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Human cord blood CD34$^+$ cells and behavioral recovery following focal cerebral ischemia in rats

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Abstract. The present study investigated effects of human umbilical cord blood derived CD34$^+$ cells on sensorimotor, cognitive, and histological outcome in rats following focal cerebral ischemia. Halothane anesthetized adult male Wistar rats were subjected to transient or permanent occlusion of the middle cerebral artery (MCAO) followed by intravenous administration of CD34$^+$ cells (5×10^5 or 2×10^6) after 24 h recovery. The beam-walking and cylinder tests were used to assess sensorimotor function, and Morris water-maze examined cognitive performance during a 25 day follow-up period. Subsequently, rats were perfused for measurement of infarct volumes and detection of CD34$^+$ cells in the brain by immunohistochemistry (MAB1281). MCAO rats showed minor or no spontaneous recovery in sensorimotor function during the follow-up. The recovery profile was similar in MCAO controls and in MCAO rats that received CD34$^+$ cells, although CD34$^+$ cells seemed to improve the use of impaired forelimb. There was also a trend toward improved water-maze performance by CD34$^+$ cells in transient MCAO rats. Infarct volumes assessed from Nissl-stained sections on postoperative day 25 did not differ between the experimental groups. MAB1281-positive cells were not detected in the brain of MCAO rats that received CD34$^+$ cells. The present study suggests that CD34$^+$ cells might improve functional outcome in MCAO rats after systemic administration, but do not significantly provide neuroprotection.

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Key words: behavioral outcome, cord blood stem cell, focal cerebral ischemia, infarct size, transplantation

INTRODUCTION

Stroke is the major cause of adult disability in the westernized countries and causes a huge burden to patients, their relatives and society (Asplund et al. 1998). As a calculated cost per patient, stroke is the third most costly neurological disorder (Truelsen et al. 2005). There are currently no effective treatments except rehabilitation to enhance impaired motor functions following stroke. One promising approach is cell-based therapies to restore functions compromised by stroke (Lindvall and Kokaia, 2004, Savitz et al. 2002). These are clinically appealing as it might be possible to initiate treatment days or weeks after the ischemic insult. A further advantage is that cell transplantation and other treatments such as intensive rehabilitative therapy can be combined to achieve an additive effect.

Human umbilical cord blood (HUCB), which is rich in adult stem and progenitor cells (both hematopoietic and non-hematopoietic), is one potential source of cells for transplantation (Newman et al. 2003, Sanberg et al. 2005). HUCB cells do not raise ethical issues, have weak immunogenicity, and are proven to be safe treatment of pediatric diseases (Sirchia and Rebulla 1999). Intravenous administration of HUCB cells is noninvasive and thus has the most immediate access to clinical applications. In rats subjected to focal cerebral ischemia, intravenous administration of HUCB cells has resulted in partial but significant improvements in behavioral recovery (Borlongan et al. 2004, Chen et al. 2001, Vendrame et al. 2004, Willing et al. 2003) indicating that cells may selectively migrate towards ischemic regions and perhaps cross the blood-brain barrier. The exact mechanisms of the cells which affect the recovery process after cerebral ischemia are poorly understood, but may include angiogenesis, enhanced neurogenesis and reduced inflammation (Chen et al. 2004, Shyu et al. 2006, Taguchi et al. 2004, Vendrame et al. 2005).

By using demanding behavioral tests, which are sensitive to detect true functional recovery, we have previously shown that intravenous administration of human cord blood mononuclear or lineage-negative (LIN^-) cells does not improve behavioral performance following transient occlusion of the middle cerebral artery (MCAO) in rats (Mäkinen et al. 2006). However, recent data suggest that $CD34^+$ cells might be a more effective fraction of cord blood cells to promote functional recovery through angiogenesis and neurogenesis (Shyu et al. 2006, Taguchi et al. 2004). Thus, the present study assessed effects of HUCB $CD34^+$ cells on behavioral and histological outcomes in MCAO rats. Since the extensive corticostriatal damage typical to the transient MCAO by a filament may mask possible therapeutic effects, $CD34^+$ cells were also administered to rats following permanent MCAO, a model which produces an ischemic damage limited to the cortical areas.

