

The Potential Therapeutic Cells in Vascular Dementia: IL-1beta Enhanced Endothelial Progenitor-like Cells

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Abstract

Vascular Dementia (VaD) is a neurodegenerative disease caused by vascular lesions that leads to reduced Cerebral Blood Flow (CBF). We investigated the potential of Endothelial Progenitor-like Cells (EPC-like cells) in mitigating dementia symptoms by improving the endothelial remodeling in the ischemic VaD mice. EPC-like cells could be differentiated from CD117 positive bone marrow cells with cytokines, e.g. VEGF, b-FGF, EGF, IL-1 β and IGF-1, and then characterized by surface markers (CD117⁺, CD31⁺, Tie2⁺ and VE-Cadherin⁺) and biological function of tube formation and LDL uptake. The feasibility in treating VaD was assessed by using the carotid artery ligation VaD mouse model. Among EPC-like cells, about 93% of cell population expressed CD117 molecules and 27% of cell population expressed CD31 molecules, which demonstrated the ability for the tube formation and phagocytosis. Half number of dementia mice in disease group was established with increased latency, which could be restored to normal latency with EPC-like cells treatment in another dementia mice group. In conclusion, the EPC-like cells improved memory function of VaD mice, which demonstrated the potential of EPC-like cells in treating VaD.

Keywords: Endothelial progenitor cell; Vascular dementia; Cell therapy; Bone marrow stem cell; CD117

Abbreviations: EPCs: Endothelial Progenitor Cells; VaD: Vascular Dementia; CBF: Cerebral Blood Flow; BM: Bone Marrow; IL: Interleukin

Introduction

Vascular Dementia (VaD) is the second most common cause of dementia after Alzheimer's disease [1]. Reduced Cerebral Blood Flow (CBF) caused by cerebrovascular lesions is a major contributor to the cognitive impairment seen in VaD patients. Current treatments for VaD may be helpful in attenuating the disease progression but are not able to reverse the injury to the brain. Therefore, recovery of cerebral blood flow *via* the repair of injured endothelial tissue could provide a therapeutic strategy to mitigate the brain damage caused by VaD and maintain the memory function in VaD patients.

In 1997, the Endothelial Progenitor Cells (EPCs) from human peripheral blood were reported to mitigate the damaged vascular vessels and promote angiogenesis in ischemic tissue [2]. EPCs could be differentiated from hematopoietic stem cells and characterized by elevated expressions of endothelial lineage markers, such as (Vascular Endothelial Growth Factor Receptor) VEGFR2/Flk-1, Tie2, CD31 (Cluster of Differentiation 31), VE-cadherin, and E-selectin [3,4]. The therapeutic effect of EPCs for treating vascular diseases has been demonstrated in many preclinical studies, including myocardial infarction, critical limb ischemia, and ischemic stroke [4,5].

To study the therapeutic effect of EPCs on VaD, cells were generated from Bone Marrow (BM)-derived stem cells, and then evaluated in an animal model of VaD.

Materials and Methods

Differentiation of BM-derived CD117⁺ cells toward endothelial cells *in vitro*

The male C57BL/6 mice aged 6 to 8 weeks were purchased from BioLASCO (Taipei, Taiwan). The protocol for animal study was approved by the Institutional Animal Care and Use Committee at the Development Center of Biotechnology. The BM-derived mononuclear cells were isolated by a ficoll-paque density gradient centrifugation method (Ficoll-Paque™ PREMIUM, GE Healthcare, PA, USA) after filtration of 40 μ m cell strainer (Corning, NY, USA). The CD117⁺ cells were purified from BM-derived mononuclear cells using the CD117 microbeads combined with a magnetic cell sorting system (Miltenyi Biotec, CA, USA). Purified CD117⁺ cells (3 \times 10⁶ per well) seeded on a fibronectin-coated six-well plate were cultured in basal MCDB131 medium (Thermo Fisher Scientific, MA, USA) containing 10% fetal bovine serum (Hyclone™, UT, USA), 1 μ g/ml ascorbic acid (Sigma, MO, USA), 50 ng/ml hEGF (PeproTech, NJ, USA), 0.5 ng/ml VEGF-165 (BioLegend, CA, USA), 10 ng/ml hb-FGF (PeproTech, NJ, USA), 20 ng/ml R3 IGF-1 (BioVision, CA, USA), and 10 ng/ml IL-1 β (PeproTech, NJ, USA) in 5% CO₂ at 37°C for 29 days.

