

Research article

HUMAN ADIPOSE-DERIVED STEM CELLS FOR THE TREATMENT OF INTRACEREBRAL HEMORRHAGE IN RATS VIA FEMORAL INTRAVENOUS INJECTION

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Abstract: Human adipose-derived stem cells (huADSC) were generated from fat tissue of a 65-year-old male donor. Flow cytometry and reverse transcription polymerase chain reaction (RT-PCR) analyses indicated that the huADSC express neural cell proteins (MAP2, GFAP, nestin and β -III tubulin), neurotrophic growth factors (BDNF and GDNF), and the chemotactic factor CXCR4 and its corresponding ligand CXCL12. In addition, huADSC expressed

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Abbreviations used: ADAS – adipose-derived adult stem; ADSC – adipose-derived stem cell; FCS – fetal calf serum; hAdMSC – adipose derived mesenchymal stem cell; hASC – adipose tissue-derived stem cell; hATSC – adipose tissue stromal cell; ICH – intracerebral hemorrhage; MCAO – middle cerebral artery occlusion; MSC – mesenchymal stem cell; SVF – stromal-vascular fraction

the characteristic mesenchymal stem cell (MSC) markers CD29, CD44, CD73, CD90, CD105 and HLA class I. The huADSC were employed, via a right femoral vein injection, to treat rats inflicted with experimental intracerebral hemorrhage (ICH). Behavioral measurement on the experimental animals, seven days after the huADSC therapy, showed a significant functional improvement in the rats with stem cell therapy in comparison with rats of the control group without the stem cell therapy. The injected huADSC were detectable in the brains of the huADSC treated rats as determined by histochemistry analysis, suggesting a role of the infused huADSC in facilitating functional recovery of the experimental animals with ICH induced stroke.

Key words: Adipose-derived stem cells, Adult stem cells, Adipose tissue, Intracerebral hemorrhage, Intravenous stem cell injection, Neural stem cells, Regenerative medicine, Stem cell therapy, Stroke, Stromal cells

INTRODUCTION

Intracerebral hemorrhage accounts for approximately 10 to 15% of all strokes [1], manifests as bleeding in the brain parenchyma (most commonly in the striatum), and is associated with high morbidity and mortality [2]. Current medical therapy for ICH patients is limited, as most patients receive primarily supportive treatment [2] and only selected patients are subjected to surgical treatment [3]. Most ICH survivors are left with profound and persistent disability which causes a substantial economic burden [2]. Following ICH, dynamic and complex processes of multiple mechanisms are involved in the injury. The most important mechanisms involved in ICH injury consist of early hematoma growth, which is the accumulation of toxic blood components [4] that causes mechanical destruction or displacement, and peri-hematoma injury resulting mainly from the inflammation around the blood clot, contributing to delayed neuronal death [5, 6]. Brain edema formation and BBB (blood-brain barrier) disruption, caused by both hematoma and peri-hematoma injuries, have also been reported to be an important component of brain injury after ICH [7-12].

Mesenchymal stem cells generated from murine or human adipose tissues have been well documented [13-22] and have been named as adipose-derived stem cells [14, 15, 19, 20], adipose-derived adult stem cells [21], adipose derived stromal cells [16], adipose derived mesenchymal stem cells [18], adipose tissue-derived stem cells [13], and adipose tissue stromal cells [17]. Systemic transplantation of human adipose-derived stem cells to attenuate cerebral inflammation and to facilitate motor recovery of rats from cerebral degeneration induced by ICH has been attempted [23].

We found previously that transplantation of the Nurr1-positive neuronal stem cells derived from the wisdom tooth of a young adult facilitated a significant recovery from neurological dysfunction in rats with an MCAO induced stroke [24]. We wonder whether the huADSC generated from a senior individual would

be beneficial in the treatment of rats with brain injury resulting from an ICH procedure. We think successful therapeutic effect of a senior's huADSC may mean huADSC from younger individuals would function equally well under similar circumstances. Here we report the therapeutic effect of the huADSC generated from a 65-year-old male donor on treating rats inflicted with induced ICH via a femoral intravenous injection of the stem cells. We selected huADSC from a senior individual in order to evaluate whether adipose-derived stem cells from an old person have potential for stroke therapy.

