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Biodistribution of Infused Human Umbilical Cord Blood Cells in Alzheimer's Disease-Like Murine Model

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Abstract

Human umbilical cord blood cells (HUCBCs), a prolific source of non-embryonic or adult stem cells, have emerged as effective and relatively safe immunomodulators and neuroprotectors, reducing behavioral impairment in animal models of Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, traumatic brain injury, spinal cord injury, and stroke. In this report, we followed the bioavailability of HUCBCs in AD-like transgenic PSAPP mice and nontransgenic Sprague–Dawley rats. HUCBCs were injected into tail veins of mice or rats at a single dose of 1×10^6 or 2.2×10^6 cells, respectively, prior to harvesting of tissues at 24 h, 7 days, and 30 days after injection. For determination of HUCBC distribution, tissues from both species were subjected to total DNA isolation and polymerase chain reaction (PCR) amplification of the gene for human glycerol-3-phosphate dehydrogenase. Our results show a relatively similar biodistribution and retention of HUCBCs in both mouse and rat organs. HUCBCs were broadly detected both in the brain and several peripheral organs, including the liver, kidney, and bone marrow, of both species, starting within 7 days and continuing up to 30 days posttransplantation. No HUCBCs were recovered in the peripheral circulation, even at 24 h posttransplantation. Therefore, HUCBCs reach several tissues including the brain following a single intravenous treatment, suggesting that this route can be a viable method of administration of these cells for the treatment of neurodegenerative diseases.

Keywords

Human umbilical cord blood cells (HUCBCs); Biodistribution; Polymerase chain reaction (PCR); Mice; Rats; Neurodegenerative diseases

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INTRODUCTION

Alzheimer's disease (AD), the most prevalent progressive form of age-related dementia, is pathologically characterized by the deposition of amyloid-β-peptide (Aβ) as amyloid plaques in the brain parenchyma as well as neurofibrillary tangles within neurons. Aβ is known to be produced via the amyloidogenic pathway, involving the sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases within the cell membrane (24,25,30). Due to widespread cellular atrophy that occurs in both cortical and subcortical brain regions, AD patients suffer cognitive and emotional dysregulation that progresses to an inability to independently and safely perform acts of daily living (ADL) (21,22). The dementia patient population, according to the World Alzheimer Report 2010, has reached a record high of 35.6 million and is expected to increase to 65.7 million by 2030 and 115.4 million by 2050. Development of more effective treatments or prophylaxes is therefore crucial.

Previous studies from our laboratory and others have shown that multiple small intravenous administrations of human umbilical cord blood cells (HUCBCs), specifically cells derived from the mononuclear fraction (HUCBMNCs), reduce cognitive impairment, Aβ levels, β-amyloid plaques, amyloidogenic APP processing, and reactive microgliosis in mouse models of AD (PSAPP and Tg2576 mice) (4,13,20). At a single high dose, HUCBC treatment can even extend the life span of AD mice over-expressing human Swedish APP695 (7). Additional studies indicate that HUCBCs improve therapeutic outcomes in rodent models of Parkinson's disease (PD) (6), amyotrophic lateral sclerosis (ALS) (11,12), types 1 and 2 diabetes (8,9), lupus (10), traumatic brain and spinal cord injury (5,11,18), and stroke (3,11). While the therapeutic consequence of these HUCBCs is of unquestionable medical merit, it is equally important to characterize which organs these cells migrate to and how long they maintain a presence in these tissues. In this report, we followed the biodistribution of HUCBCs following a single intravenous treatment in PSAPP mice and non-transgenic Sprague–Dawley rats. Elucidating the homing proclivity and longevity of HUCBCs following this route of administration is useful for elucidating its potential effectiveness and mechanism of action in the treatment of neurodegenerative disorders.

MATERIALS AND METHODS

Animals and Institutional Approvals

All procedures described herein were in accordance with the animal protocol approved by the University of South Florida (USF) Institutional Animal Care and Use Committee. PSAPP transgenic mice and Sprague–Dawley rats, both at 7 months of age, were obtained from the Jackson Laboratory (Bar Harbor, ME, USA), maintained on a 12-h light/12-h dark cycle at ambient temperature and humidity and allowed standard rodent chow and water ad libitum. Saneron used de-identified cord blood donations from commercial sources for the processing of the U-CORD-CELL™.