The expression of cell surface antigens [CD117 (Miltenyi Biotec, CA, USA), CD31, VE-cadherin, and Tie2 (BioLegend, CA, USA)] was quantified by flow cytometry. The *in vitro* angiogenesis activity of differentiated cells was assessed by tube formation as previously described by Hur *et al.* with a slight modification [6]. In brief, cells were cultured in the MCDB131 medium containing 10% FBS, and seeded on BD Matrigel Matrix Growth Factor Reduced (BD Biosciences, NJ, USA). In Dil-Ac-LDL uptake activity assays, cells were incubated with 10 μ g/ml 1,1'-dioctadecyl-3,3',3'-Tetramethylindocarbocyanine-labeled acetylated low-density lipoprotein (Dil-Ac-LDL, Invitrogen, MA, USA), and the uptake

activity was measured as the percentage of fluorescent cells using a fluorescence microscope.

Therapeutic efficacy of IL-1 β enhanced CD117⁺ differentiated cells in a mouse model of vascular dementia

Twelve male C57BL/6 mice aged 6 to 8 weeks were purchased from BioLASCO (Taipei, Taiwan). The protocol for animal study was approved by the Institutional Animal Care and Use Committee at the Development Center of Biotechnology. Mice were randomly divided into three groups: normal, disease, and treatment groups (n=4 per group). In the disease and treatment groups, cerebrovascular cognitive impairment was induced through permanent bilateral common carotid artery ligation to reduce the CBF as previously described by Wang *et al* [7]. The treatment group intravenously received one shot of 3×10^5 IL-1 β enhanced CD117⁺ differentiated cells 3 days after carotid artery ligation. After 32 days of cell treatment, the Morris water maze was used to assess memory function following 3 days of continuous training. The maximum escape latency was recorded as 120 s when latency time was exceeded [8].

Statistical analysis

Data for Morris water maze were expressed as mean \pm SEM and the statistical analysis was compiled with the one-way ANOVA method for the significant difference between groups. A value of $p < 0.05$ was considered statistically significant.

Results

The IL-1 β enhanced CD117⁺ differentiated cells acquired the characteristics of endothelial cells

In this study, purified BM-derived CD117⁺ cells were cultured in a basal differentiation medium comprising a mixture of growth factors and additional IL-1 β for 29 days, and characterized using the expression levels of CD117, CD31, Tie2, and VE-cadherin. The data showed a slight increase in CD117⁺ cell population (79.6% to 92.8%) and dramatic increase in the population of VE-cadherin (0.2% to 72.1%) and Tie2 (4.0 to 34.3%), respectively, in those differentiated cells (Figure 1A). It was also observed that CD31-expressed cells were slightly down-regulated from 31.4% to 26.9%.

In the functional assay, the differentiated cells showed phenomena of vascularization on the Growth Factor Reduced (GFR) matrigel (Figure 1B), and the length of tube formation was measured as 11.3 ± 1.32 mm (mean \pm SD) per field. Around 99% of cells showed the engulfment ability of Dil-Ac-LDL with positive fluorescent cells.

Improvement of memory function in mice with VaD

Surgical ligation of the carotid artery was performed to induce VaD in mice. Using the Morris water maze test, a half number of dementia mice in disease group were observed with maximum escape latency of 120 s that resulted in around 1.7 fold increase in latency (68.6 ± 30.0 s) compared to normal mice (40.0 ± 26.9 s) (Figure 2). Mice treated with the IL-1 β enhanced CD117⁺ differentiated cells showed a lower latency (28.4 ± 6.1 s) compared with the normal group.