MATERIALS AND METHODS

Generation of the huADSC from adipose tissue

Fat tissues derived from a cosmetic blepharoplasty procedure performed on a healthy 65-year-old individual, stored at 4°C within 36 hours of collection, were washed with a large quantity of normal saline to remove blood cells and then homogenized in a sterile Petri dish with scissors. In a 50 ml sterile tube, 2 ml of the homogenized fat material was mixed with Medium 199 (Invitrogen, Grand Island, N.Y., USA) supplemented with 20% fetal calf serum (FCS; Biological Industries, Kibbutz Beit Haemek, Israel) in a 1:20 ratio. The mixture was centrifuged at 600 g for 10 minutes to remove lipid droplets and floating debris in the supernatant fraction. The remaining stromal-vascular fraction (SVF) in the pellet was treated with trypsin-EDTA (Gibco, Carlsbad, CA, USA), washed twice by centrifugation with 10 ml of Medium 199 containing 20% FCS and cultured immediately in a 25 cm² tissue culture flask with the same media in a humidified 37°C incubator supplied with 5% CO₂. Every 3 to 4 days, spent culture medium was replaced with fresh Medium 199 containing 20% FCS until the adherent cells reached 85% confluence, at which time the huADSC were ready for characterization, expansion, cryopreservation or cellular therapy.

Reverse transcription PCR

mRNA was extracted from the huADSC at 85% confluence in a 75T flask using the RNeasy kit (Qiagen, GmbH, Hilden, Germany). The mRNA was reverse transcribed using Omniscript RT (Qiagen, GmbH, Hilden, Germany), and 1/10 of the cDNA products was subjected to 30 rounds of amplification (ABI PRISM 9700, Perkin-Elmer/Applied Biosystems) with reaction conditions of 30 PCR cycles (95°C for 30 s, 55°C for 30 s and 72°C for 60 s) after initial denaturation (95°C for 10 min). Controls consisted of amplifications without reverse transcription and reactions without addition of the cDNA template. The authenticity and size of the PCR products were confirmed by gel analysis. The primers used and the size of expected PCR products are listed in Table 1. The mRNA levels were normalized to the housekeeping GAPDH gene.

Table 1. Primer sequences for PCR.

| Primer name | Primer sequence | *Target mRNA and size of PCR product |
|-----------------------|-----------------------------------|--------------------------------------|
| Nurr1 Forward | 5'- ACCCGGGATCTCTCCACAACCTT -3' | NM_006186 |
| Nurr1 Reverse | 5'- CACCCCATTGCAAAAGATGA -3' | 971 bp |
| GAPDH Forward | 5'- CCATGTCGTCATGGGTGTGAACCA -3' | NM_002046 |
| GAPDH Reverse | 5'- GCCAGTAGAGGCAGGGATGATGTTC -3' | 251 bp |
| NFM Forward | 5'- TCTGTAACCGTCACTCAAAAGG -3' | NM_001105541 |
| NFM Reverse | 5'- CCGTTCTGTTTTGAAGCTGCC -3' | 204 bp |
| nestin Forward | 5'- GAGAGGGAGGACAAAAGTCCC -3' | NM_006617 |
| nestin Reverse | 5'- TCCCTCAGAGACTAGCGCAT -3' | 197 bp |
| GFAP Forward | 5'- TCCTCAGGGGAGATGATGGTG -3' | NM_002055 |
| GFAP Reverse | 5'- AAGCCAGCATTGAGTGCCCC-3' | 140 bp |
| βIII tubulin Forward | 5'-TGAACACCTTCAGCGTCGTG-3' | NM_001197181 |
| βIII tubulin Reverse | 5'-TTGAGGGTGCGGAAGCAGAT-3' | 157 bp |
| BDNF Forward | 5'- AAACATCCGAGGACAAGGTG- 3' | NM_170735 |
| BDNF Reverse | 5'- CCTGCAGCCTTCTTTTGTGT-3' | 520 bp |
| GDNF Forward | 5'- CAGTGCTTCCTAGAAGAGAG -3' | NM_000514 |
| GDNF Reverse | 5'- TGTCACTCACCAGCCTTCTA -3' | 267 bp |
| CXCR4 Forward | 5'- ACCAGAAGAACTGAGAAGC -3' | NM_003467 |
| CXCR4 Reverse | 5'- ATTGGGGTAGAAGCGGTCAC-3' | 383 bp |
| CXCL12 Forward | 5'- AACGCCAAGGTCGTGGTCGT -3' | NM_199168 |
| CXCL12 Reverse | 5'- GCTTTCTCCAGGTACTCCTG -3' | 254 bp |
| PPAR γ Forward | 5'- GCTGTGCAGGAGATCACAGA -3' | NM_138712 |
| PPAR γ Reverse | 5'- GGGCTCCATAAAGTCACCAA -3' | 225 bp |

* NCBI accession numbers of the target mRNAs are listed.

