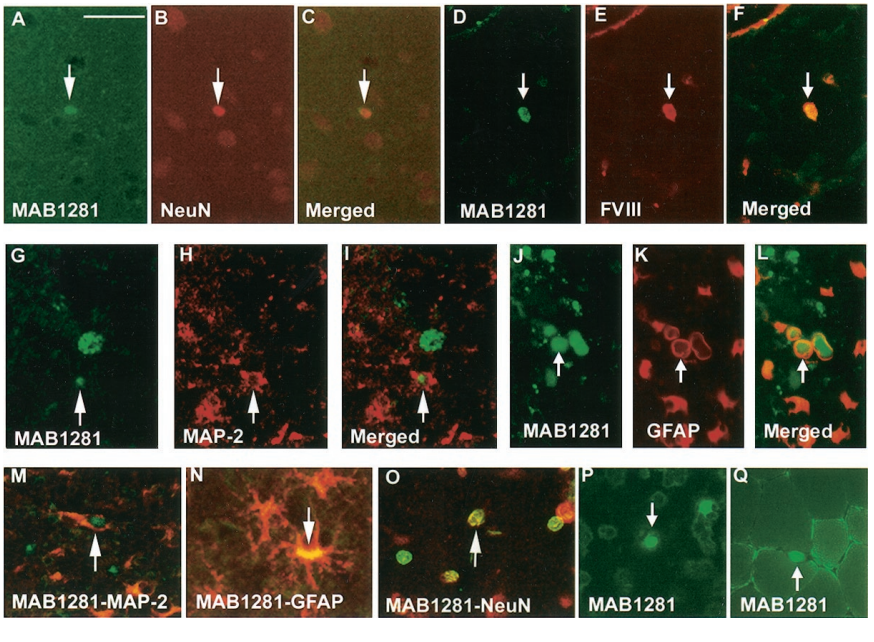

Figure 3. Immunohistochemical staining from a recipient rat. Photomicrographs show the morphological characteristics of group with HUCBC treatment at 24 hours after MCAO. Double immunohistochemical responses of cells derived from HUCBC are shown (A through L). Immunofluorescent FITC (green) shows that MAB1281-reactive HUCBC (A, D, G, J) express phenotypes of neuronal markers NeuN (B) and MAP-2 (H), astrocytic marker GFAP (K), and endothelial cell marker FVIII (E) in the recipient rat brain. Use of immunofluorescent FITC (green) shows that a few MAB1281-reactive cells (HUCBC) are present in the bone marrow (P) and muscle (Q) of the recipient rat. LSCM image shows that colocalization of immunofluorescent labels MAP-2 and MAB1281 (M), GFAP and MAB1281 (N), and NeuN and MAB1281 (O) was observed. Arrows indicate MAB1281-reactive cells. Bar=15 μm.

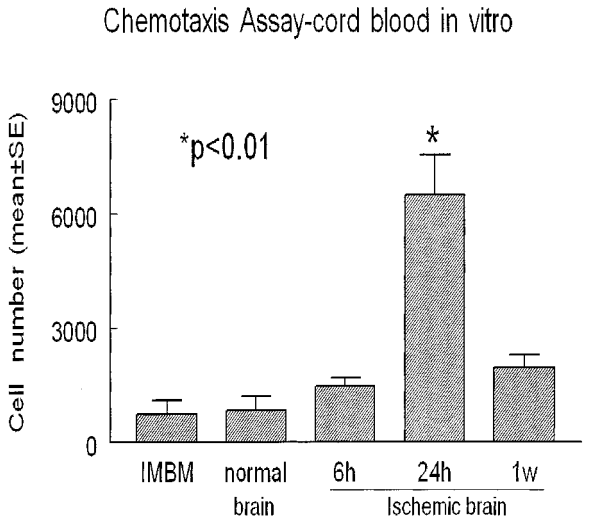

Figure 4. Ischemic brain tissue extract assay on HUCBC migration. A significant increase in HUCBC migration activity was detected in the presence of ischemic cerebral tissue harvested at 24 hours after the onset of stroke ($P<0.01$) (mean±SE). *$P<0.01$.