HUCBC Preparation

HUCBCs (95–98% mononuclear cells) were graciously donated by Saneron CCEL Therapeutics Inc. (Tampa, FL, USA). Briefly, the mononuclear fraction of HUCBCs (U-CORD-CELL™) was obtained from donated cord blood using a Ficoll-Paque density gradient solution (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) and stored at −210°C. Prior to transplantation, HUCBCs were thawed at 37°C for 4 min, washed in phosphate-buffered saline (PBS), assessed for viability (>93%) using the trypan blue (Sigma-Aldrich, St. Louis, MO, USA) exclusion method (hemocytometer), and suspended in PBS to achieve a final desired cell concentration of $1.0–2.2 \times 10^6$ cells per 100 µl.

HUCBC Infusion

Seven-month-old PSAPP mice ($n = 22$; 11 males, 11 females) or nontransgenic Sprague–Dawley rats ($n = 22$; 11 males, 11 females) were randomly injected in the right tail vein with HUCBCs at 1.0×10^6 or 2.2×10^6 cells/100 µl, respectively, or 100 µl PBS (Fisher Scientific, Pittsburgh, PA, USA) as control. At 24 h, 7 days, or 30 days after injection ($n = 6$ for each time point, $n = 4$ control), animals were anesthetized with pentobarbital (Sigma-Aldrich) (50 mg/kg), and the hindlimbs and peripheral blood were harvested for determination of HUCBCs in bone marrow and blood. Transcardiac perfusion with ice-cold PBS was then performed, followed by harvesting of the liver, lung, spleen, kidney, heart, spinal cord, brain, and gonads. These organs were immediately snap frozen in liquid nitrogen for polymerase chain reaction (PCR) analysis for the presence of human DNA.

PCR Analysis

The presence of human DNA in murine and rodent tissues was used as a screen to determine the biodistribution of HUCBCs. Total purified DNA was obtained from tissue samples using a DNeasy Blood and Tissue kit (Qiagen, Valencia, CA, USA) and quantified using a NanoDrop 2000C spectrophotometer (ThermoFisher, Wilmington, DE, USA). DNA was replicated using nested PCR techniques (total volume 25 µl), specifically for the presence of the human glycerol-3-phosphate dehydrogenase (HG3PDH) gene using appropriate primers (sense: 5′-GGCTGGGACTCATGGAGAT-3′ and antisense: 5′-CG GGTAAGTCGTTGA-GAAAG-3′) (28) An additional nested PCR was performed with primers (sense: 5′-TC TTGGAGAGCTGTGGTGTTG-3′ and antisense: 5′-GT TACCTGAAAGGACTGC-3′) specific to the product of the first PCR reaction to further account for any nonspecific binding and PCR products (28). DNA presence in the various organs was resolved using a 1% agarose gel (Invitrogen, Grand Island, NY, USA), stained with ethidium bromide (Sigma-Aldrich) and visualized using UV translumination.

RESULTS

In order to determine the biodistribution of HUCBCs after a single intravenous injection, the presence of HG3PDH DNA in the blood, bone marrow, brain, spinal cord, spleen, kidney, liver, heart, lung, and gonads was determined at 24 h, 7 days, and 30 days after the HUCBC administration in PSAPP mice and Sprague–Dawley rats. Within 24 h, HG3PDH DNA was observed by PCR analysis in every organ except blood for mice and remained in the bone marrow, brain, kidney, liver, heart, and lungs after 30 days (Table 1). For rats, the human

DNA was observed in the brain, spinal cord, spleen, kidney, liver, and heart within 24 h and remained in the brain, spinal cord, kidney, and liver after 30 days (Table 2). Interestingly, for rats, the human DNA was first detectable in the bone marrow at 30 days and in gonads at 7 days. All animals survived for the entirety of the transplantation periods with no indication of aberrant cell growth or tumor formation. Overall, these results indicate that HUCBCs distribute to several organs, including the brain and spinal cord, within 24 h after a single intravenous injection and can remain in these organs for up to 30 days.

DISCUSSION

In this report, we show that HUCBCs distribute widely throughout the body within 24 h after a single intravenous injection and can remain in several tissues, including the brain, even after 30 days. Since an AD mouse model (PSAPP mice) was used in these studies, data also indicate that peripherally administered HUCBCs offer a viable treatment for this neurodegenerative disease. Previous studies have shown that PSAPP mice treated from 7 months of age with infused bone marrow mesenchymal stem cell (BM-MSC) suspensions biweekly for 1 month exhibited reduced amyloid-β deposition and rescued memory deficits (15). Further studies from our laboratory indicated that multiple monthly low-dose injections of HUCBCs into PSAPP mice reduce cognitive impairment, Aβ levels/β-amyloid plaques, amyloidogenic APP processing, and reactive microgliosis (4). The PSAPP transgenic mouse model of amyloid-β deposition has shown numerous benefits in characterizing the role of Aβ in cognitive impairment and pathogenesis in Alzheimer's disease (16,31). In order to determine the biodistribution of peripherally administered HUCBCs, and thus elucidate their mechanism of action, the present study determined the expression of HG3PDH DNA in several tissues after a single intravenous injection. The results indicate that this route of administration can be sufficient to have a direct therapeutic effect in several tissues, including the brain, for up to 1 month after administration.