Immunocytochemistry analysis

Identification of neural specific antigens was performed as previously described [24]. Briefly, the huADSC, seeded overnight in a 24-well culture plate (1×10^4 /well), were fixed with 10% formaldehyde for 20 minutes at 4°C. Nonspecific binding was blocked with normal serum from the species in which the secondary antibodies were raised (10% in PBS containing 0.25% Triton X-100). The fixed huADSC were incubated with the primary antibodies diluted in PBS containing 0.25% Triton X-100 at 25°C for 2 hours. After rinsing in PBS, the cells were incubated in the dark with FITC- or rhodamine-conjugated secondary antibodies (1:100, Santa Cruz) for one hour at room temperature and observed with an Axiovert 200M (Zeiss) fluorescence inverted microscope. Images were taken and processed with Metamorph (Universal Imaging Co, Ver 6.0 rev 5) equipped with a CoolSnap HQ CCD camera. In the controls, all procedures were processed in the same manner except the primary antibodies were omitted. The

primary antibodies used in this study were as follows: anti-nestin (1:200; Chemicon), anti- β -III tubulin (1:400; Chemicon), anti-GFAP (1:200; Sigma) and anti-MAP2 (1:200; Chemicon).

Adipogenic differentiation of the huADSC

For the adipogenic induction experiment, 1×10^4 huADSC with 1 ml of the induction medium [100 ml low glucose DMEM (Invitrogen, UK) supplemented with 10% FCS, 50 μ g/ml ascorbate-2 phosphate, 10^{-7} M dexamethasone and 50 μ g/ml indomethacin] were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 12 to 14 days. Induction media replacements every 3 to 4 days were performed. Induced adipocytes were stained by Oil O-red to stain lipid droplets.

Osteogenic differentiation of the huADSC

For the osteogenesis induction experiment, 5×10^4 huADSC with 1 ml of the induction medium [45 ml low glucose DMEM (Invitrogen, UK) supplemented with 10% FCS, 50 μ M ascorbate-2 phosphate, 10^{-7} M dexamethasone and 10 mM β -glycerophosphate] were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 21 days. Induction media replacements every 3 to 4 days were carried out as scheduled. Osteogenesis was examined by alizarin S staining for calcium deposition.

Bisbenzimidazole labeling of the huADSC

Twenty-four hours prior to femoral intravenous injection of the huADSC, huADSC culture medium was replaced with 10 ml of fresh Medium 199 containing 10% FCS and 1 μ M bisbenzimidazole (Sigma, U.S.A.).

Induction of ICH and huADSC administration in rats

Experimental protocols on the animal study were approved by the Animal Care and Use Committee of the Buddhist Tzu Chi University. The committee accords with the guidelines set by the National Institutes of Health for care and use of laboratory animals. All efforts were made to minimize suffering of the experimental animals and to reduce the number of animals used. In total, 15 ICH induced male Sprague-Dawley rats (200-250 g) were randomly assigned without bias to two groups: the ICH + vehicle group (6 rats) and the ICH + huADSC treatment group (9 rats). After the rats were anesthetized with chloral hydrate (0.4 g/kg) intraperitoneally, body temperatures of the rats were maintained automatically with a heating pad (CMA-150, CMA Microdialysis, Sweden) at $37.5 \pm 0.5^\circ\text{C}$. The heads of the rats were fixed on a Stoelting stereotaxic instrument (Stoelting Co., Illinois, U.S.A.). ICH induction was performed by microinfusion of bacterial collagenase type VII-S into the striatum of the rats [25, 26]. Briefly, a craniotomy was performed for insertion of a needle (30-G) into the striatum (0.0 mm and 3.0 mm lateral to the bregma, 5.0 mm below the dura surface), with the needle attached to a microinfusion syringe (Hamilton Co., Nev., U.S.A.). Bacterial collagenase (type VII-S; Sigma, MO, U.S.A.; 0.23 U in 1 μ l volume of normal saline) was infused into the striatum at a rate of

0.1 $\mu\text{l}/\text{min}$ for 10 min with an infusion pump (CMA-100, CMA Microdialysis, Sweden). The needle remained in place for another 10 min after completion of the infusion to prevent reflux. The craniotomies were sealed with bone wax at the end of the procedure. The rats were allowed to recover in separate cages at room temperature. They had free access to food and water under a 12-h light-dark cycle. Twenty-four hours after the ICH insult, the huADSC (at 5th to 6th passages; 1×10^6 of cells in 0.2 ml normal saline) were injected slowly for 5 minutes into the blood circulation of the rats, via the right femoral vein, in the ICH + huADSC treatment group. An equal amount of normal saline was administered to rats in the ICH + vehicle group in the same manner.

