Molecular and Cellular Neuroscience 59 (2014) 76–84

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

Monocytes are essential for the neuroprotective effect of human cord blood cells following middle cerebral artery occlusion in rat

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ARTICLE INFO

Article history:
Received 26 November 2012
Revised 17 January 2014
Accepted 20 January 2014
Available online 25 January 2014

Keywords:
Cord blood
Cerebral ischemia
Monocytes
Behavior
Infarct
Microglia

ABSTRACT

Systemic administration of human umbilical cord blood (HUCB) mononuclear cells (MNC) following middle cerebral artery occlusion (MCAO) in the rat reduces infarct size and, more importantly, restores motor function. The HUCB cell preparation is composed of immature T-cells, B-cells, monocytes and stem cells. In this study we examined whether the beneficial effects of HUCB injection were attributable to one of these cell types. Male Sprague Dawley rats underwent permanent MCAO followed 48 h later by intravenous administration of HUCB MNC preparations depleted of either $CD14^+$ monocytes, $CD133^+$ stem cells, $CD2^+$ T-cells or $CD19^+$ B cells. Motor function was measured prior to MCAO and 30 days post-stroke. When $CD14^+$ monocytes were depleted from the HUCB MNC, activity and motor asymmetry were similar to the MCAO only treated animals. Monocyte depletion prevented HUCB cell treatment from reducing infarct size while monocyte enrichment was sufficient to reduce infarct size. Administration of monocyte-depleted HUCB cells did not suppress Iba1 labeling of microglia in the infarcted area relative to treatment with the whole HUCB preparation. These data demonstrate that the HUCB monocytes provide the majority of the efficacy in reducing infarct volume and promoting functional recovery.

Introduction

Despite great progress in the prevention, diagnosis and understanding of the pathophysiological mechanisms of stroke, it is the fourth leading cause of death and the leading cause of disability worldwide (Roger et al., 2011). Still, our advancement toward developing new therapeutic agents has been limited. Currently, only the thrombolytic agent, tissue plasminogen activator (TPA), is approved by the U.S. Food and Drug Administration (FDA) for the acute (urgent) treatment of ischemic stroke, which accounts for 85% of all strokes. TPA is only effective within 4.5 h of the onset of stroke and resolves ischemia by dissolving the clot. With this narrow therapeutic window, only 2–3% of all stroke patients are able to benefit from the use of TPA.

Cell therapy has garnered attention over the last 20 years, and could substantially expand the treatment window. The earliest studies used fetal tissue to examine the ability of transplanted cells to repair stroke-damaged brain by replacing the dead neurons (Mampalam et al., 1988; Tonder et al., 1989). The first cell therapy to reach clinical trials for a treatment of lacunar ischemic strokes was the hNT or LBS neurons, a cell-line developed from a teratocarcinoma (Kondziolka et al., 2000). Since that time much of the focus has been on stem cell therapies encompassing embryonic, neural (and other somatic stem cells), and, more recently, induced pluripotent stem cells (see (Sladek and Bjugstad, 2011) for a recent commentary). Our understanding of the repair mechanisms that underlie the therapeutic benefits associated with cell therapy have evolved from simple neural repair to include trophic support (Kern et al., 2011), inhibition of inflammation (Yang et al., 2010), as well as stimulation of angiogenesis and endogenous neurogenesis (Taguchi et al., 2004).

The first published report of HUCB MNC intravenous administration as a treatment for experimental stroke was by Chen and associates (Chen et al., 2001). They found that delivering 3×10^6 cells 24 h post-MCAO significantly improved motor function, as determined with the modified neurological severity score (mNSS) and rotorod tests, while having little effect on infarct size. Since that time there have been a number of reports demonstrating that HUCB cells can repair damaged brain in rodent models of cerebral hypoxia and ischemia. We found that systemic administration of these cells significantly decreases infarct size, and reduces the pro-inflammatory cells and cytokines associated with stroke (Hall et al., 2009; Jiang et al., 2010; Leonardo et al., 2010; Vendrame et al., 2005). Systemic administration is the preferable route, producing more sustained behavioral improvements compared to direct intraparenchymal administration (Willing et al., 2003). This was confirmed by another research group that showed that HUCB cells do not need to enter the CNS to produce their reparative effects