Interestingly, we observed the disappearance of HUCBC DNA in the rat kidney at 7 days and reemergence of a positive signal at the later 30-day time point. This disappearance and reemergence of the HUCBC signal is most likely due to the unique properties of these cells. Initially, a small population of HUCBCs migrated from circulation into the kidney and were detectable using PCR techniques, at the 24-h time point. These cells remain proximal to the vessels that they extravasate from and possess the ability to reenter the circulation at later time points (12). Sometime between the 24-h and 7-day time point, a majority of the cells either reemerged into circulation or were degraded in the microenvironment of the kidney. Furthermore, at some point between the 7 days and 30 days, a few of the remaining HUCBCs emerged back into circulation and migrated from the blood into the kidney. This results in the return of a positive signal for the presence of HUCBCs in the rat kidney. Since they do not migrate very far from the vessels, another possibility is that we may simply be detecting the DNA of these cells that have died in the tissue. The dead/dying cells or their DNA may be expelled back into circulation and filtered out by the kidney for clearance. As a result, we may be detecting the HG3PDH DNA from these dead or dying cells.

HUCBCs have been verified as a prolific source of non-embryonic or adult stem cells and have shown therapeutic potential in animal models of AD, multiple sclerosis (MS), ALS,

age-related macular degeneration, PD, traumatic brain injury (TBI), spinal cord injury (SCI), and stroke (3–5,11–14,18,20). HUCBCs can be safely cryopreserved and remain viable for years. Apart from retaining a primitive ontogeny, HUCBCs have not been exposed to immunologic challenge, thus retaining an immunologically immature phenotype (27). It is also reported that HUCBC progenitors have as much as four times as many $CD34^+$ cells and up to an eightfold proliferative capacity compared to similar cells in the bone marrow (1). Moreover, these cells form larger colonies, possess higher cell cycle rates, and have fairly long telomere lengths, confirming their immature status. Furthermore, with greater availability, weak immunogenicity, and reduced probability of arbitrating viral transmission, HUCBCs may represent the best alternative for cell-based therapy (17).

The therapeutic benefits of HUCBC treatment seem to evoke modulation of inflammatory processes both peripherally and centrally (2,23,26). In studies that employed the middle cerebral artery occlusion (MCAO) animal stroke model, it was observed that the spleen is one of the primary vessels of immune cells that become activated and migrate in response to the progression of stroke pathology, eventually leading to cell death in the ischemic penumbra due to chronic inflammation. When HUCBCs are administered after the ischemic MCAO event, the immune cell activation and migration, as well as the massive delayed cell death observed in the penumbra, can be prevented (28,29). In vitro, it was shown that the supernatant from cultured HUCBCs promotes survival of NT2 neural cells cultured under cell stress conditions (5). Moreover, it has been shown in animal studies that HUCBC infusion mediates recovery after brain injury within days (19,23), a time period too short for reinnervation, suggesting transplanted cells may not act at the site of brain injury by rejuvenating new cells, but may confer therapeutic effects through paracrine factors.

In conclusion, we have demonstrated that a single low dose of HUCBCs can be sufficient in promoting therapeutic benefits without the possibility of concomitant aberrant cell growth or tumor formation. However, due to the low dose of HUCBCs administered and eventual loss of these cells in key organs, such as the spleen, the use of multiple low-dose infusions may be necessary to prolong the therapeutic benefit of these cells (4), while still benefiting from a lower chance of ectopic cell growth. The exact mechanism of efficacy with multiple low-dose HUCBC infusions in AD patients remains to be elucidated. In addition, further studies investigating which specific HUCBC cell type(s) and/or possible secreted factors that are capable of modulating the neuroinflammation observed with AD, stroke, ALS, and MS are essential.