Neurological deficit evaluation of the experimental rats

Neurological deficit evaluation of the experimental rats was performed, in a blind setting, by a colleague with no knowledge of the experimental animals involved in the vehicle or the therapeutic group. Modified Neurological Severity Score (mNSS) of the rats was determined on day 0 and on day 1, 3, 7, and then weekly for three weeks after the cell administration. Neurological and behavioral functions were graded on a scale of 0-18 (normal score, 0; maximal deficit score, 18). The mNSS was a composition of motor, reflex, balance and sensory tests [27]. The entire procedure for the animal experiment is summarized in Supplemental Fig. 1 in Supplementary material at <http://dx.doi.org/10.2478/s11658-012-0016-5>.

Histochemistry analysis of brain sections of the experimental rats

One ICH treated rat was sacrificed on day 3 and two on day 7 after the huADSC infusion. The rest of the ICH treated rats were sacrificed at the end of the neurological deficit evaluation. The rats were anesthetized with chloral hydrate (Sigma-Aldrich, USA) (0.4 g/kg intraperitoneally) and perfused with 250 ml of normal saline and 200 ml of 4% paraformaldehyde (Sigma-Aldrich, U.S.A.). The brains of the rats were fixed with 4% paraformaldehyde overnight and dehydrated with a 30% sucrose solution for 48 h. The dehydrated brains were embedded in OCT embedding medium and sectioned by cryomicrotome (Leica CM3050S) into 20 μm and 8 μm thick sections for histochemistry staining as follows: the brain sections were rinsed with 1x PBS with 0.3% Triton X-100 and then incubated with propidium iodide (Invitrogen Molecular Probe, U.S.A.; 1 $\mu\text{g}/\text{ml}$) for 5 minutes, followed by rinsing with 1x PBS containing 0.1% Tween-20 and mounting with 70% glycerol. Fluorescence images were observed by confocal laser scanning microscopy (LSM 510; Zeiss) and by fluorescence inverted microscopy.

RESULTS

Generation of the huADSC from adipose tissue

After seeding of the blepharon SVF, initially, the majority of the cells settled to the floor of the tissue culture flask after overnight incubation. In four to five

days, adhering spindle shape fibroblast-like cells resembling MSCs [28] began to appear. Supplemental Fig. 2 shows morphology of the huADSC in tissue culture. However, after subsequent subculture, the huADSC gradually showed a mixture of cells displaying spindle features and cells resembling neural cells with triangular morphology (Supplemental Fig. 3). Spontaneous maturation of the huADSC into neuron-like cells was occasionally found without differentiation induction in the routine tissue culture environment (Supplemental Fig. 4).

Characteristics of the huADSC

Phenotypically, the huADSC expressed immunophenotypes of CD29, CD44, CD73, CD90, CD105, and HLA class I that are characteristic markers of MSCs. The huADSC also expressed CD34 very weakly, as reported by others [29, 30]. The huADSC did not express pan-hematopoietic stem cell marker CD45 or HLA-DR molecules (Supplemental Fig. 5), in agreement with the general criteria for MSCs [31]. Additionally, RT-PCR analysis indicated the expression of intrinsic neurological genes β -III tubulin, nestin, GFAP, NFM, Nurr1, and neurotrophic factors BDNF and GDNF. Furthermore, mRNAs of adipogenic PPAR γ as well as chemotactic factor CXCR4 and its corresponding ligand CXCL12 genes were also detected (Fig. 1). Identification of neural specific

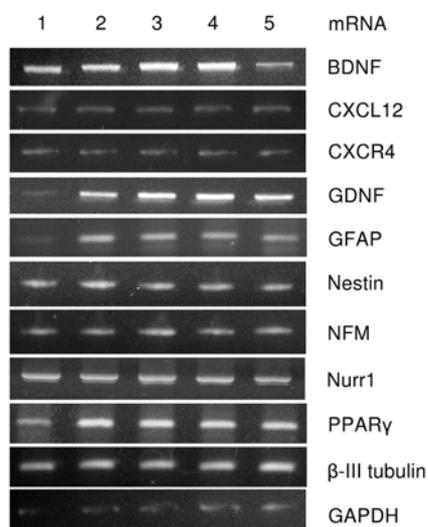


Fig. 1. RT-PCR assay of the huADSC. Five randomized huADSC cell lines were investigated for the presence of mRNAs for cell markers and neural proteins. Visible bands demonstrated on the electrophoresis gel suggest presence of the mRNA for BDNF, CXCL12, CXCR4, GDNF, GFAP, nestin, NFM, Nurr1, PPAR γ , β -III tubulin, and GAPDH.

antigens expressed on the huADSC was demonstrated by immunocytochemistry as shown in Fig. 2. We confirmed our RT-PCR findings that the huADSC express astrocyte specific GFAP protein, neural protein MAP2, and neuronal