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http://dx.doi.org/10.1016/j.mcn.2014.01.004

(Borlongan et al., 2004). When delivered systemically at 48 h following MCAO, these cells have their optimal effect on decreasing infarct volume and enhancing behavioral recovery (Newcomb et al., 2006; Vendrame et al., 2004). This work has been replicated by other research groups (Boltze et al., 2006, 2011). Other studies have focused on $CD34^+$ hematopoietic stem cells from HUCB as the active cell type (Boltze et al., 2008; Liu et al., 2006; Taguchi et al., 2004). Although both $CD34^+$ and $CD34^-$ HUCB cells had similar behavioral effects, neither were as effective as MNC (Boltze et al., 2012). Still others have focused on developing HUCB derived neural cell lines (Kozłowska et al., 2007; Xiao et al., 2005) for the treatment of stroke.

We have routinely employed the MNC fraction of HUCB in our studies. It is a mixed population of cells composed predominantly of immature T-cells, B-cells, monocytes, and stem cells. Although we have repeatedly shown the neuroprotective and anti-inflammatory effects of HUCB cells following MCAO, it is unclear which of the cell populations within the MNC is responsible for these effects. All of the major components of the HUCB MNCs are immunologically immature and do not respond to stimulation in the same way mature T cells, B cells or monocytes respond; as such they may passively interfere with the ongoing inflammatory responses that occur after MCAO. Alternatively, their more limited repertoire of responses may skew their responses toward one aspect of the cells' function. For example, HUCB derived monocytes are not as responsive to stimulation with hepatocyte growth factor (Jiang et al., 2001), interleukin (IL)-4, or granulocyte and macrophage colony stimulating factor (GM-CSF) (Liu et al., 2001) and therefore produce fewer dendritic cells than adult monocytes as well as producing less IL-1β, tumor necrosis factor alpha (TNF-α) and adhesion molecules (Brichard et al., 2001; Jiang et al., 2001; Le et al., 1997). As we begin to develop a HUCB-based cell therapy for stroke, it becomes even more important to characterize the active component of the cell preparation in order to provide only those cells that are necessary for the anti-stroke effect, thereby enhancing the therapeutic potency and reducing potential adverse reactions. Therefore, in this study, specific cellular components of the HUCB MNC were depleted (monocytes ($CD14^+$), stem cells ($CD133^+$), T-cells ($CD2^+$) or B cells ($CD19^+$)) and the ability of the resulting cell preparations to induce behavioral recovery and minimize infarct size was measured in male Sprague Dawley rats after MCAO. $CD133^+$ stem cells were examined , instead of the $CD34^+$ hematopoietic stem cells studied by other groups, because these cells can give rise to both hematopoietic and non-hematopoietic cells. Moreover, administration of CD14 enriched fractions confirmed the effects of this specific cell population on infarct size.

Results

Removing $CD14^+$ monocytes from the HUCB MNC reversed the behavioral improvement observed with MNC administration