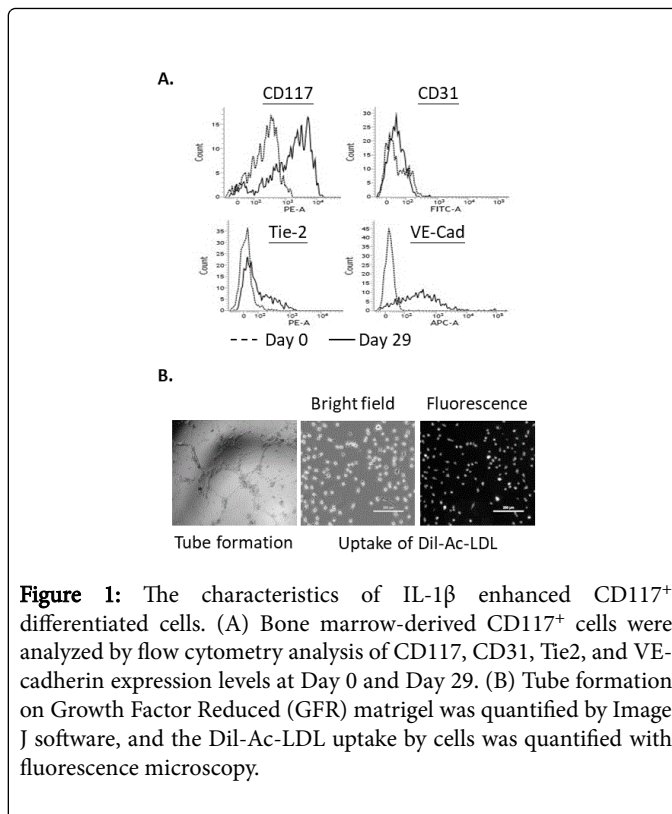


Figure 1: The characteristics of IL-1 β enhanced CD117⁺ differentiated cells. (A) Bone marrow-derived CD117⁺ cells were analyzed by flow cytometry analysis of CD117, CD31, Tie2, and VE-cadherin expression levels at Day 0 and Day 29. (B) Tube formation on Growth Factor Reduced (GFR) matrigel was quantified by Image J software, and the Dil-Ac-LDL uptake by cells was quantified with fluorescence microscopy.

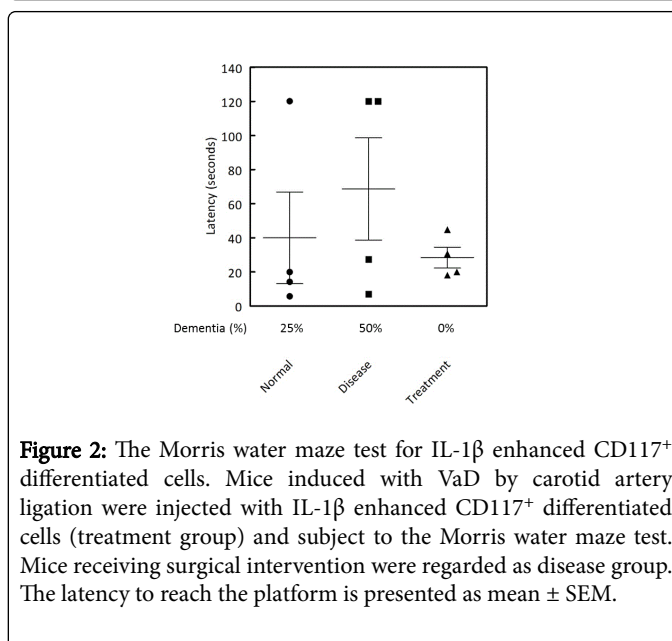


Figure 2: The Morris water maze test for IL-1 β enhanced CD117⁺ differentiated cells. Mice induced with VaD by carotid artery ligation were injected with IL-1 β enhanced CD117⁺ differentiated cells (treatment group) and subject to the Morris water maze test. Mice receiving surgical intervention were regarded as disease group. The latency to reach the platform is presented as mean \pm SEM.

Discussion

In this study, we investigated how the IL-1 β improves the differentiation of EPC-like cells that Tie2 and VE-cadherin were expressed in accordance with the acquired abilities in tube formation and Ac-LDL uptake. In dementia animals, those EPC-like cells could improve the memory of dementia mice in a water maze test. It is suggested that a potential therapeutic strategy for treating dementia could be developed by improving the blood circulation.

The effect of IL-1 β on EPCs had previously been studied *in vitro*, and the data showed that viability, proliferation, colony formation, and angiogenic activity of EPCs could be enhanced in the presence of IL-1 β [9,10]. However, the *in vivo* therapeutic potential of IL-1 β enhanced EPCs was unclear till now. It is evidenced that the IL-1 β enhanced EPC-like cells were observed to improve the memory of dementia mice in a water maze test in our study.