METHODS

Thirty-nine male Wistar rats (3–4 months at the time of operation) were used in the present study. The animals had free access to food and water and were housed in individual cages in a temperature-controlled environment (20 ± 1°C), with lights on from 7:00 A.M. to 07:00 P.M. Experimental procedures were conducted in accordance with the European Community Council directives 86/609/EEC and the study was approved by the Ethics Committee of the University of Kuopio and the Provincial Government of Kuopio.

The study design is presented in Fig. 1. A modified version of the limb-placing test (Jolkkonen et al. 2000, Puurunen et al. 2001) was used to assess successful MCA occlusion 24 h after operation. Only the rats with total scores less than 10, indicating a severe ischemic damage, were included into the study. The test scores were also used to assign MCAO rats to behaviorally equivalent experimental groups.

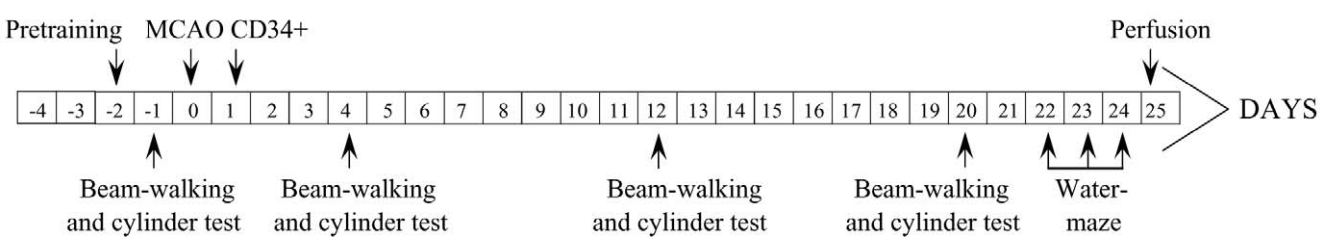

Fig. 1. Study design. The arrows indicate the timing of middle cerebral artery occlusion (MCAO), $CD34^+$ cell administration, and behavioral testing.

Transient occlusion of the middle cerebral artery (tMCAO) was induced using the intraluminal filament technique (Longa et al. 1989). Anesthesia was induced in a chamber using 3% halothane in 30% O_2/70% N_2O. A surgical depth of anesthesia was maintained throughout the operation with 0.5 to 1% halothane delivered through a nose mask. To occlude blood flow to the right MCA territory, a heparinized nylon filament (∅ 0.25 mm, rounded tip) was advanced 1.9 to 2.1 cm into the internal common carotid artery until resistance was felt. The filament was held in place by tightening the suture around the internal common carotid artery and placing a microvascular clip around the artery. Body temperature was monitored and maintained at 37°C using a heating pad connected to a rectal probe. After 120 min occlusion, the filament was removed and the external carotid artery was permanently closed by electrocoagulation. At the end of the operation and on postoperative days when necessary, supplementary 0.9% NaCl solution was administered (i.p.) to the animal because of dehydration.

For permanent MCA occlusion (pMCAO), a small craniectomy was drilled between the eye and external auditory canal through which the distal MCA was exposed and electrocoagulated (Chen et al. 1986). Subsequently both common carotid arteries were exposed and occluded with miniature clips for 60 min. Then, the clips were removed, the cervical incisions were closed with silk sutures, and the animals were returned to their home cages. Sham-operated rats underwent the same surgical procedures excluding electrocoagulation of the MCA. At the end of the operation and on postoperative days when necessary, supplementary 0.9% NaCl solution was administered (i.p.) to the animal.

