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Systemic delivery of umbilical cord blood cells for stroke therapy: A review

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Abstract

Purpose—This review paper summarizes relevant studies, discusses potential mechanisms of transplanted cell-mediated neuroprotection, and builds a case for the need to establish outcome parameters that are critical for transplantation success. In particular, we outline the advantages and disadvantages of systemic delivery of human umbilical cord blood (HUCB) cells in the field of cellular transplantation for treating ischemic stroke.

Methods—A MEDLINE/PubMed systematic search of published articles in peer-reviewed journals over the last 25 years was performed focusing on the theme of HUCB as donor graft source for transplantation therapy in neurological disorders with emphasis on stroke.

Results—Ischemic stroke remains a leading cause of human death and disability. Although stroke survivors may gain spontaneous partial functional recovery, they often suffer from sensory-motor dysfunction, behavioral/neurological alterations, and various degrees of paralysis. Currently, limited clinical intervention is available to prevent ischemic damage and restore lost function in stroke victims. Stem cells from fetal tissues, bone marrow, and HUCB has emerged in the last few years as a potential cell transplant cell source for ischemic stroke, because of their capability to differentiate into multiple cell types and the possibility that they may provide trophic support for cell survival, tissue repair, and functional recovery.

Conclusion—A growing number of studies highlight the potential of systemic delivery of HUCB cells as a novel therapeutic approach for stroke. However, additional preclinical studies are warranted to reveal the optimal HUCB transplant regimen that is safe and efficacious prior to proceeding to large-scale clinical application of these cells for stroke therapy.

Keywords

Cerebral ischemia; adult stem cells; transplantation; neuroprotection; neurorestoration

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1. Introduction

Stroke is one of the leading causes of death and a primary health care burden in both the developed and developing countries in the world (Truelsen et al., 2006; Lavados et al., 2007). While we have an escalating increase in our understanding of the biological and molecular underpinnings of the pathological cascade that occurs following stroke, there are no effective treatments (Brainin et al., 2007; Suwanwela & Koroshetz, 2007). Currently tPA (thrombolytic tissue-plasminogen activator) is the most widely used for acute stroke treatment but even in those fewer patients its effectiveness is limited (Luders 2007; Ali & Saver, 2007). Given the inability to effectively mitigate the devastating effects of stroke, it is imperative that novel therapeutic strategies are developed to both minimize the initial CNS trauma as well as repair the damaged brain once the pathological cascade of stroke has run its course.

Transplantation of stem cell has been proposed as a means of treating stroke. Stem cell therapy is an emerging and relatively young field of research, which may provide the opportunity to further our understanding of the extent and limitation of the uses of stem cells in the cellar therapy (Peterson, 2004). Stem cells derived from human umbilical cord blood (HUCB) can be differentiated into all the major cellular phenotypes of the brain including neurons, oligodendrocytes, and glial cells (Sanchez-Ramos et al., 2001; Ha et al., 2001; Bicknese et al., 2002; Buzanska et al., 2002). This multipotent feature of HUCB cells make them a potential source of transplantable neuronal cells capable of reconstituting the damaged neuronal circuitry that occurs in numerous diseases including stroke. The growing evidence from studies on HUCB cells following systemical transplantation suggests that these cells preferentially survive and differentiate into neurons in the damaged brain, and promote behavioral recovery in preclinical stroke (Chen et al., 2001; Willing et al., 2003a,b; Taguchi et al., 2004; Vendrame et al., 2004; Borlongan et al., 2004a; Xiao et al., 2005; Boltze et al., 2005; Lobel DA et al., 2003; Nystedt et al., 2006; see Table 1). The goal of this article is to provide an update on the preclinical use of systemically HUCB cell transplantation for stroke and focus on the mechanisms, feasibility, and determinants for the efficacies as well as to define the advantages and disadvantages of pursuing clinical application of this cell therapy in stroke.

2. Potential mechanisms underlying HUCB functional effects

Understanding how systemically transplanted HUCB cells affect the brain, and vice versa, in model systems is important before proceeding to clinical trials. The following mechanisms may be responsible for systemically transplanted HUCB cell-mediated recovery in ischemic stroke.

2.1. Cell replacement in the stroke brain

In contrast to a neurodegenerative disorder such as Parkinson's disease, which destroys a relatively homogenous population of neurons, stroke affects multiple different neuronal phenotypes. For example, an infarct might involve the thalamus, hippocampus, and striate visual cortex, affecting three or more very different neuronal populations including neurons, oligodendrocytes, astrocytes, and endothelial cells (Savitz et al., 2003; Savitz et al., 2004).

Reconstitution of the complex and widespread neuronal-glial-endothelial interrelationships may require access to a broader array of lineages than more committed phenotypes. Cells for transplantation may need to initially remain immature and phenotypically plastic to differentiate into appropriate neuronal, glial, and endothelial cell types depending on the ectopic site (Savitz et al., 2003; Savitz et al., 2004).