VE-cadherin is an adhesion molecule that plays an important role in the vascularization of the blood vessel [6]. High expression of VE-cadherin in EPC-like cells posed a capable of angiogenesis, which was beneficial to improving the memory of dementia mice in this study. It is deduced that it may be contributed to remodel the vascularization and increase the blood flow to the brain.

According to Alzheimer's Disease International, patients were first diagnosed for dementia in every 3 s [11]. Unfortunately, there are no approved medicines to improve patient's cognitive activity. Here, our preliminary data from dementia mice treated with endothelial progenitor-like cells showed a benefit to cognitive behavior, which might be contributed to a remodeling of injured with vascularization to increase the cerebral blood flow. Some studies have been reported to improve dementia by increasing the blood flow to the brain, for examples, constructed exercises or medicines used for cardiovascular disease also show some benefits in improvement of dementia to support our observation in this study [12-14]. The cause of Alzheimer's disease may also be contributed with low cerebral blood flow that might be regarded as target of improvement [15].

Conclusion

In this study, the improvement of memory in dementia animals had been demonstrated by IL-1 β enhanced EPC-like cells. In conclusion, the vascular damage-caused dementia can be rescued by remodeling the blood vessel of therapeutic cells.

Acknowledgement

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Disclosure of Potential Conflicts of Interest

Authors declare that they have no conflicts of interest.

References

1. O'Brien JT, Thomas A (2015) Vascular dementia. *Lancet* 386: 1698-1706.
2. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, et al. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964-967.
3. Zhao YH, Yuan B, Chen J, Feng DH, Zhao B, et al. (2013) Endothelial progenitor cells: therapeutic perspective for ischemic stroke. *CNS Neurosci Ther* 19: 67-75.
4. Asahara T, Kawamoto A, Masuda H (2011) Concise review: Circulating endothelial progenitor cells for vascular medicine. *Stem cells* 29: 1650-1655.
5. Li YF, Guo G, Cannella LA, Chernaya V, Samuel S, et al. (2015) Endothelial progenitor cells in ischemic stroke: an exploration from hypothesis to therapy. *J Hematol Oncol* 8: 33.
6. Hur J, Yoon CH, Kim HS, Choi JH, Kang HJ, et al. (2004) Characterization of two types of endothelial progenitor cells and their different contributions to neovascularization. *Arterioscler Thromb Vasc Biol* 24: 288-293.
7. Wang J, Fu X, Jiang C, Yu L, Wang M, et al. (2014) Bone marrow mononuclear cell transplantation promotes therapeutic angiogenesis via upregulation of the VEGF-VEGFR2 signaling pathway in a rat model of vascular dementia. *Behav Brain Res* 265: 171-180.
8. Higaki A, Mogi M, Iwanami J, Min LJ, Bai HY, et al. (2018) Predicting outcome of Morris water maze test in vascular dementia mouse model with deep learning. *PLoS One* 13: e0191708.
9. Qin SL, Li TS, Takahashi M, Hamano K (2006) In vitro assessment of the effect of Interleukin-1 β on angiogenic potential of bone marrow cells. *Circ J* 70: 1195-1199.
10. Rosell A, Arai K, Lok J, He T, Guo S, et al. (2009) Interleukin-1 β augments angiogenic responses of murine endothelial progenitor cells in vitro. *J Cereb Blood Flow Metab* 29: 933-943.
11. C Patterson (2018) World Alzheimer Report 2018: The State of the art of dementia research: new frontiers. Alzheimer's Disease International.
12. Barnes JN (2015) Exercise, cognitive function, and aging. *Adv Physiol Educ* 39: 55-62.
13. Ölmestig JNE, Marlet IR, Hainsworth AH, Kruse C (2017) Phosphodiesterase 5 inhibition as a therapeutic target for ischemic stroke: A systematic review of preclinical studies. *Cell Signal* 38: 39-48.
14. Sheng M, Lu H, Liu P, Li Y, Ravi H, et al. (2017) Sildenafil improves vascular and metabolic function in patients with Alzheimer's disease. *J Alzheimers Dis* 60: 1351-1364.
15. Sweeney MD, Kisler K, Montagne A, Toga AW, Zlokovic BV (2018) The role of brain vasculature in neurodegenerative disorders. *Nat Neurosci* 21: 1318-1331.