Umbilical cord blood was collected from informed and consenting donors at the Helsinki University Central Hospital, Department of Obstetrics and Gynecology. The permit to collect and use donated stem cells was obtained from the ethics board of the Helsinki University Central Hospital and the ethics board of the Finnish Red Cross Blood Service. Cord blood collections were performed *ex utero* using a standardized procedure. All cord blood units tested negative for human immunodeficiency virus, hepatitis C virus, hepatitis B virus, human T-cell lymphotropic virus and syphilis. The used cord blood units measured between 60–110 ml in volume and were processed within 24 hours from delivery. Mononuclear cells were isolated from diluted cord blood by Ficoll-Paque PLUS density gradient (GE Healthcare, Piscataway, USA). $CD34^+$ cells were enriched from mononuclear cells through positive magnetic selection using a two-column system with the Direct CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany) and MACS columns type LS+ and MS+ (Miltenyi Biotec) according to the manufacturer's instructions. We have previously shown that two successive column separations result in an at least 77 % pure $CD34^+$ population and the selected $CD34^+$ cells exhibit a clonogenic capacity of 84.5 per 1000 cells (Kekarainen et al. 2006). The selected $CD34^+$ cells were frozen in 90% fetal bovine serum (FBS, Gibco/Invitrogen, Paisley, UK) and 10 % dimethyl sulfoxide (DMSO) and stored in liquid nitrogen until use. Immediately before use in cell transplantation, the $CD34^+$ cells were thawed in a 37°C water bath and gently dissolved in excess warm Iscove's Modified Dulbecco's Medium (Gibco), which was supplemented with 10% FBS and 0.002% DNaseI (Sigma-Aldrich). The cells were allowed to rest for 5 min at room temperature and were subsequently washed twice in excess phosphate buffered saline (PBS). Cell number and viability was determined by Trypan blue staining and counting in a Bürker chamber. The cell viability was always concluded to be above 90%. The cells were dissolved in 500 µl 0.9% NaCl for the injections and were administered in the femoral vein 24 h after recovery. tMCAO rats received 5×10^5 $CD34^+$ cells/animal. pMCAO rats received 2×10^6 $CD34^+$ cells pooled from two cord blood units ($1 \times 10^6 + 1 \times 10^6$)/animal. Control rats received only vehicle. $CD34^+$ cells from one cord blood unit were always administered to at least two individual rats.

The tapered/ledged beam was used to test sensorimotor function of hindlimbs (Schallert and Woodlee 2005, Zhao et al. 2005). The pretrained animals were tested before surgery and on postoperative days 4, 12, and 20 (Fig. 1). The beam-walking apparatus consisted of a tapered beam with underlying ledges on each side to permit foot faults without falling. The end of the beam was connected to a black box (20.5 × 25 × 25 cm) with a platform at the starting point. A bright light was placed above the start point to motivate the rats to traverse the beam. The rats' performance was videotaped and later analyzed by calculating the slip ratio of the impaired (contralateral to lesion) hindlimb. Steps onto the ledge were scored as a full slip, and a half slip was given if the limb touched the side of the beam. The slip ratio was calculated as: (full slips + 1/2 half slips)/(total

steps) ×100%. The mean of three trials was used for statistical analyses. All behavioral analysis was carried out by an observer blind to the experimental groups.

The cylinder test was used to assess forelimb use asymmetry (Schallert and Woodlee 2005). For the test, the rat was placed in a transparent cylinder (∅ 20 cm) and videotaped before operation, and on postoperative days 4, 12, and 20 (Fig. 1). A mirror was placed at 45° angle beneath the cylinder so that behavior could be filmed from below the cylinder. Exploratory activity for 1 to 3 min was analyzed by using a video recorder with slow motion capabilities. Number of contacts by both forelimb and by either impaired or nonimpaired forelimb was counted. Cylinder score for impaired forelimb was calculated as: (contralateral contacts + 1/2 bilateral contacts) /(total contacts) × 100%.

Spatial learning was assessed using a modified version of the Morris water-maze task (Puurunen et al. 2001). Rats were given five daily trials on postoperative days 22–24 (Fig. 1). The pool was divided into four quadrants of equal surface area. The starting locations were called north, south, east, and west, and were located arbitrarily at equal distances on the pool rim. The platform was located in the middle of the south-west quadrant 25 cm from the pool rim. The swim paths were monitored by a video camera connected to a computer through an image analyzer (HVS image). If the rat failed to find the hidden platform within 70 s, it was placed on the platform. The animal was allowed to remain on the platform for 10 s and to rest for either 30 s (after trials 1, 2, and 4) or 1 min (after trials 3 and 5 of the third day). Trials 1, 3, and 4 of each day were started from one of the points located farthest from the platform. The start point was changed after each trial. Escape latency (time to reach the platform) and path length the animal swam to find the platform were used to assess acquisition of the water-maze task. Swimming speed (path length/escape latency) was used to assess the motoric activity of rats in this task.