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References

1. Broxmeyer HE, Cooper S, Etinne-Julan M, Wang XS, Ponnazhagan S, Braun S, Lu L, Srivastana A. Cord blood transplantation and the potential for gene therapy. Gene transduction using a

recombinant adeno-associated viral vector. Ann NY Acad Sci. 1995; 770:105–115. [PubMed: 8597353]

2. Cairns K, Finklestein SP. Growth factors and stem cells as treatments for stroke recovery. Phys Med Rehabil Clin N Am. 2003; 14(Suppl 1):S135–142. [PubMed: 12625643]
3. Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke. 2001; 32:2682–2688. [PubMed: 11692034]
4. Darlington D, Deng J, Guinta B, Hou H, Sanberg CD, Kuzmin-Nichols N, Zhou HD, Mori T, Ehrhart J, Sanberg PR, Tan J. Multiple low-dose infusions of human umbilical cord blood cells improve cognitive impairments and reduce amyloid-β-associated neuropathology in Alzheimer mice. Stem Cells Dev. 2012; 3:412–421.
5. El-Badri NS, Hakki A, Saporta S, Liang X, Madhusodanan S, Willing AE, Sanberg CD, Sanberg PR. Cord blood mesenchymal stem cells: Potential use in neurological disorders. Stem Cells Dev. 2006; 4:497–506.
6. Ende N, Chen R. Parkinson's disease mice and human umbilical cord blood. J Med. 2002; 33:173–180. [PubMed: 12939116]
7. Ende N, Chen R, Ende-Harris D. Human umbilical cord blood cells ameliorate Alzheimer's disease in transgenic mice. J Med. 2001; 32:241–247. [PubMed: 11563821]
8. Ende N, Chen R, Mack R. NOD/LtJ type 1 diabetes in mice and the effect of stem cells (Berashis) derived from human umbilical cord blood. J Med. 2002; 33:181–187. [PubMed: 12939117]
9. Ende N, Chen R, Reddi AS. Transplantation of human umbilical cord blood cells improves glycemia and glomerular hypertrophy in type 2 diabetic mice. Biochem Biophys Res Commun. 2004; 321:168–171. [PubMed: 15358230]
10. Ende N, Czarneski J, Raveche E. Effect of human cord blood transfer on survival and disease activity in MRL-lpr/lpr mice. Clin Immunol Immunopathol. 1995; 75:190–195. [PubMed: 7704978]
11. Garbuzova-Davis S, Willing AE, Saporta S, Bickford PC, Gemma C, Chen N, Sanberg CD, Klasko SK, Borlongan CV, Sanberg PR. Novel cell therapy approaches for brain repair. Prog Brain Res. 2006; 157:207–222. [PubMed: 17046673]
12. Garbuzova-Davis S, Willing AE, Zigova T, Saporta S, Justen EB, Lane JC, Hudson JE, Chen N, Davis CD, Sanberg PR. Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: Distribution, migration, and differentiation. J Hematother Stem Cell Res. 2003; 3:255–270.
13. Giunta B, Fernandez F, Nikolic WV, Obregon D, Town T, Tan J. Inflammaging as a prodrome to Alzheimer's disease. J Neuroinflammation. 2008; 5:51. [PubMed: 19014446]
14. Henning RJ, Burgos JD, Ondrovic L, Sanberg P, Balis J, Morgan MB. Human umbilical cord blood progenitor cells are attracted to infarcted myocardium and significantly reduce myocardial infarction size. Cell Transplant. 2006; 15:647–658. [PubMed: 17176616]
15. Lee JK, Jin HK, Bae JS. Bone marrow-derived mesenchymal stem cells attenuate amyloid β-induced memory impairment and apoptosis by inhibiting neuronal cell death. Curr Alzheimer Res. 2010; 6:540–548.
16. Lemere CA, Spooner ET, Leverone JF, Mori C, Iglesias M, Bloom JK, Seabrook TJ. Amyloid-beta immunization in Alzheimer's disease transgenic mouse models and wildtype mice. Neurochem Res. 2003; 7:1017–1027.
17. Lewis ID. Clinical and experimental uses of umbilical cord blood. Intern Med J. 2002; 12:601–609.
18. Lu D, Sanberg PR, Mahmood A, Li Y, Wang L, Sanchez-Ramos J, Chopp M. Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. Cell Transplant. 2002; 11:275–281. [PubMed: 12075993]
19. Newman MB, Davis CD, Kuzmin-Nichols N, Sanberg PR. Human umbilical cord blood (HUCB) cells for central nervous system repair. Neurotox Res. 2003; 5:355–368. [PubMed: 14715454]
20. Nikolic WV, Hou H, Town T, Zhu Y, Guinta B, Sanberg CD, Zeng J, Luo D, Ehrhart J, Mori T, Sanberg PR, Tan J. Peripherally administered human umbilical cord blood cells reduce

parenchymal and vascular beta-amyloid deposits in Alzheimer mice. Stem Cells Dev. 2008; 3:423–439.