In an effort to define the phenotype of HUCB (Sanchez-Ramos et al., 2001), cells were cultured for seven days in a proliferation media supplemented with N2 (neuronal proliferating component), epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Additional cells were cultured in the differentiation media consisting of all-tans-retinoic (RA) and nerve growth factor (NGF). Immunocytochemistry showed RA and NGF increased the expression of Musashi-1 (an early marker of neuronal precursors), β-tubulin III (a specialized tubulin found in neurons-TUJ1) and glial fibrillary acidic protein (a marker for astrocytes-GFAP) in cultured HUCB cells. Quantitative real time transcription polymerase chain reaction (RT-PCR) analysis confirmed the presence of Musashi-1 and mRNA for nestin in RA and NGF treated cord blood cells. In addition, the GFAP mRNA was identified in both cell culture conditions.

Another study extending these results reported by Ha et al. (2001), showed β-mercaptoethanol promoted HUCB cells to differentiate toward a neuronal phenotype, which was confirmed by immunocytochemical expression of neuronal nuclei (NeuN), neurofilament, and GFAP and RT-PCR mRNA for nestin, neurofilament, and MAP2 (microtubule-associated protein 2). A multipotent HUCB cell subset that does not express CD14, CD34, and CD45 was identified by Bicknese et al. (2002). These cord blood cells cultured in a low glucose and more acidic media and supplemented with bFGF and hEGF differentiated and expressed both GFAP and TUJ1 after at least seven days. Using a magnetic cell sorter and subfraction, Buzanska et al. (2002) excluded CD 34^+ and $CD45^+$ cells from cord blood. The remaining cells cultured in Dulbecco's modified Eagle's medium (DMEM) with fetal bovine serum (FBS) and EGF, showed a very high commitment (30–40%) to neuronal and astrocytic fates and modest proportion of oligodendrocytes (11%). These clone-forming cells expressed nestin, but did not produce hematopoietic colonies. After exposure to RA and brain-derived neurotrophic factor (BDNF), the cells were immuno-positive for the expressed TUJ1, MAP2, GFAP, and GalC (oligodendrocyte marker). BDNF seemed to increase the number of astrocytes, but RA treated cells alone had more neuronal expression. In addition, the cells that were co-cultured with rat cortical cells for four days showed all three types of neural phenotypes (Buzanska et al., 2002). This suggested that endogenous neurotrophic factors may be required to promote HUCB cells towards neuronal lineages. These same investigators (Buzanska et al., 2006a, b) recently reported that after two years in culture, HUCB-derived neuronal stem cells continue to display normal chromosomal patterns, proliferation, and self-renewal properties. High performance liquid chromatography (HPLC) analysis confirmed that the stem cells produced and secreted serotonin (5-HT) and dopamine (DA) metabolites. Differentiated HUCB neuronal stem cells also exhibit electrophysiological properties indicative of neurons including hyperpolarization-activated inward and outward currents (Sun et al., 2005).

Recently, Chen et al. (2005) characterized in vitro two different subpopulations of mononuclear (MNC) HUCB cells adherent and floating. The results showed there was a significant number of progenitor/stem and neuronal cell antigen expressions in the floating population. The adherent cell population mainly contained lymphocytes (over 50%) expressing hematopietic antigen. These data suggested that nonhematopoietic subpopulation of cells exists within MNC HUCB cells and seems to have potential to become neuronal cells.

To date, there are comparatively few vivo studies demonstrating that HUCB cells express neuronal phenotypes after transplantation. In the study by Chopp and colleagues (Chen et al., 2001), HUCB cells survived and were found in ipsilateral cortex, subcortex, and striatum in damaged rodent brain when the cells were administered intravenously at 24 hours after experimental stroke. Immunocytochemical analysis showed that the HUCB-derived cells in damaged brain express neuronal markers NeuN, and MAP2, the astrocytic marker GFAP and endothelial marker FVβ. In one recent study, Xiao and colleagues (Xiao et al., 2005) intravenously injected one million nonhematopoietic HUCB cells into rats 48 hours after transient unilateral middle cerebral artery occlusion. Histological analysis of brain tissue revealed the expression of human nuclei. Some human nuclei-positive cells were also co-labeled for NeuN. However, cells expressing human nuclei marker within the brain were scant, which suggested that the restorative effects of HUCB cells may be mediated other than cell replacement. Another recent similar report by Boltze et al. (2005) extended and supported this in vivo study, by demonstrating that HUCB cells were found in the ipsilateral hemisphere of the lesion even at four weeks following transplantation when $CD34^-$ or $CD34^+$ HUCB cells were administered intravenously at eight and 10 hours after permanent middle artery occlusion in spontaneously hypertensive rats. In our study (Borlongan et al., 2004a), we did not detect intravenously administered HUCB cells (a sub-therapeutic dose of 2×10^5/10 μl) in the brains of animals at three days after stroke. These cells were co-infused with a blood-brain barrier (BBB) permeabilizer (mannitol) immediately after stroke induction. Although the combined HUCB-mannitol treatment significantly reduced cerebral infarcts and improved behavioral functions, immunohistochemical analyses with specific human antigens failed to detect any positive HUCB cells in the transplanted stroke brain. Our data show that central nervous system availability of grafted cells is not a prerequisite for acute neuroprotection.

Although these in vitro studies provide compelling evidence that HUCB cells differentiate and express neuronal phenotypes under appropriate media conditions, HUCB their functionality has not been unequivocally confirmed. Based on the limited in vivo studies, the data reveal the absence or the detection of only a small number of transplanted HUCB cells surviving and expressing neuronal phenotypes in the post-stroke brain, suggesting that cell replacement might not be the main mechanism responsible for the functional recovery in these transplanted stroke animals.