On postoperative day 25 after behavioral follow-up, rats were perfused transcardially with 0.9% NaCl followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The brains were removed from the skulls, postfixed in the same fixative, and cryoprotected in phosphate buffered 30% sucrose. Frozen sections (35 μm) were cut with a sliding microtome and stored in a cryoprotectant tissue-collection solution at –20°C. One series of sections was stained for detection of HUCB cells. Briefly, sections were rinsed in 0.1 M phosphate buffered saline (PBS) and endogenous peroxide was removed with 10% hydrogen peroxide and 10% methanol in 0.1 M PBS. Nonspecific binding was blocked with 5% normal goat serum, prior to incubating in 1:1000 diluted monoclonal antibody, against human nuclei (MAB1281, Chemicon International), for 4 days at 4°C. Sections were rinsed in PBS and then incubated in 1:500 diluted horseradish peroxidase (HRP) conjugated secondary antibody (AP181P, Chemicon International) for 1 hour. Triton X-100 (0.25%) was used to increase tissue antibody penetration. After washing the secondary antibody, TrueBlue peroxidase substrate (KPL) was used to visualize MAB1281-positive cells. Slides were dried overnight at 37°C, cleared in xylene and mounted. Negative control sections were processed as the experimental tissue, but the primary antibody was omitted. Postfixed human cortex was used as positive control.

Infarct volumes were measured from Nissl-stained sections collected at 0.525-mm intervals. Analysis was performed using the MCID image analysis system by an observer blind to experimental conditions. The image of each section was stored as a 1280 × 1024 matrix of calibrated pixel units. The digitized image was then displayed on a video screen and areas of surviving grey matter were outlined. The difference between the size of an intact area in the contralateral hemisphere and its respective residual area in the ipsilateral hemisphere was defined as the infarcted area (Swanson et al. 1990). Total infarct volumes were calculated by multiplying the infarct area by the distance between sections and summing together the volumes for each brain.

Beam-walking, cylinder and water-maze (path length, escape latency, swimming speed) data were analyzed for the overall group effect using analysis of variance (ANOVA) for repeated measures. Comparisons between groups were made using the LSD *post-hoc* test. Statistical differences in infarct volumes between groups were analyzed using Student *t*-test.

RESULTS

Transient MCAO rats showed a corticostriatal infarct, whereas permanent MCAO produced primarily cortical damage (Fig. 2). There were no signifi-

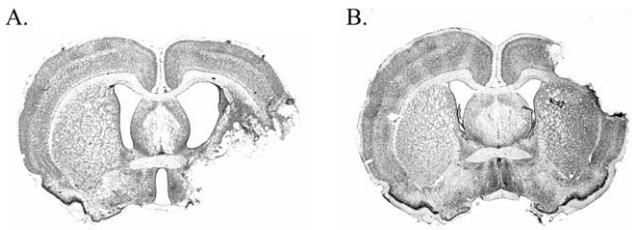

Fig. 2. Nissl-staining showing typical infarct 25 days following transient (A) or permanent (B) occlusion of the middle cerebral artery (MCAO) in rats.

cant differences in infarct volumes between tMCAO rats receiving vehicle (cortex 53.9 ± 24.9 mm^3; striatum 25.9 ± 2.7 mm^3) or $CD34^+$ cells (cortex 68.0 ± 32.2 mm^3; striatum 24.7 ± 4.7 mm^3). Cortical infarct size was also similar in pMCAO rats receiving vehicle (100.9 ± 23.4 mm^3) or $CD34^+$ cells (92.2 ± 3.2 mm^3).

Hindlimb function was assessed by beam-walking test in MCAO rats (Fig. 3). After tMCAO, slip ratios for impaired (contralateral to lesion) hindlimb increased. There was a significant overall group effect in impaired hindlimb function (P<0.01), but this was due to difference between sham-operated and tMCAO rats (P<0.01) on postoperative days 4, 12, and 20. An increase in hindlimb slip ratios following pMCAO did not reach statistical significance.