colony stimulating factor-1 is a growth factor in the central nervous system.[33] Thrombopoietin has proliferation effects on hematopoietic cells, suppresses apoptosis, and functions as a survival factor. Cytokines are survival and/or differentiation factors for murine hippocampal neuronal progenitor cells,[34] and they may play an important role in proliferation or differentiation of neural tissue. In contrast, in regard to the field of stem cell transplantation in which the implanted stem cells are expected to integrate into and to replace the damaged tissue, therapeutic benefit derived from HUCBC treatment may derive from stimulation of endogenous brain recovery mechanism. In our study a small percentage of HUCBC expressed proteins phenotypic of neural-like cells. Functional recovery was found within days after administration of HUCBC. It is highly unlikely that these cells integrate into the cerebral tissue and make appropriate connections within days after transplantation. In addition, the number of cells that enter tissue is relatively small, and the replacement of tissue would constitute less than a cubic millimeter. Thus, it is far more likely that these cells act as sources of trophic factor production.

More HUCBC were found in the lesioned hemisphere than in the intact hemisphere, which is consistent with data that ischemic brain tissue extract induced migration of HUCBC and which suggests that ischemia-induced chemotactic factors facilitate HUCBC migration. Blood-brain barrier permeability increases at 3 hours of reperfusion after MCAO, reaches a maximum at 48 hours, and decreases from 4 days after stroke.[35–37] Significantly more HUCBC are present in the ipsilateral hemisphere when treatment is initiated at 24 hours than at 7 days after stroke. Disruption of the blood-brain barrier may facilitate selective entry of HUCBC into ischemic brain. In addition, other mechanisms may promote migration of HUCBC into brain. Ischemic brain elicits a strong inflammatory response. Cerebral ischemic tissues express chemotactic proteins, eg, monocyte chemoattractant protein-1 (MCP-1/JE),[38] and adhesion molecules, eg, intercellular adhesion molecule (ICAM)[39] and vascular adhesion molecule-1 (VCAM) on endothelial cells. ICAM-1, VCAM, and E-selectin are significantly increased in microvascular endothelial cells in acute ischemic brain.[39,40] In addition, HUCBC express several adhesive-related antigens: integrin subunits ($\alpha 4$ and $\alpha 5$), integrins ($\alpha v\beta 3$ and $\alpha v\beta 5$), and ICAM. Among them, VLA4 ($\alpha 4v\beta 1$), a critical homing integrin, participates in mononuclear trafficking to sites of target through interaction with an inducible vascular ligand, VCAM.[41] This interaction likely facilitates migration of HUCBC across the blood-brain barrier into ischemic target.

HUCBC treatment at 24 hours after MCAO significantly improved functional recovery (motor rotarod test and mNSS scores) after stroke. Treatment with HUCBC at 7 days after MCAO resulted in functional recovery only on the mNSS test after MCAO. Thus, the treatment benefit of HUCBC depends on the time of treatment and may be related to the migration activity of HUCBC. A significant increase in HUCBC migration activity was detected in the presence of ischemic cerebral tissue harvested at 24 hours and not at 7 days after MCAO. Early treatment with HUCBC after stroke may promote HUCBC migration into ischemic brain and facilitate functional recovery after MCAO.

In conclusion, we have shown that intravenously administrated HUCBC survive, migrate, and improve functional recovery after stroke. Since HUCBC are widely available and have been used clinically, they potentially may be an excellent source of cells for treatment of early stroke. Furthermore, treatment with HUCBC has a therapeutic window of days compared with the present 3-hour window for the treatment of stroke with recombinant tissue plasminogen activator.

Acknowledgments

This work was supported by CCEL Biotherapies, Inc, a subsidiary of CRYO-CELL International, Inc (Clearwater, Fla), and by the State of Florida 14 Corridor Grant. The authors thank Cecylia Power, Cynthia Roberts, and Lijie Zhang for their technical assistance and Deborah Jewell for secretarial support.

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