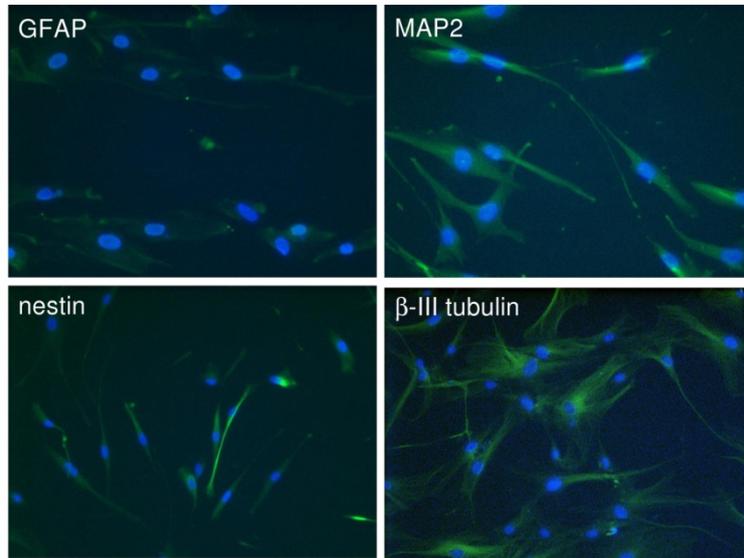


Fig. 2. Immunocytochemistry study of surface proteins expressed on the huADSC. GFAP, MAP2, nestin and β -III tubulin surface proteins expressed on the huADSC were visualized under a fluorescence inverted microscope.

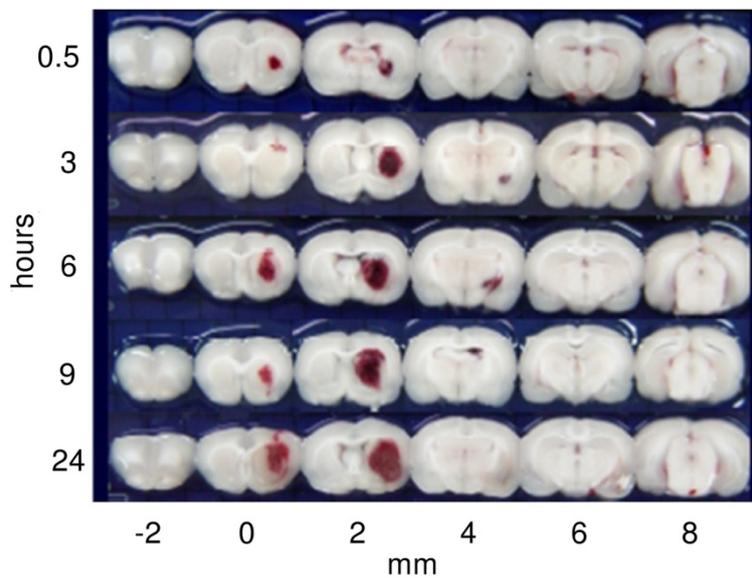


Fig. 3. Representative observations of hemorrhage areas on 2 mm^3 thickness unstained brain sections of rats that were subjected to ICH insult at 0.5, 1, 3, 6 and 24 hours after collagenase injections.

specific molecules nestin and β -III tubulin (Fig. 2). Under the adipogenic induction procedure, some huADSC differentiated into adipocytes and produced lipid droplets as expected (Supplemental Fig. 6). The capacity of huADSC for adipogenesis was also supported by the presence of PPAR γ mRNA by RT-PCR, as shown in Fig. 1. Under the osteogenic induction condition, by the same token, some huADSC were found to generate calcium deposits, as visualized by the alizarin S staining protocol (Supplemental Fig. 7).

Induction of ICH in the experimental rats

Fig. 3 shows brain hemorrhage areas of the rats inflicted with the ICH procedure at different time intervals. The brain hemorrhages caused by the injection of type VII-S collagenase were observed at 0.5 hour to 24 hours after injections. The areas of brain hemorrhage were localized in the striatum of the injected right hemisphere of the ICH induced rats and the lesions expanded from hour 1 and thereafter, with 24 hours after the injection having the largest area of hemorrhage.

Therapeutic potential of the huADSC in the rats induced with ICH

To test the therapeutic potential of the huADSC in the rats with brain injury induced by ICH, twenty-four hours after the insult, the rats received a dose of 1×10^6 cells in 0.2 ml of normal saline via a right femoral vein injection. Comparison of mNSS scores 24 h before and 24 h after the ICH induction indicated that the brain hemorrhage indeed caused neurological and motional deficits in the rats.