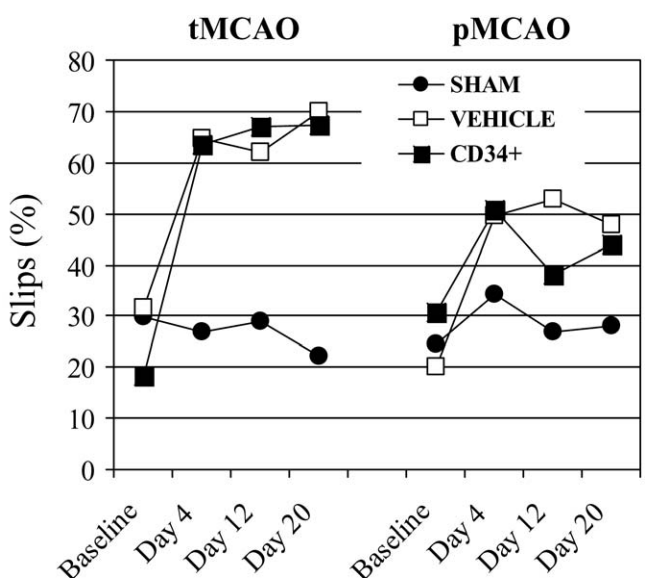

Fig. 3. Hindlimb performance of rats in the beam-walking test following transient or permanent occlusion of the middle cerebral artery (MCAO) and intravenous $CD34^+$ cell administration. Data are expressed as slips ratio for impaired hindlimb (contralateral to lesion): (full slips + 1/2 half slips)/(total steps) × 100%.

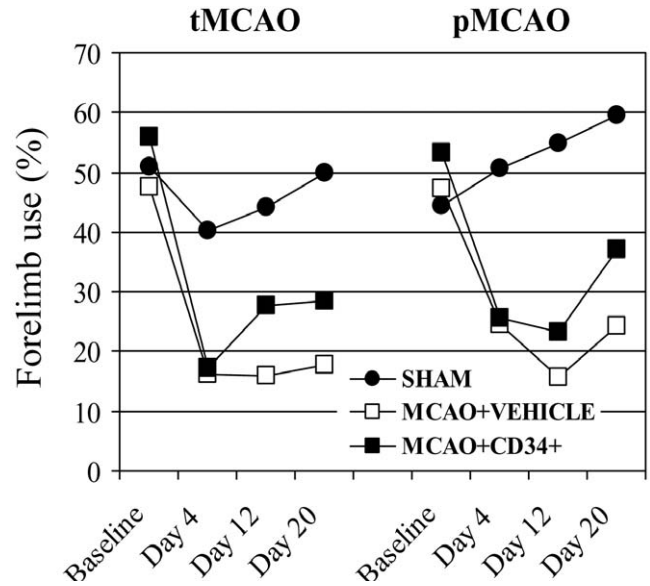

Fig. 4. Forelimb asymmetry in the cylinder test following transient or permanent occlusion of the middle cerebral artery (MCAO) and intravenous $CD34^+$ cell administration. Cylinder score was calculated as: (contralateral contacts + 1/2 bilateral contacts)/(total contacts) ×100%.

Use of impaired forelimb was assessed by cylinder tests in MCAO rats (Fig. 4). Before operation the animals exhibited equal forelimb use. There was a decrease in use of impaired (contralateral to lesion) forelimb and a parallel increase in reliance on nonimpaired forelimb after tMCAO and pMCAO. Statistical analysis showed a significant group effect in impaired forelimb use (P<0.001) in both stroke models. This was due to differences between sham-operated and MCAO rats on postoperative test days. However, $CD34^+$ cells seemed to increase somewhat the use of impaired forelimb, but this was not statistically significant.

The water-maze data for length and latency on postoperative days 22–24 are presented in Fig. 5. There was no significant group effect in water-maze length (P=0.097) and latency (P=0.210) in rats subjected to tMCAO. However, rats receiving $CD34^+$ cells seemed to perform better compared to vehicle treated rats. Water-maze performance was not affected in pMCAO rats. Swimming speed was not affected by MCAO.

Human nuclei specific MAB1281-positive cells were not detected in the ipsilateral or contralateral hemisphere of MCAO rats when assessed after behavioral follow-up on postoperative day 25.