2.2. Neuronal rescue of the host ischemic penumbra

Immediately after stroke onset, various phenotypes of neurons, glial and endothelial cells are damaged. Acute delivery of stem cells may avert these acute pathological conditions, by

reducing lesion size and inhibiting cell death in the penumbra. Because neuronal differentiation of exogenously delivered stem cells and functional reconstruction of neuronal or glial network have not been fully achieved within a few days, the robust recovery after transplantation suggests neuroprotective effects rather than a cellular replacement mechanism. Neurotrophic factors such as glial cell-derived neurotrophic factor (GDNF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) are likely to contribute to this neuroprotective mechanism during the early period after transplantation of stem cells in the ischemic brain (Borlongan et al., 2004a, b; Lobel et al., 2003; Horita et al., 2006; Nomura et al., 2005; Pisati et al., 2007; Zhu et al., 2005; Yasuhara et al., 2006; Cairns et al., 2003). Of note, exogenous application of these trophic factors has been shown to provide neuroprotective effects in the ischemic brain. The participation of trophic factors in stroke recovery is further demonstrated by the observation that intravenous administration of stem cells increased the levels of trophic factors including VEGF, FGF, GDNF, NGF and BDNF in the ischemic brain (Taguchi et al., 2004; Borlongan et al., 2004; Lobel et al., 2003). Interestingly, in our study (Borlongan et al., 2004a), stroke animal models that received HUCB cell grafts, but pre-exposed to a cocktail of antibodies against GDNF, BDNF, NGF failed to exhibit any measurable neuroprotection of behavioral recovery. This set of data suggests that tropic factors secreted by HUCB cells appeared to play a direct role in the reduction of ischemic damage and improvement in behavioral recovery.

The highly acute therapeutic window to rescue the ischemic cells has been challenged in recent years. Indeed, the widely accepted initiation of therapeutic intervention in stroke has been the acute phase, i.e., 3 hours after onset, as seen with tPA. This early timing post-stroke is the target of neuroprotective treatments. In contrast, the advent of cell therapy has significantly prolonged this therapeutic window (i.e., > 3 hours) after stroke. Here, cell therapy corresponds to neurorestorative processes that are either a result of cell replacement (i.e., grafted cells directly replacing the dead or dying cells) or via a bystander effect (i.e., grafts secreting trophic factors and rescuing the injured but still viable cells) (see our recent reviews about these CNS repair mechanisms; Hess and Borlongan, 2008a and 2008b; Borlongan et al., 2008). Accumulating scientific evidence provides solid data that support this latter therapeutic benefit of grafted cells in several CNS animal models including stroke (see for example Borlongan et al., 2004a; Yasuhara et al., 2006; Redmond et al., 2007). Whereas the prevailing view asserts that stroke leads to an almost immediate fixation of the necrotic core, consisting of dead nonviable cells, equally compelling evidence have demonstrated that the area lining the core referred to as the ischemic border zone or the penumbra is a progressively evolving brain area that persists several hours and even days after stroke in both rodents and humans (Henninger and Fisher, 2007; Sun et al., 2007; Fisher 2004; Kidwell et al., 2003; Duval et al., 2002). Accordingly, the penumbra presents with a wider therapeutic window for cell therapy. Indeed, transplantation either directly into the penumbra or from the periphery after a delay (e.g., > 3 hours, days and even weeks after injury) post-stroke has shown robust graft survival, suggesting that the penumbra confers a conducive transplant site, accompanied by a reduction in the ischemic cell loss, and angiogenesis and neurogenesis within this brain region (Hara et al., 2007; Borlongan et al.,

1998; Shen et al., 2007; Bliss et al., 2006; Onda et al., 2008). Collectively, these results support a wider therapeutic window for cell therapy when targeting the ischemic penumbra.

2.3. Induction of host brain plasticity

An increase in endogenous brain plasticity and motor remapping after ischemia is also postulated to underlie the spontaneous recovery seen after a stroke (Carmichael 2006; Wei et al., 2005). Such plasticity-related events include an increase in afferent and efferent connections between the site of injury and both adjacent and contralateral brain regions, restoration of local synaptic activity by synaptogenesis, and probably strengthening of existing synapses as well as activation of silent synapses. Cell transplantation may enhance these endogenous repair mechanisms. Xiao et al.'s study (2005) revealed that animals with HUCB transplants exhibited significantly greater densities of biotinylated dextran amine (BDA)-positive cells in the damaged side of the brain compared to animals with intraparenchymal saline injections, indicating transplant-mediated sprouting of nerve fibers from the nondamaged hemisphere into the ischemically damaged side of the brain. These results allude to the involvement of transplants in the reorganization of host nerve fiber connections within the injured brain.

2.4. Increased neovascularization

Increased vascularization in the penumbra within a few days after stroke is associated with neurological recovery and offers another potential target for cell therapy (Chen et al., 2003). Transplanted HUCB cell-induced blood vessel formation has been reported by Taguchi et al. (2004). The findings of this study demonstrated that systemic administration of human cord blood-derived $CD34^+$ cells to immunocompromised mice subjected to stroke 48 hours earlier induces neovascularization in the ischemic zone. Behavioral recovery from stroke and injury following delivery of human cord blood-derived $CD34^+$ cells was found, but in the absence of significant number of grafted cells entering brain. These results suggest an essential role for $CD34^+$ cells in promoting directly or indirectly an environment conducive to neovascularization of ischemic brain so that neuronal regeneration can proceed.