Measurement of spontaneous activity was performed during the 12 hour dark phase of the light cycle when the animals were most active using an automated open field system from Accuscan Instruments. The animals were tested both prior to MCAO and then at 1 month after the surgical procedure. The data are expressed as percent of baseline. In Fig. 1, we present activity measured on four parameters — horizontal activity (Fig. 1A), total distance traveled in the horizontal plane (Fig. 1B), total distance traveled around the outside edges of the test cage (margin distance; Fig. 1C) and clockwise rotation (Fig. 1D). After MCAO, there was an increase in activity above baseline levels on all measured variables, consistent with previous observations (Block and Schwarz, 1996; Borlongan et al., 1995a,b; Newcomb et al., 2006; Poignet et al., 1989; Puurunen et al., 1997; Willing et al., 2002). There were no significant differences between the MCAO group and the adult human peripheral blood (HPB) group on any parameters. Administration of HUCB MNC reduced horizontal activity significantly ($p < 0.05$) but only marginally reduced activity on all other parameters examined. Animals that were treated with CD14 depleted MNC were as active as untreated MCAO animals on all activity measures and significantly more active than MNC treated animals ($p < 0.05$). Removal of the CD133 cells resulted in a significant increase from the amount of horizontal activity observed compared to the MNC group ($p < 0.05$). Removal of the $CD19^+$ B cells produced variable results, tending to increase horizontal activity and clockwise rotation but having little effect on total distance or margin distance.

The Step test of motor asymmetry was used to measure function of the contralateral forelimb. After MCAO, the number of steps taken with the contralateral paw in the MCAO only group was 33.0 ± 9.2% of the baseline number of steps (Fig. 2). Administering HPB cells actually reduced the number of steps taken even further (11.9 ± 12.2%, $p < 0.05$ compared to MCAO only). With administration of MNC, the percentage of steps taken marginally improved to 50.6 ± 9.6%. The number of steps taken significantly decreased in the CD14 depleted group (28.4 ± 5.8%) compared to the MNC treated group ($p < 0.05$). Removal of the CD2, CD133 and CD19 cells from the MNC did not alter performance on the Step test compared to the MNC treated group.

Removal of $CD14^+$ monocytes from the HUCB MNC increased infarct size after MCAO

Infarct size was determined using Nissl thionin histology (Fig. 3). There was extensive damage in the lateral striatum and overlying cortex after MCAO (Fig. 3A). Administration of HPB did not reduce infarct size (Fig. 3B, 3H). The lateral ventricle in the infarcted hemisphere was enlarged and there was some loss of striatal architecture. Infarcts were visible in all HUCB treated groups (Fig. 3C–G); all had ongoing degenerative processes as illustrated with the Nissl thionin staining and various degrees of ventricular enlargement and loss of tissue. When we analyzed infarct size, there was a significant increase in infarct size in the groups treated with CD14 depleted MNC compared to the group that received the whole MNC fraction ($p < 0.05$).

Administration of $CD14^+$ monocytes replicates the effect of HUCB MNC on infarct size after MCAO

To provide further evidence for the role of $CD14^+$ monocytes in the HUCB MNC effects, we injected a cohort of animals with either HUCB MNCs, HUCB $CD14^+$ cells or HPB $CD14^+$ cells (Fig. 4). Infarct size was decreased equally in all cell transplant groups and these groups had significantly smaller infarcts than the MCAO only group.

IBa1 labeling of cells in the infarcted hemisphere

We have previously shown that HUCB MNC modify the inflammatory response to MCAO, as indicated by changes in the number and morphology of myeloid cells (microglia, monocytes or macrophage) (Leonardo et al., 2010) and altered cytokine profile (Vendrame et al., 2005). In this study we used an antibody to IBa1 to determine if there were long term changes in these myeloid cells in response to the MCAO and HUCB treatment. Microglia within the contralateral hemisphere of the untreated MCAO only animals exhibited a resting morphology, with a small cell body and long branching processes (Fig. 4A). In the untreated MCAO animals even 30 days post-MCAO, there were many IBa1 positive (+) cells in the border zone around the infarct as well as within the core of the infarct (Fig. 5B). The cells in this region exhibited all three “microglial” morphologies — small and ramified (resting), bushy and ramified (partially activated state) or amoeboid (phagocytic/inflammatory state). In the HPB treated animals, there were many amoeboid and bushy ramified microglia within and around the infarct site (Fig. 5C). Consistent with the earlier studies, rats treated with HUCB MNC exhibited microglia with a resting phenotype throughout the infarcted hemisphere (Fig. 5D). When we examined sections from the CD14 depleted group, however, there was