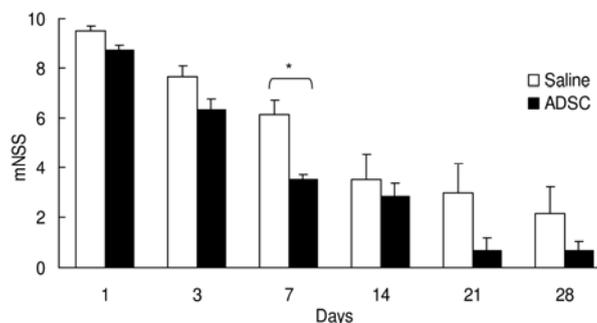


Fig. 4. Comparison of the mNSS (modified neurological severity scores; see text under the Materials and Methods section) on the experimental rats with (■) and without (□) the huADSC therapy at various time points. On day 7, as judged by the mNSS, significant functional improvement was observed in the group treated with the huADSC (6 rats) (■) over the control group (6 rats) without the huADSC treatment (□). * indicates $p < 0.005$.

Fig. 4 shows the mNSS scores in the rats with and without the huADSC administrations at various time points after the cell infusions. Both the therapeutic and the vehicle groups of the ICH induced rats showed functional recovery on day 3, day 7 and each week thereafter to day 28. On day 7, however, the rats with femoral intravenous huADSC injection showed a statistically significant recovery in neurological responses and in motional behaviours as

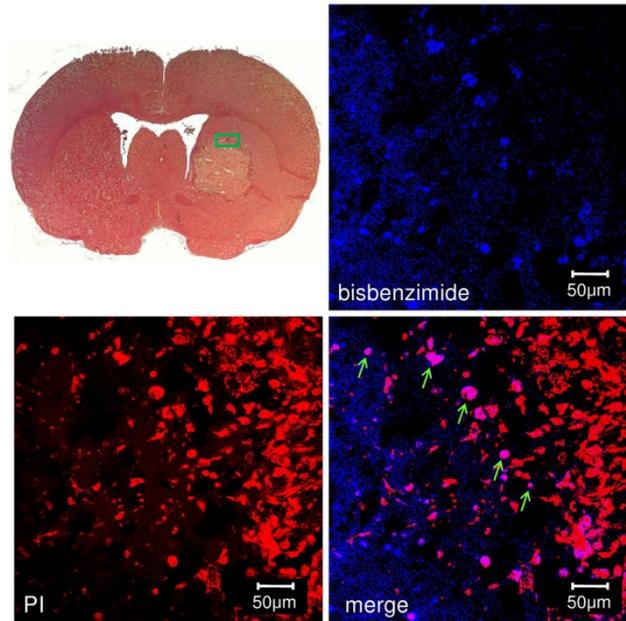


Fig. 5. Confocal microscopy showing detection of infused huADSC in the brain section of the experimental rat. Green rectangular box depicts the area of the section near the hemorrhage lesion where the staining was focused. The presence of huADSC is indicated by the green arrows.

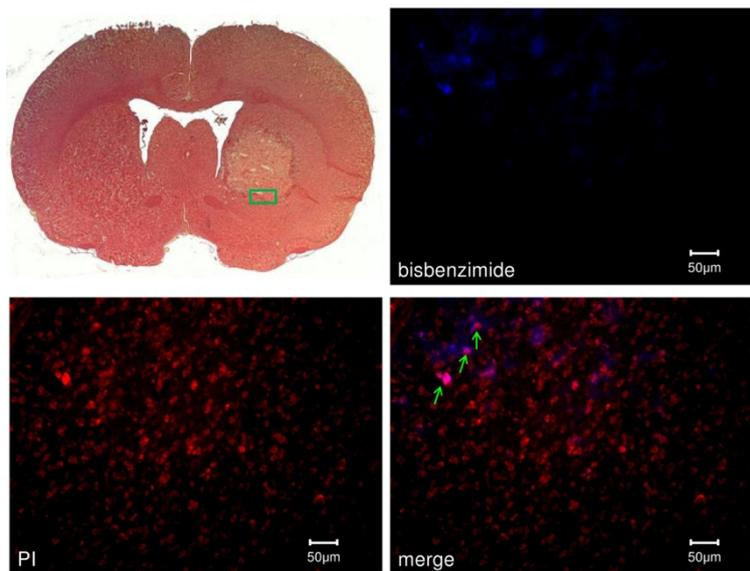


Fig. 6. Fluorescence inverted microscopy showing detection of infused huADSC in the brain section of the experimental rat. Green rectangular box depicts the area of the slide near the hemorrhage lesion where the staining was focused. The presence of huADSC is indicated by the green arrows.