In addition, transplanted stem cells from HUCB, as well as bone marrow (Chen et al., 2003; Shen et al., 2007), might increase endogenous levels of other factors such as BDNF, VEGF, FGF, and SDF-1 that could induce proliferation of existing vascular endothelial cells and mobilization with homing of endogenous endothelial progenitors.

2.5. Attenuation of inflammation

An intriguing repair mechanism is the ability of transplanted cell to attenuate the stroke-induced inflammatory/immune response. After stroke and intravenous injection of HUCB cells, there was a decrease in CD45/CD11b- and CD45/B220-positive (+) cells in the brain (Vendrame et al., 2005). This decrease was accompanied by a decrease in mRNA and protein expression of pro-inflammatory cytokines and a decrease in nuclear actor κB (NF-κB) DNA binding activity in the brain, although it is not clear whether this is a direct effect on the inflammatory response or secondary effect attributable to a reduction in infarct size. It is paradoxical that a xenotransplant would inhibit the immune response. However, there is evidence from the recent study by same researchers showing that HUCBC treatment rescued

the spleen weight, splenic $CD8^+$ T-cell counts, as well as the amount of brain injury (Vendrame et al., 2006). Additionally, splenocyte proliferation assays demonstrated that HUCBC treatment opposed MCAO-associated T-cell proliferation by increasing the production of IL-10 while decreasing IFN-γ (Vendrame et al., 2006). These results suggest that in the experimental model of stroke, HUCB cells can indirectly or directly inhibit T-cell activation.

2.6. Recruitment of endogenous progenitors

Endogenous neurogenesis is increased after stroke (Taguchi et al., 2004; Chen et al., 2003; Zhang et al., 2004). The function of this has yet to be determined but may correspond to a natural repair mechanism of the brain that could potentially be further enhanced by transplanted cells. The report by Taguchi A et al. (2004) showed that systemic administration of HUCB-derived $CD34^+$ cells to immunocompromised mice subjected to stroke 48 hours earlier induces neovascularization in the ischemic zone and also provides a favorable environment for neuronal regeneration. Endogenous neurogenesis, suppressed by an antiangiogenic agent, is accelerated as a result of enhanced migration of neuronal progenitor cells to the damaged area, followed by their maturation and functional recovery. In addition to local effects on the damaged tissue, transplanted cells from HUCB could potentially attract different progenitor cell types from other tissues possibly by releasing cytokines, such as human interleukin-8 (IL-8), monocyte chemoattractant protein-1(MCP-1), cytokine-induced neutrophic chemoattractant-1(CINC-1), which may aid in the recovery of the injured brain (Newman et al., 2006). As mentioned above, transplanted cells could mobilize endogenous endothelial progenitors into the circulation to enhance vascularization. However, whether transplanted cells enhance endogenous hematopoietic or mesenchymal cell mobilization after ischemia remains poorly understood.

The endothelial cell proliferation in the stroke brain coincides with a robust recruitment of neuronal progenitor cells from the neurogenic sites subventricular zone and dentate gyrus. Even in the absence of any therapeutic intervention, the ischemic insult recruits neuronal and endothelial progenitor cells, and triggers neurogenesis and angiogenesis (Teng et al., 2008; Liu et al., 2007; Liu et al., 2007; Chen et al., 2005), which likely represent an endogenous compensatory mechanism against stroke. Of note, cell transplantation amplifies these endogenous repair mechanisms (Zacharek et al., 2007; Chen et al., 2003; Zhang et al., 2004; Chen et al., 2003).

With all the aforementioned studies, unequivocal conclusions on whether the neuroprotective or neurorestorative mechanism being measured is a primary or a secondary effect of transplanted HUCB cells warrants additional studies. The full characterization of these mechanisms, either acting singly or in tandem, will allow development of strategies that are designed to optimize the functional outcome of HUCB transplantation in stroke.

3. Lab-to-clinic translation of systemic delivery of HUCB cells in stroke

Despite a growing number of studies demonstrating that peripherally administered HUCB cells could improve recovery from stroke, the factors responsible for the success of this cell therapy have not been elucidated. Researchers have used different cell lines from cord

blood, transplanted at varying times after stroke, and employed a battery of behavioral tests to assess the efficacy of the transplant (see Table 1). Because of these uncontrolled variables, the optimal condition for HUCB cell transplant therapy in stroke. Here we discuss some critical issues that need to be considered for further preclinical development of systemically transplanting HUCB cell therapy in stroke.

3.1. Timing of transplantation

The optimal time to transplant HUCB after stroke is not known. The brain environment changes dramatically over time after ischemic injury. In the acute phase there is an increase in excitatory amino acid release, peri-infarct depolarization, and reactive oxygen species release (Dirnagl et al., 1999). This is followed by an inflammatory/immune response and cell death, which, in the penumbra, can last up to several weeks. Brain repair and plasticity after the acute phase take place over several weeks to months.