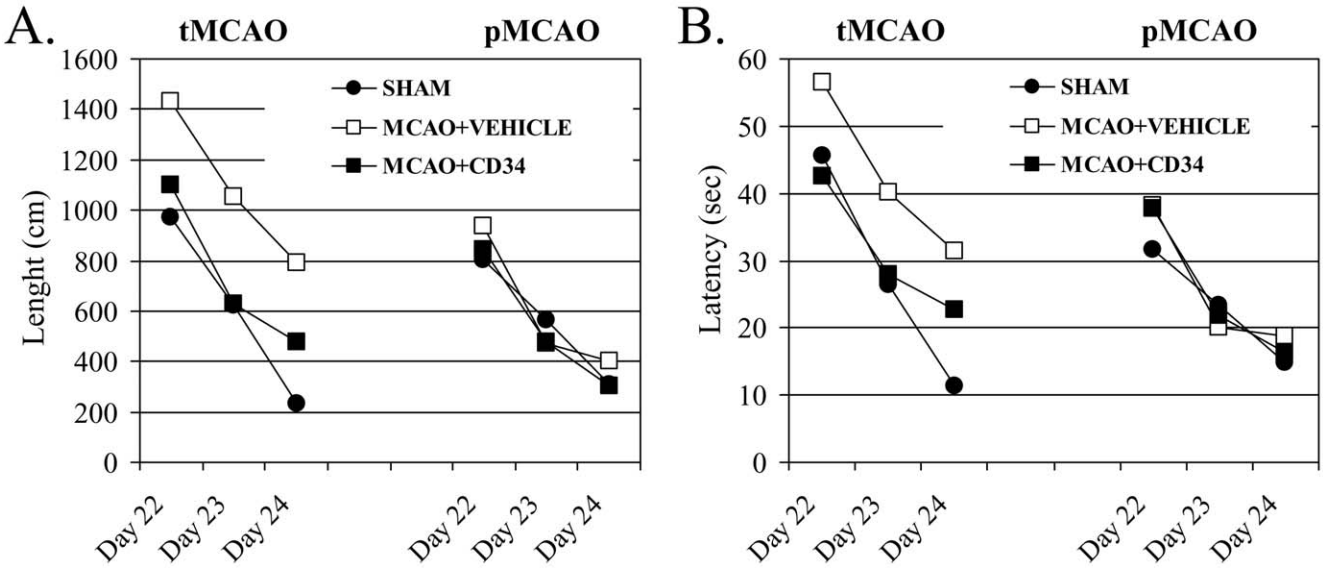

Fig. 5. The path length (A) and escape latency (B) of rats in the Morris water-maze following transient or permanent occlusion of the middle cerebral artery (MCAO) and intravenous $CD34^+$ cell administration.

DISCUSSION

The present study assessed functional and histological outcome following intravenous administration of HUCB $CD34^+$ cells in two focal cerebral ischemia models characterized by different brain pathology. A battery of demanding behavioral tests sensitive to the brain damage and treatment effects was applied for assessment of functional recovery. The data indicated that there was a trend towards improved sensorimotor and cognitive performance by $CD34^+$ cells in MCAO rats. This suggests that $CD34^+$ cells might be a more potential alternative for future studies compared to unselected mononuclear cells or Lin^- cells (Mäkinen et al. 2006). Used stroke models, doses, timing of administration, and administration route may also contribute to contrasting data. Rats subjected to transient and permanent MCAO behave in a similar manner after cell administration and thus different brain pathology is unlikely to explain the lack of therapeutic effect. Based on previous literature, the used cell doses were high enough to produce behavioral effects (Chen et al. 2001, Vendrame et al. 2004). In addition, we explored the strategy to use cells from two unrelated HUCB units in transplantation in the pMCAO model. It has recently been clinically shown that despite the theoretical risk of bidirectional immune rejection, cell therapy with two unrelated HUCB units have proven to achieve engraftment in all patients tested so far, and with a better speed than with single unit transplants (Barker et al. 2005). It appears that one unit acts as a "fertilizer" to the other, but that only one unit finally will engraft and continue to contribute to haematopoiesis. However, this did not provide significant benefit in pMCAO rats. Timing of cell administration 24 h after ischemia was selected to target subacute ischemic process (i.e. inflammation, delayed cell death). The recent study, however, suggests that the effective therapeutic window for recovery-enhancing effect by cell-based therapies may be substantially extended (Shen et al. 2006). Delayed administration may possibly increase cell survival and more specifically targets brain repair mechanisms. The most critical issue, however, might be the administration route. We previously showed using SPECT that following intravenous administration, HUCB cells remain in lung, liver, spleen, and kidney at least for 24 h (Mäkinen et al. 2006). A similar biodistribution pattern after systemic administration has also been shown for other cell populations (Gao et al. 2001, Noort et al. 2002). This is not to say that HUCB cells are not effective, but that trophic factors secreted by these cells may come into play, instead of engraftment of cells in the ischemic hemisphere. This is also supported by data showing that central nervous system entry of HUCB cells is not a prerequisite for neuroprotection (Borlongan et al. 2004).

