Neuroscience Letters 450 (2009) 136–141

Contents lists available at ScienceDirect

Neuroscience Letters

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

Bone marrow-derived mesenchymal stem cells reduce brain amyloid-β deposition and accelerate the activation of microglia in an acutely induced Alzheimer's disease mouse model

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ARTICLE INFO

Article history:
Received 25 June 2008
Received in revised form
14 November 2008
Accepted 14 November 2008

Keywords:
Acutely induced Alzheimer's disease model
Bone marrow-derived mesenchymal stem cell
Amyloid-β
Microglia
Transplantation

ABSTRACT

The therapeutic potential of bone marrow-derived mesenchymal stem cells (BM-MSCs) has recently been explored in various pathological conditions of the central nervous system (CNS). However, the application of BM-MSCs in acutely induced Alzheimer's disease (AD) has not yet been reported. Herein the feasibility of using the BM-MSCs, as a therapeutic agent for AD has been tested. To assess this possibility, an acutely induced AD model induced by injecting amyloid-β (Aβ) into the dentate gyrus (DG) of hippocampus of C57BL/6 mice was used. Intracerebral transplantation of BM-MSCs into the brain of an induced AD model reduced their Aβ levels when compared to sham-transplanted animals. The diminution of Aβ deposits was accompanied by the activation of microglia. In addition, the activated microglia was located near the Aβ deposits, and their morphology was changed from ramified to ameboid as a sign of microglial phagocytosis. This study provides evidence that BM-MSCs can promote the reduction of Aβ through the microglial activation in this acutely induced AD brain, suggesting a potential therapeutic agent against AD.

Currently, different sources of adult stem cells are being explored for potential use in repairing neurodegenerative disorders. Among them, the therapeutic potential of bone marrow-derived mesenchymal stem cells (BM-MSCs) has recently been studied in various pathological conditions of the central nervous system (CNS), taking advantage of their high degree of accessibility and plasticity [8,23,24]. Alzheimer's disease (AD) is an age-related progressive neurodegenerative disorder characterized by memory loss and severe cognitive decline. The neuropathological hallmarks of AD include the presence of amyloid-β (Aβ) peptides in the form of amyloid plaques in the brain parenchyma, particularly in the hippocampus and cerebral cortex, leading to neuronal loss. Aβ has been suggested to play an etiological, pivotal and likely causal role in the pathogenesis of AD [7,16]. Therefore, a reduction of brain Aβ would have the potential to prevent and treat AD [22].

BM-MSCs have successfully been used in intracerebral engraftment strategies in animal models with neurodegenerative disorders [3,6]. Our recent studies have also shown that BM-MSCs can be an effective therapeutic vehicle for preventing neurodegenerative disorders [1,2,9]. Therefore, it was hypothesized that BM-MSCs transplantation may have beneficial effects in AD patients. In this study, acutely induced mouse AD models were used to explore the possibility that BM-MSCs might be applied in AD therapy. Interestingly, we found that BM-MSCs promoted microglial activation, and substantially reduced the Aβ deposits of acutely induced AD mice. These results suggest that BM-MSCs may be a useful therapeutic agent against AD.

The acute Aβ-induced model was generated according to a previously described protocol [17,18]. Eight-week-old C57BL/6 male mice were purchased from the Charles River Laboratories Inc., Japan. Briefly, aggregated Aβ was prepared from a solution of 10 mM of soluble Aβ1-42 (Sigma–Aldrich, St. Louis, MO) in 0.01 M PBS, pH7.4. The solution was incubated at 37 °C for 3 days to form the aggregated Aβ and stored at −70 °C. Animals were anesthetized intraperitoneally with a combination of 100 mg/kg ketamine and 10 mg/kg xylazine, and the injection of aggregated Aβ was made bilaterally into the dentate gyrus (DG) of hippocampus using a glass capillary (1.2 mm × 0.6 mm) connected to a microsyringe. The

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doi:10.1016/j.neulet.2008.11.059

animals were subjected to stereotaxic surgery with the incisor bar set at the following coordinates: 2 mm posterior to the bregma, 2 mm bilateral to the midline, and 2 mm ventral to the skull surface. The volume of the injection was 1 μl of aggregated Aβ or 1 μl of PBS. Experimental procedures for handling the mice followed NIH Guideline for Use of Laboratory Animals. Animals were housed in a room maintained under controlled temperature and on a 12 h/12 h light/dark cycle.