Depending on the mechanism underlying the therapeutic benefits of HUCB, the timing of transplantation will need to be adjusted to enhance the potential for this cell therapy in treating stroke. If a treatment strategy focuses on neuroprotective mechanisms, acute delivery of the HUCB cells will be critical. Most of the studies examining the transplantation of HUCB have systemically delivered the cells within 12 to 72 hours (Chen et al., 2001; Willing et al., 2003a, b; Taguchi et al., 2004; Vendrame et al., 2004; Borlongan et al., 2004a; Xiao et al., 2005; Boltze et al., 2005; Lobel DA et al., 2003; Nystedt et al., 2006). In a vitro study by Newman et al. (2006), HUCB cells migrate toward the extract of ischemic brain in a temporal pattern. Here, the critical factor postulated in determining the extent of migration was the time period when brains were harvested after stroke in the rats. The optimal time was determined to be from 48 to 72 hours in both hippocampal and striatal ischemic tissues, producing peak levels of cytokine-induced neutrophil chemoattractant-1 and monocyte chemoattractant protein-1 at 48 hours after stroke. These results suggested that thei three-hour therapeutic window for the treatment of stroke victims, using the approved anticoagulant treatment, may be extended with the use of HUCB cell therapy extending the window up to 24–72 hours after stroke. If the cells acted to enhance endogenous repair mechanisms (e.g., plasticity, angiogenesis, and neurogenesis) or required these events in order to survive and integrate, then early delivery would be pertinent because these events are most prevalent in the first two to three weeks after ischemia. If cell survival is important, then transplanting late, after inflammation has subsided, could be beneficial, in that delayed administration may possibly increase cell survival and more specifically target brain repair mechanisms (e.g., synaptic formation, neuronal circuitry restoration).

A systemic analysis of transplantation timing and its effect on functional recovery was done by Chen et al. (2001). This study showed that intravenous delivery of HUCB cells at 24 hours after stroke significantly improved functional recovery. Treatment with HUCB cells at seven days after stroke resulted in functional recovery only on neurological, but not motor test. A significantly higher number in surviving HUCB cells was detected in the presence of ischemic cerebral tissue when treatment is initiated at 24 hours than at seven days after stroke. These data suggested that early treatment with HUCB cells might promote HUCB cell migration into ischemic brain and facilitate functional recovery after stroke. The

literature reports a wide timing interval between stroke and transplantation, demonstrating functional recovery when transplantation was performed within the first three days after ischemia (Chen et al., 2001; Willing et al., 2003a,b; Taguchi et al., 2004; Vendrame et al., 2004; Borlongan et al., 2004; Xiao et al., 2005; et al., 2005; Lobel et al., 2003; Nystedt et al., 2006). However, therapeutic benefits have not been reported with systemic delivery of HUCB cells at longer post-stroke delay (i.e., more than seven days after injury). At this time, based on the scientific evidence, acute stroke patients appear to be the target population for systemic HUCB therapy. We wish to caution, however, that the time course of neurological recovery remains up for debate, in that it is not clear whether rodents and humans recover at different rates after a stroke. We also do not have firm evidence that a time window of opportunity in a rodent differs from a human. To this end, a careful examination of translating rodent studies into clinical application is warranted.

3.2. HUCB migration to the stroke brain: The status of the blood-brain barrier

Theoretically, the recruitment of systemically transplanted HUCB cells to the site of tissue damage is thought important for the treatment of stroke. In a previous study by Chen et al. (2001), a significant number of HUCB cells were found in ischemic brain. However, in a recent study by Nystedt et al. (2004), rats subjected to transient or permanent ischemic stroke, then 24 hours after intravenously transplanted with HUCB cells displayed no detectable HUCB cells when assessed at 25 day after transplant. Despite the absence of surviving HUCB cells in the stroke brain, the transplanted rats exhibited improvement in the use of impaired forelimb, with a trend toward better performance in the water-maze task. Another study by Mäkinen (2006) reported similar results in that HUCB cells did not improve functional recovery or histological outcome in stroke rats after systemic administration because of limited migration of cells into the ischemic brain. These findings raise the concern that while the stroke brain has long been perceived as presenting with a compromised blood brain barrier (BBB), the injury may not be that conducive for attracting systemically injected cells such as HUCB to hone towards the damaged site. Indeed, this less favorable feature of the BBB was implicated in the observed low survival of cells at damaged site and only partial behavioral recovery in stroke rats systemically transplanted with HUCB cells (Shoichet & Winn, 2000).

In our desire to circumvent the BBB, we investigated the use of the BBB permeabilizer, mannitol (1.1 M), to enhance intracerebral versus intra-arterial injection of HUCB cells within one hour after stroke (Lobel et al. 2003; Borlongan et al., 2004). The intracerebral transplantation of HUCB cells reduced the volume of the infarct and ameliorated neurobehavioral deficits in stroke rats. In the intra-arterial groups, only those animals that received the BBB permeabilizer had a reduced infarct size. Elevated levels of GDNF were found in both the intracerebral transplanted group and the intra-arterial transplanted group that received the BBB permeabilizer. Quantitative histological analysis determined that the volume of infarction was reduced by 37%in animals receiving mannitol plus intra-arterial HUCB cells and by 30% in those receiving mannitol plus intra-striatal HUCB cells. Levels of GDNF were elevated by 88% and 51%, respectively, in these groups. Stroke induced motor deficits were reduced by 18% following intra-striatal and by 15% following intra-arterial HUCB cell delivery. These studies revealed the possibility that modulation of the

BBB could be used to enhance the rescue of ischemic penumbra and the behavioral improvement from stroke deficits produced by systemically delivered HUCB cells.