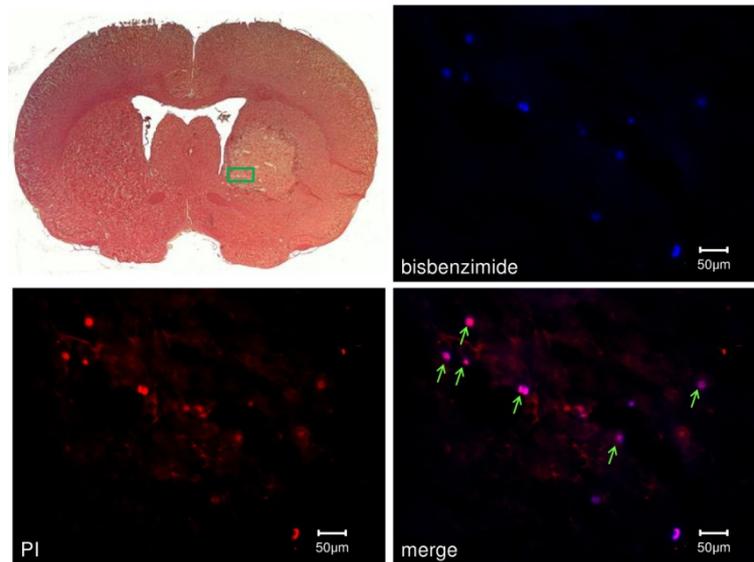


Fig. 7. Fluorescence inverted microscopy showing detection of infused GFAP expressing huADSC in the brain section of the experimental rat. Green rectangular box depicts the area of the slide near the hemorrhage lesion where the staining was focused. The presence of huADSC is indicated by the green arrows.

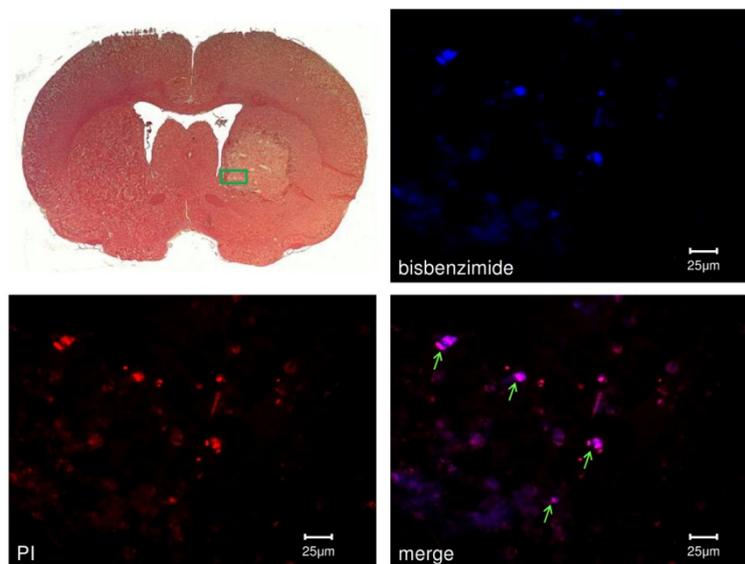


Fig. 8. Fluorescence inverted microscopy showing detection of infused MAP2 expressing huADSC in the brain section of the experimental rat. Green rectangular box depicts the area of the slide near the hemorrhage lesion where the staining was focused. The presence of huADSC is indicated by the green arrows.

determined by the mNSS scores ($p < 0.005$). The huADSC injected via the femoral vein were detectable in the brain sections of the experimental animals by histochemistry analysis using confocal laser scanning microscopy (Fig. 5) and fluorescence inverted microscopy (Fig. 6). Fig. 5 and Fig. 6 depict several bisbenzimidazole labeled huADSC in the brain sections, close to the sites of the hemorrhage areas, with fluorescence in blue light. When merged with propidium iodide stained nuclei in red, the blue and the red lights were superimposed on each other, showing the presence and localization of the infused huADSC. Furthermore, Fig. 7 and Fig. 8 show that huADSC in the brain sections were stained by rhodamine conjugated anti-GFAP and anti-MAP2 antibodies, confirming the presence of the infused huADSC near the hemorrhage sites.

DISCUSSION

The plasticity of stem cells has been explored for therapeutic purposes for several years. Many studies have indicated that human adipose-derived adult stem cells are a population of mesenchymal stem cells and demonstrated differentiation of stem cells into adipocytes, chondrocytes, myocytes, osteoblasts and neural progenitor cells after chemical inductions [14, 16, 18, 20, 32]. Transplantation of matured adipocyte-derived cells and dedifferentiated fat cells to promote functional recovery from spinal cord injury-induced motor dysfunction in rats was reported [20] and an attempt of cellular therapy employing human adipose tissue-derived stromal cells for rats with cerebral ischemia after MCAO resulted in significant functional improvement [17].