$CD34^+$ cells, when administered 24 h after MCAO, did not reduce infarct size in either model. A decrease in infarct volumes has been previously demonstrated

even when HUCB cells are administered 48 h after MCAO (Borlongan et al. 2004, Newcomb et al. 2006, Vendrame et al. 2004). The permanent MCAO model used in those studies has, however, a particular feature producing delayed neuronal death, whereas both transient and permanent MCAO models used in the present study induce severe necrotic damage, which might be more difficult to protect. In addition, the recent studies in MCAO rats applied short occlusion time (45 min), which may favor neuroprotection by intravenous administration of bone marrow cells (Iihoshi et al. 2004) and human bone marrow-derived mesenchymal cells (Honma et al. 2006). In these cases HUCB cells may reduce inflammation and provide neuroprotection against delayed cell death, which in turn is translated to improved functional outcome. One has to note that the lack of neuroprotection does not exclude possible stimulation of brain own repair mechanisms.

$CD34^+$ cells were not detected in the ipsilateral or contralateral hemisphere of MCAO rats that received cell infusion. This is in contrast to previous work showing a significant number of HUCB cells in ischemic brains (Chen et al. 2001). We used for immunohistochemistry: (1) MAB1281 antibody which is specific for human nuclei, (2) TrueBlue as chromogen which gives less nonspecific staining particularly in ischemic boarder zone compared to diaminobenzidine (DAB) or fluorescent labeled secondary antibodies, and (3) human cortical sections as a positive control in all staining series. Thus, we are confident that at this late time point after transient or permanent MCAO, the survival rate of $CD34^+$ cells in the brain (if any) is very low. It is possible, however, that expression of human specific antigen of $CD34^+$ cells is depressed below the detection level of staining used. This is not supported by the fact that we were unable to amplify human genomic DNA by PCR from rat brain tissue, and that HUCB cells administered by intracerebral injection seem to disappear within few days, indicating that they are rapidly removed by macrophages (data not shown).

CONCLUSION

The present results showed that HUCB $CD34^+$ cells when administered intravenously do not provide neuroprotection against necrotic cell death, but might improve functional recovery in rats subjected to focal cerebral ischemia. Only a low number of $CD34^+$ cells may eventually enter the ischemic brain contributing to the lack of substantial therapeutic effect.

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REFERENCES

Asplund K, Stegmayr B, Peltonen M (1998) From the twentieth to the twenty-first century. A public health perspective on stroke. In: Cerebrovascular Disease. Pathophysiology, Diagnosis, and Management (Ginsberg MD, Bogousslavsky J, eds). Blackwell Science, p. 901–918.

Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, Miller JS, Verfaillie CM, Wagner JE (2005) Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood 105:1343–1347.

Borlongan CV, Hadman M, Sanberg CD, Sanberg PR (2004) Central nervous system entry of peripherally injected umbilical cord blood cells is not required for neuroprotection in stroke. Stroke 35: 2385–2389.

Chen ST, Hsu CY, Hogan EL, Maricq H, Balentine JD (1986) A model of focal ischemic stroke in the rat: Reproducible extensive cortical infarction. Stroke 17: 738–743.

Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M (2001) Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke 32: 2682–2688.

Chen J, Li Y, Zhang R, Katakowski M, Gautam SC, Xu Y, Lu M, Zhang Z, Chopp M (2004) Combination therapy of stroke in rats with a nitric oxide donor and human bone marrow stromal cells enhances angiogenesis and neurogenesis. Brain Res 1005: 21–28.

Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI (2001) The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cell Tiss Org 169: 12–20.

Honma T, Honmou O, Iihoshi S, Harada K, Houkin K, Hamada H, Kocsis JD (2006) Intravenous infusion of immortalized human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. Exp Neurol 199: 56–66.

Iihoshi S, Honmou O, Houkin K, Hashi K, Kocsis JD (2004) A therapeutic window for intravenous administration of autologous bone marrow after cerebral ischemia in adult rats. Brain Res 1007: 1–9.

Jolkkonen J, Puurunen K, Rantakömi S, Härkönen A, Haapalinna A, Sivenius J (2000) Behavioral effects of the α2-adrenoceptor antagonist, atipamezole, after focal cerebral ischemia in rats. Eur J Pharmacol 400: 211–219.