3.3. Subsets of mononuclear cells within HUCB

The population of cells within cord blood has been used analyzed by the expression of CD antigens coupled with self-renewal proliferation assays, which have been historically used to delineate hematopoetic stem and progenitor cells from the other cellular components of cord blood. Cells positive for CD 34 (a type I transmembrane glycophosphoprotein) are routinely used to identify and sort cells. CD 34 is believed to identify hematopoetic stem cells (Cardoso et al., 1993), but the expression of this protein alone does not reliably distinguish hematopoetic stem cells from progenitor cells. A more reliable sorting technique to isolate and identify hematopoetic stem cells uses combinations of cell surface markers. For example, $CD34^+$ positive cells co-express $CD38^+$ are a more committed progenitor cells (Erythroid and granulocyte) (Mitsui et al., 1993). Although CD34 have been extensively used to identify hematopoiec stem and progenitor cells, studies have suggested there may be subset of stem and progenitor cells within cord blood that are CD- and lineage-(Goodell et al., 1997; Osawa et al., 1996). It is worth mentioning that within the hematopoietic stem cell population there is a subset of $CD133^+$ cells that co-express strongly with CD34 bright cells (Miraglia et al., 1997; Yin et al., 1997; Potgens et al., 2001). In vitro studies confirmed that $CD133^+$ positive cells are a more primitive hematopoietic progenitor/stem cells than the $CD34^+$ positive and lineage-negative cell of HUCB (Yin et al., 1997; Potgens et al., 2001; Uchida et al., 2000). $CD133^+$ positive cells are consistently found in 90–95% of neurosphere-derived cells, whereas these cells express neither CD34 nor CD45 (Uchida et al., 2000). However, the importance of this cell type in relation to cell transplantation requires further investigation.

A vis-a vis transplant study on CD34 positive and negative cells was done by Boltze et al. (2005), comparing the efficacy of these cell subsets following their systemic injection between eight to 10 hours after permanent stroke in spontaneously hypertensive rats. The results demonstrated that $CD34^+$ cells were nearly as effective as $CD34^-$ cells in that both groups of transplanted animals displayed a significant improvement in neuromotoric function. Immunocytochemical analysis showed fluorescence dye labeled HUCB cells in the ipsilateral hemisphere of lesion but these human cells did not express a neuronal phenotype in the damaged brain of animals receiving either $CD34^+$ or $CD34^-$ cells. However, recent reports demonstrated that $CD34^+$ cells might be a more effective fraction of HUCB cells to promote functional recovery through angiogenesis and neurogensis (Vendrame et al., 2004; Shyu et al., 2006). Additional studies are needed to clearly reveal the therapeutic effects of HUCB, either as an unsorted cell population, or whether harvesting the subsets of CD34 positive or negative cells is required to achieve the optimal functional outcome.

3.4. Lesion location and size

Lesion location and size appears as another important factor in determining efficacy of HUCB cells transplantation. To date, most experimental studies showing cell-enhanced recovery used a stroke model that damages the striatum (with some damage extending to the cortex), and the cells are often injected into striatum or by systemic route. Only a few

studies have investigated cell therapy for lesions that primarily damage the cortex, and most of these have used primary fetal tissue blocks, with varying efficacy (Dunnett et al., 1987; Gates et al., 2000). Cortical lesions involving the white matter are more problematic. A proliferation of transplanted cells in the cortex may not repair underlying axonal damage. At this time, there is little evidence to support cell therapy in patients with pure white matter infarcts, which may require an entirely different therapeutic strategy (Bliss et al., 2007), since most of the transplant studies have used a stroke model with lesion mostly limited to the gray matter. Accordingly, a direct comparison between the two types of lesions is required before a conclusive statement can be made.

Precise anatomic location of the lesion, and its functional implication, as well as lesion size, will be critical determinants to define the therapeutic efficacies of transplanted cells in the stroke animal, as well as in establishing the criteria for selecting stroke patients as candidates for stroke therapy. When contemplating with intracerebral delivery of cells, the size and extent of infarction involving major arterial territories will play a significant role in patient selection, in that targeting the brain region should avoid damaging the arterial supply. In a systemic delivery approach, however, the proximity of the ischemic region to an artery may be beneficial in that cells will likely use this blood supply to migrate into the brain. In both intracerebral and peripheral transplant routes, the ideal scenario is for a limited number of cells and/or their secreted growth factors to reach the ischemic area. With this in mind, stroke patients with widespread brain damage will require a large number of grafted cells to restore function. This cell dose controversy, including the need to extrapolate from animal to human brain size and weight, or to base on the extent of stroke volume, location and size, has been debated over many years that needs to be resolved if cell therapy is to be pursued in the clinic.