21. Patterson MB, Mack JL, Neundorfer MM, Martin RJ, Smyth KA, Whitehouse PJ. Assessment of functional ability in Alzheimer disease: A review and a preliminary report on the Cleveland Scale for Activities of Daily Living. Alzheimer Dis Assoc Disord. 1992; 6:145–163. [PubMed: 1485930]
22. Potkin SG. The ABC of Alzheimer's disease: ADL and improving day-to-day functioning of patients. Int Psychogeriatr. 2002; 14(Suppl 1):7–26.
23. Sanberg PR, Willing AE, Garbuzova-Davis S, Saporta S, Liu G, Sanberg CD, Bickford PC, Klasko SK, El-Badri NS. Umbilical cord blood-derived stem cells and brain repair. Ann NY Acad Sci. 2005; 10:855–857.
24. Sinha S, Lieberburg I. Cellular mechanisms of β-amyloid production and secretion. Proc Natl Acad Sci USA. 1999; 96:11049–11053. [PubMed: 10500121]
25. Steiner H, Capell A, Haass C. Proteolytic processing and degradation of Alzheimer's disease relevant proteins. Biochem Soc Trans. 1999; 27:234–242. [PubMed: 10093740]
26. Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Sterm DM, Naritomi H, Matsuyama T. Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest. 2004; 114:330–338. [PubMed: 15286799]
27. Van de Ven MO, Engels RC, Kerstjens HA, Cairo MS. The potential of umbilical cord blood multipotent stem cells for nonhematopoietic tissue and cell regeneration. Exp Hematol. 2007; 12:1753–1765.
28. Vendrame M, Cassady J, Newcomb J, Butler T, Pennypacker KR, Zigova T, Sanberg CD, Sanberg PR, Willing AE. Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. Stroke. 2004; 10:2390–2395.
29. Vendrame M, Gemma C, de Mesquita D, Collier L, Bickford PC, Sanberg CD, Sanberg PR, Pennypacker KR, Willing AE. Anti-inflammatory effects of human cord blood cells in a rat model of stroke. Stem Cells Dev. 2005; 5:595–604.
30. Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, Carter DB, Tomasselli AG, Parodi LA, Heinrikson RL, Gurney ME. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature. 1999; 402:533–537. [PubMed: 10591213]
31. Zhu Y, Hou H, Rezai-Zadeh K, Guinta B, Ruscin A, Gemma C, Jin J, Dragicevic N, Bradshaw P, Rasool S, Glabe CG, Ehrhart J, Bickford P, Mori T, Obregon D, Town T, Tan J. CD45 deficiency drives amyloid-β peptide oligomers and neuronal loss in Alzheimer's disease mice. J Neurosci. 2011; 4:1355–1365.

Table 1

Presence of Human G3PDH DNA in PSAPP Mice

	Presence of Human DNA		
Organs/Tissues	**24 Hours**	**7 Days**	**30 Days**
Blood	−	−	−
Bone marrow	+	+	+
Brain	+	+	+
Spinal cord	+	+	−
Spleen	+	+	−
Kidney	+	−	+
Liver	+	+	+
Heart	+	+	+
Lung	+	+	+
Gonad	+	+	−

PSAPP double transgenic mice were given one single injection of 1×10^6 HUCBCs via the tail vein. After tissue collection, DNA isolation, and PCR replication of human G3PDH DNA was detectable in virtually all organs tested, except blood, 24 h after HUCBC administration. By 30 days, bone marrow, brain, kidney, liver, heart, and lungs were found to contain human DNA, while blood, spinal cord, spleen, and gonad were negative (n = 6 per group).

Table 2

Presence of Human G3PDH DNA in Sprague–Dawley Rats

	Presence of Human DNA		
Organs/Tissues	**24 Hours**	**7 Days**	**30 Days**
Blood	−	−	−
Bone marrow	−	−	+
Brain	+	+	+
Spinal cord	+	+	+
Spleen	+	+	−
Kidney	+	+	+
Liver	+	+	+
Heart	+	−	−
Lung	−	−	−
Gonad	−	+	+

Nontransgenic Sprague–Dawley rats were given a single injection of 2.2×10^6 HUCBCs via the tail vein. Twenty-four hours after injection, human DNA was detectable in the brain, spinal cord, spleen, kidney, liver, and heart, but not in blood, bone marrow, lung, or gonad. By 30 days, human DNA was found in the bone marrow, brain, spinal cord, kidney, liver, and gonad, while the blood, spleen, heart, and lung were negative ($n = 6$ per group).