Kekarainen T, Mannelin S, Laine J, Jaatinen T (2006) Optimization of immunomagnetic separation for cord blood-derived hematopoietic stem cells. BMC Cell Biology 7: 30 [Epub ahead of print].

Lindvall O, Kokaia Z (2004) Recovery and rehabilitation in stroke. Stem cells. Stroke 35: 2691–2694.

Longa EZ, Weinstein PR, Carlson S, Cummins R (1989) Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20: 84–91.

Mäkinen S, Kekarainen T, Nystedt J, Liimatainen T, Huhtala T, Närvänen A, Laine J, Jolkkonen J (2006) Human umbilical cord blood cells do not improve sensorimotor or cognitive outcome following transient middle cerebral artery occlusion in rats. Brain Res 1123: 207–215.

Newcomb JD, Ajmo CT Jr, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE (2006) Timing of cord blood treatment after experimental stroke determines therapeutic efficacy. Cell Transplant 15: 213–223.

Newman MB, Davis CD, Kuzmin-Nichols N, Sanberg PR (2003) Human umbilical cord blood (HUCB) cells for central nervous system repair. Neurotox Res 5: 355–368.

Noort WA, Kruisselbrink AB, in't Anker PS, Kruger M, van Bezooijen RL, de Paus RA, Heemskerk MH, Lowik CW, Falkenburg JH, Willemze R, Fibbe WE (2002) Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34(+) cells in NOD/SCID mice. Exp Hematol 30: 870–878.

Puurunen K, Jolkkonen J, Sirviö J, Haapalinna A, Sivenius J (2001) Selegiline combined with enriched-environment housing attenuates spatial learning deficits following focal cerebral ischemia in rats. Exp Neurol 167: 348–355.

Sanberg PR, Willing AE, Garbuzova-Davis S, Saporta S, Liu G, Sanberg CD, Bickford PC, Klasko SK, El-Badri NS (2005) Umbilical cord blood-derived stem cells and brain repair. Ann N Y Acad Sci 1049: 67–83.

Savitz SI, Rosembaum DM, Dinsmore JH, Wechsler LR, Caplan LR (2002) Cell transplantation for stroke. Ann Neurol 52: 266–275.

Schallert T, Woodlee MT (2005) Orienting and placing. In: Behavior of the Laboratory Rat. A Handbook with Tests (Whishaw IQ, Kolb B, eds). Oxford University Press, New York, p. 129–140.

Shen LH, Li Y, Chen J, Zacharek A, Gao Q, Kapke A, Lu M, Raginski K, Vanguri P, Smith A, Chopp A (2006) Therapeutic benefit of bone marrow stromal cells administered 1 month after stroke. J Cereb Blood Flow Metab: Apr 5 [Epub ahead of print].

Shyu WC, Lin SZ, Chiang MF, Su CY, Li H (2006) Intracerebral peripheral blood stem cell (CD34+) implantation induces neuroplasticity by enhancing beta1 integrin-mediated angiogenesis in chronic stroke rats. J Neurosci 26: 3444–3453.

Sirchia G, Rebulla P (1999) Placental/umbilical cord blood transplantation. Haematologia 84: 738-747.

Swanson RA, Morton MT, Tsao-Wu G, Savalos RA, Davidson C, Sharp FR (1990) A semiautomated method for measuring brain infarct volume. J Cereb Blood Flow Metab 10: 290–293.

Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T (2004) Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest 114: 330–338.

Truelsen T, Ekman M, Boysen G (2005) Cost of stroke in Europe. Eur J Neurol 12: 78–84.

Vendrame M, Cassady J, Newcomb J, Butler T, Pennypacker KR, Zigova T, Sanberg CD, Sanberg PR, Willing AE (2004) Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. Stroke 35: 2390–2395.

Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE (2005) Anti-inflammatory effects of human cord blood cells in a rat model of stroke. Stem Cells Dev 14: 595–604.

Willing AE, Lixian J, Milliken M, Poulos S, Zigova T, Song S, Hart C, Sanchez-Ramos J, Sanberg PR (2003) Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. J Neurosci Res 73: 296–307.

Zhao C-s, Puurunen K, Schallert T, Sivenius J, Jolkkonen J (2005) Effect of cholinergic medication, before and after focal photothrombotic ischemic cortical injury, on histological and functional outcome in aged and young adult rats. Behav Brain Res 156: 85–94.

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