3.5. Immunosupression of HUCB transplant recipients

Intracerebral and intravenous delivery of HUCB cells with or without chronic immunosupression using cyclosporine A (CSA) decreased infarct volume and led to behavioral improvement (Willing et al., 2003a,b; Vendrame et al., 2004; Xiao et al., 2005). These data suggested that immunosuppressive therapy may not be necessary for HUCB cells to exert their therapeutic benefits. The immaturity of cord blood cells has been postulated as the reason for this low incidence of graft-versus-host diseases (Gluckman et al., 1997). However, to date, there exists no study examining the effects of immunosuppression, or lack thereof, in the long-term following HUCB transplantation in stroke. The incidence of graft-versus-host diseases remains uncertain. Indeed, one recent study by Kozłowska et al. (2007) showed there is a possibility of development of a severe adverse host reaction to alien donor cells after intra-cerebral delivery of HUCB cells, even when co-administered CSA. The authors reported that while HUCB cells were robustly detected at one week after stroke, only minimal HUCB cell survival was apparent at one month post-injury. Moreover, acute rejection of grafted cells was recognized despite CSA immunosuppression. These observations warrant additional studies in characterizing the immunological response evoked by HUCB transplantation in stroke. Furthermore, equal considerations need to be given to immunossupressants, such as CSA and methylprednisolone, which by themselves have been shown to exert neuroprotection against ischemia (Akdemir et al., 2005).

3.6. Effective HUCB dose

Intravenously delivered HUCBC have been previously shown to improve functional recovery of stroked rats at a cell dose of 2×10^5 to 5×10^7 (Sanchez-Ramos et al., 2001; Ha et al., 2001; Bicknese et al., 2002; Buzanska et al., 2002; Chen et al., 2001;Willing et al., 2003a,b; Taguchi et al., 2004; Vendrame et al., 2004; Borlongan et al., 2004; Xiao et al., 2005). In one study (Vendrame et al., 2004), transplantation of HUCBC at 24 hours after a permanent stroke in rats revealed that the dose of 10^6 or more is the threshold to promote functional recovery. Infarct volume was also shown to depend on the HUCBC dose, but requiring more cells (10^7 cells) to obtain robust reduction of the histological damage. Surviving HUCBC cells were detected by immunohistochemistry and PCR analysis only in the injured brain hemisphere and spleen. These results revealed that the HUCBC dose directly impacted on both behavioral and histological benefits in the stroke model.

4. Advantages of HUCB as donor cells for transplantation

Numerous reports have outlined the several advantages of HUCB as cell source for transplantation therapy. First, these cells are easily accessible in unlimited supply without jeopardizing the mother or infant, avoiding logistical and ethical concerns (Newman et al., 2003 and 2004). Second, cryopreservation does not seem to affect capacities of proliferation or differentiation of the stem or progenitor cell from cord blood, even stored for 15 years (Broxmeyer et al., 2003). Interestingly, cryopreserved cord blood cells may allow better transduction of retroviral vectors more than fresh cells (Orlic et al., 1997). Third, HUCB yields higher numbers of hematopoeitic progenitor cells with a better proliferation rate and expansion potential than adult bone marrow (Hows et al., 1992). Fourth, HUCB has a low incidence of graft versus host disease when compared to that of adult bone marrow, even in children that received one antigen HLA-mismatch (Wagner et al., 1992; Wagner et al., 1995). As noted above, the immaturity of cord blood cells may lead to their low rejection rate in the transplant recipient (Vaziri et al., 1994; Gluckman et al., 1997), thereby circumventing the need for chronic immunosuppression. Fifth, the option to possibly deliver the cells peripherally and still afford therapeutic benefits in the stroke brain allows a minimally invasive procedure for cell therapy. Indeed, systemic administration of HUCB cells has a successful clinical history in the hematopoietic field (Lu et al., 1996; Newman et al., 2003 and 2004).

5. Genetic modification of HUCB to enhance trophic factor secretion

As discussed above, HUCB has the capacity to secrete trophic factors, such as GDNF, FGF, NGF, and BDNF (Taguchi et al., 2004; Borlongan et al., 2004a, b; Lobel et al., 2003; Horita et al., 2006; Nomura et al., 2005; Pisati et al., 2007; Zhu et al., 2005; Yasuhara et al., 2006; Cairns et al., 2003), supporting the hypothesis that the delivery of these growth factors to the stroke brain greatly influences the HUCB functional effects. Based on this trophic factor-mediated action of grafted cells, previous studies have explored genetically modifying the donor cells to enhance growth factor secretion in an effort to further improve the beneficial outcome of cell therapy. For example, transplantation of human bone marrow stromal cells genetically modified to secrete BDNF has been shown effective in experimental stroke (Horita et al., 2006). Along this line, our group is currently investigating the potentially

improved benefits of HUCB $CD133^+$ cells genetically modified to secrete GDNF over non-manipulated HUCB $CD133^+$ cells when delivered intravenously in spontaneously hypertensive rats after transient ischemic stroke. The long-term goal is to eventually regulate the secretion of GDNF in HUCB cells following transplantation; from a basic science standpoint, this will provide insights into the direct role of the trophic factor in cell therapy, but more importantly from a translational view, the modulation of cell therapy during the disease progression should improve HUCB safety and efficacy outcomes. We acknowledge, however, the risks of uncontrolled regulation of the gene, including tumor or ectopic tissue formation, associated with genetic manipulation of HUCB as opposed to using cells alone. In the end, utmost consideration must be given on the risks and benefits of enhancing HUCB's therapeutic potential via gene delivery versus the risks entailed with introducing a gene into the cell without the proper control mechanism for regulating gene expression.