We found that the huADSC consist of innate neural stem cells in addition to MSCs based on our observation that the huADSC expressed neural β -III tubulin, nestin, GFAP and MAP2 proteins (Fig. 2) and based on our discovery by RT-PCR analysis of the expression of intrinsic neural genes NFM, Nurr1, β -III tubulin, nestin, GFAP, and neurotrophic factors BDNF and GDNF (Fig. 1). In addition, we demonstrated that the huADSC carry characteristic MSC markers CD29, CD44, CD73, CD90, CD105, and HLA class I (Supplemental Fig. 5). Evidently, the presence of MSCs in the huADSC culture allowed us to show adipogenesis and osteogenesis inductions from the huADSC (Supplemental Fig. 6 and Fig. 7), as reported previously by others [14, 16, 18, 20, 32].

We used the huADSC generated from the fat tissue of a 65-year-old donor to treat ICH inflicted rats and evaluated functional behavior of the rats for 28 days. We found 7 days after the cellular therapy that the stem cell infusion facilitated statistically significant neurological and motional recovery of the rats with brain injury, as evidenced by the significant improvement of the mNSS scores on day 7 ($p < 0.005$) (Fig. 4), although the rats had a natural ability to recover from behavioral deficits gradually after ICH injury without cell therapy. Interestingly, using marrow stromal cells cultured with neurotrophic factor to treat rats with traumatic brain injury (TBI), Mahmood *et al.* also observed functional benefit in TBI rats on day 7 after transplantation of the marrow stromal cells [32].

The injected huADSC were detectable in the brain sections of the ICH treated rats receiving huADSC therapy in histochemistry analysis by confocal laser scanning microscopy and by fluorescence inverted microscopy observations (Fig. 5 – Fig. 8). We assume that the injected huADSC may have functioned in promoting motional recovery of the ICH treated rats by a mechanism that has yet to be precisely elucidated. One of the possibilities is a mechanism involving the effects of neurotrophic growth factors and/or cytokines generated by the endogenous neural stem cells and the MSC in the huADSC. The infused huADSC may be involved in eliciting repair of tissue damage or regeneration of lost nerve cells to facilitate motional improvement of the ICH induced rats, as speculated by other investigators previously [14, 33, 34]. Alternatively, the huADSC may participate in angiogenesis or neoangiogenesis, providing better circulation to regenerate lost brain tissue.

One advantageous property of huADSC in the treatment for brain hemorrhage of rats that we observed in this study is probably the migratory ability of the huADSC whereby the cells were detectable in the brains in areas of the injured sites after the cells were infused into the animals via a femoral vein injection. Intravenous delivery of cells has been asserted to be advantageous due to its ability to cope with high volume infusion and ease of access [35], although it has been questioned regarding its ability to transport a critical number of cells to the area of injury [36]. Another investigation demonstrated that venous administration indeed allows a significant number of cells to reach the traumatically injured brain and to modulate significant functional recovery [37]. The migration ability of the huADSC may be attributed to the chemotactic factor CXCR4 and its corresponding ligand CXCL12 that were expressed on the surface of the huADSC [38]. Undoubtedly, the migratory potential of the huADSC is especially valuable and essential for clinical application of cellular therapy for brain injury or brain diseases when the intravenous route of cell administration is considered in order to avoid injury to brain tissue caused by direct injection of cells into the striatum of patients in clinical trials.

Pluripotent stem cells have gained tremendous interest in recent years, primarily driven by the hope of finding cures for debilitating diseases through cell therapy. While embryonic stem cells have been proven to exhibit unlimited potential, their application is limited by ethical, religious, legal and political concerns as well as the issue of teratoma. Studies have indicated that stem cells are found within adult tissues and organs, but the most accessible and abundant source of adult stem cells is the adipose tissue, which may be acquired through relatively noninvasive procedures. We found that huADSC express the *Nurr1* gene, which is known to be functionally essential for the development and differentiation of midbrain neurons [39]. Therefore, the huADSC expressing neuronal *Nurr1* may be potentially useful in cell therapy to regenerate lost neurons due to brain injuries. In addition, expression of neurotrophic growth factors GDNF and BDNF further suggests the potential of the huADSC in cell-based therapy for neurological diseases. Indeed, the future of adipose-derived stem cells for

treatment of neural diseases and brain injuries, whatever name it is given, warrants further full scale investigations. Lastly, in a future study, we aim to determine whether MSC and neural stem cell populations in the huADSC may be isolated separately. We hope to find out whether each subpopulation would function effectively and independently in ICH cellular therapy and what effect each component would contribute to the behavioral recovery of ICH inflicted animals.

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