6. Perspective

The systemic delivery of HUCB cell transplantation therapy for stroke holds great promise. However, the impressive benefits documented in the studies described above were obtained from a single dose and at a single time point post-stroke. Due to the low survival rate of cells at the damage or injury site and partial behavioral recovery, strategies need to be developed to further improve the beneficial effects of HUCB transplantation in stroke. The optimization of cell number, therapeutic window and repeated dosing, and identification of more potent and selective subsets of HUCB cells and BBB agents, are likely to lead to greater histological and behavioral benefits. Furthermore, long-term studies are required to determine whether the cell-enhanced recovery is sustained and also to determine any adverse effects, i.e., turmorigenic potential of the cells. Standardization of outcome parameters, especially for characterizing the functional outcome, is also needed for direct comparisons between studies. The translation of HUCB to the clinic requires further laboratory investigations.

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Table 1

BBBP: Blood-brain barrier permeabilizer; EBST: elevated body swing test; GFAP & FVIII: markers for astrocytes and neuronal progenitors; HUCB: human umbilical cord blood; IA: intra-artery delivery; IC: intra-cerebral delivery; IV: intravenous delivery; Lin: lineage negative; MCAO: middle cerebral artery occlusion; NeuN, MAP-2 and MAB1281: neuronal markers; ND, not determined; PBPc: peripheral blood progenitor cells; NSS: neurological severity score; PT: post-transplantation; SHR: spontaneously hypertensive rat; CsA. Cyclosporine 10 mg/kg, i.p., daily throughout test period

Reference	Cell type	Stroke model	Time of delivery	Dose and route	Effect on lesion size	Survival of HUCB in the brain	Phenotype	Functional recovery
Chen et al., 2001	HUCBC used immediately after thawing	Transient MCAO in adult male rats	24 h or 7 days after MCAO	3×10^6 IV	Decreased	14 and 35 day PT, but more cells in 24 h than 7 day group	Some cells positive for NeuN, MAP-2, GFAP & FVIII	Motor neurological severity scores improved but rotarod recovery seen in 24h group only
Willing et al., 2003a	HUCB used immediately after thawing	Permanent MCAO adult male rats	24 h after MCAO	$1.1.1 \times 10^6$ IV + CsA $2.2.5 \times 10^6$ IC + CsA	ND	2 mos PT more cells from IC group when compared to IV	Human nuclear staining did not clearly demonstrate HUCB	Both groups had functional improvement on a number of behavioural tests, including EBST
Willing et al., 2003b	HUCB and PBPC used immediately after thawing	Permanent MCAO in adult male rats	24 h after MCAO	1×10^6 IV + CsA	ND	ND	ND	Improved spontaneous activity and motor symmetry (EBST)
Taguchi et al., 2004	$CD34^+$ vs $34CD^-$ HUCB cells	Permanent MCAO in SCID mice	48 h after MCAO	5×10^5 CD 34+ vs CD 34^- cells IV	Modest but significant increase in cortical thickness in $CD34^+$ transplanted mice	ND	ND	Locomotion, rearing, and startle behaviours normalized in CD+34, but not CD34− transplant recipients
Borlongan et al., 2004	HUCB used immediately after thawing+mannitol (BBBP)	Transient MCAO in adult male rats	During occlusion	2×10^5 HUCB cells IV	Decreased at day 3	No labeled cells found between 1h and day 3.	ND	Recovery on EBST and passive avoidance test, only seen with HUCB+ mannitol
Xiao et al., 2005	HUCB	Permanent MCAO in adult rats	48 h after MCAO	1×10^7 HUCB cells IV	Decreased	Human nuclei-positive cells found	Co-labeled with neuronal marker NeuN	Improved behavioral performance
Vendrame et al., 2005	HUCB used immediately after thawing	Permanent MCAO in adult rats	24 h after MCAO	1×10^4 up to 3 to 5×10^7 HUCB IV	Decreased at the higher HUCB doses	Human nuclei-positive cells found	Co-labeled with neuronal	All dose (except 1×10^4) improved behavioral performance at 4 weeks PT

Reference	Cell type	Stroke model	Time of delivery	Dose and route	Effect on lesion size	Survival of HUCB in the brain	Phenotype	Functional recovery
							marker NeuN marker NeuN	
Boltze et al., 2005	$CD34^+$ vs $34CD^-$ HUCB	Permanent MCAO in SHR	8 to 10 h after MCAO	1×10^6 CD 34+ vs CD 34− IV	ND	HUCB were found at 29 day in both groups PT	Human neuronal marker (NF-L) negative	Improved rotarod, beamwalk and NSS in both groups at 4 weeks PT
Nystedt et al., 2006	$CD34^+$ HUCB cells	Permanent and transient MCAO in adult rats	24 h after MCAO	5×10^5 CD 34+ cells IV or 2×10^6 CD 34+ IV	Not decreased in both models	HUCB not detected	ND	Improved in hindlimb use and a trend of recovery in water-maze performance
Mäkinen et al., 2006	HUCB sorted as mononuclear or Lin negative	Transient MCAO in adult rats	24 h after MCAO	$1–5 \times 10^7$ HUCB IV	ND	Sporadic HUCB found	ND	No improvement in a range of motor and cognitive tasks