Tibias and femurs were dissected from 4- to 6-week-old C57BL/6 mice. Bone marrow was harvested, and single-cell suspensions were obtained using a 40-μm cell strainer (Becton–Dickinson Labware, Franklin Lakes, NJ). Approximately 10^6 cells were plated in 25-cm^2 flasks containing MesenCult™ MSC Basal Medium and Mesenchymal Stem Cell Stimulatory Supplements (StemCell Technologies Inc., Vancouver, Canada) with antibiotics according to our previous reports [1,2,9]. Cell cultures were grown for 2 weeks, and the plastic-adherent population (BM-MSC) was used for subsequent experiments. Most BM-MSCs expressed CD29 and CD90 (Supplemental Fig. 1). In contrast, the majority of adherent cells were negative for CD34, CD45 and CD117. A small fraction of adherent cells expressed CD71.

Mice ($n = 4$ of each group) were anesthetized and fixed into a stereotaxic apparatus (David Koft Instruments, Tujunga, CA) for the BM-MSCs transplantation at 12 days after injection of aggregated Aβ. Two microliters of the cell suspension (approximately 1×10^6 cells) were injected into the DG of the hippocampus bilaterally. The cell suspension was delivered at a rate of 0.5 μl/min. As controls, either 2 μl of PBS or 2 μl of PBS containing 1×10^6 nonviable BM-MSCs rendered by repeated freezing and thawing was implanted. The non-viability of the cells was confirmed by trypan blue staining and failure to observe growth after replating. After transplantation, the scalp was closed by suture and the animals recovered from the anesthesia before they were returned to their cage.

Mice were sacrificed at 7 and 30 days after BM-MSC transplantation. Mice were anesthetized with 2.5% avertin in PBS. Animals were immediately cardiac perfused with 4% paraformaldehyde in PBS. After perfusion, brains were removed, postfixed overnight at 4 °C, and incubated in 30% sucrose at 4 °C until equilibrated. Sequential 30 μm coronal sections were taken on a cryostat (CM3050S; Leica, Wetzlar, Germany) and stored at −20 °C.

To visualize plaques, thioflavin S (Sigma–Aldrich) was applied for 10 min at a concentration of 0.5% dissolved in 50% ethanol. Sections were washed twice with 50% ethanol for 5 min each and once with tap water for 5 min, and mounted with mounting medium. For immunostaining, sections were incubated for 1 h in PBS containing 5% normal goat serum, 2% BSA, and 0.4% Triton X-100. With the use of same buffer solution, the sections were then incubated for 24 h in primary antibodies at 4 °C. Primary antibodies anti Iba-1 were used to detect microglia/macrophages and activated monocytes in the brain sections (rabbit, diluted 1:500, Wako, Osaka, Japan). For visualization, sections were incubated in secondary antibodies for 1 h at room temperature followed by washes. Alexa Fluor 594 (diluted 1:1000, Molecular Probes, Carlsbad, CA) was used as secondary antibody. After final washes in PBS, sections were mounted and cover slipped with Vectashield (Vector Laboratories, Burlingame, CA) for fluorescent microscopic analysis. For double immunofluorescent staining with Aβ and microglia, brain sections were incubated with 0.5% thioflavin S (Sigma–Aldrich) to detect Aβ deposition in 50% ethanol for 10 min.

Sections were rinsed several times in 50% ethanol, distilled water, Tris buffered saline (TBS) with 0.1% Triton X-100, followed by an incubation in TBS containing 0.1% Triton X-100 and 2% BSA for 30 min. Sections were then incubated with primary antibodies and analyzed with a laser scanning confocal microscope equipped with Fluoview SV1000 imaging software (Olympus FV1000, Japan). To quantify the number of Iba-1^+ cells, the images from individual sections were acquired using Olympus FV1000 confocal microscopy equipped with Metamorph 6.2r4 Universal Imaging Corporation (Downingtown, PA). Confocal images were acquired by sequential scanning using a two-frame Kalman filter and a z-separation of 1 μm. The images were then processed to enhance contrast and sharpness using Adobe Photoshop 6.0.

Aβ aggregate-induced mouse AD models were used to explore the possibility that BM-MSCs might be applied for therapy of AD. Aggregated Aβ was injected near the DG of the hippocampus of mice brains, and the injected mice were allowed 12 days to develop AD-like features (Fig. 1A). As indicated by immunostaining with thioflavin S (Fig. 1B), which identified the locations of the aggregated Aβ, the Aβ deposits were formed near the sites of injection. But 42 days after the injection of aggregated Aβ, the deposits of Aβ in all mice were undetectable (Fig. 1B). These results show that injection of pre-incubated Aβ peptides can acutely induce an AD mice model. In this model, aggregates are maintained for 42 days as small clusters of Aβ suggesting that pathological effects might remain for long-term.

To determine whether BM-MSCs can modulate the appearance of the Aβ deposits and activation of microglia, BM-MSCs (1×10^6 cells) were bilaterally transplanted into the DG of the hippocampus of an acutely induced AD model. The mice were sacrificed, and their brains were harvested at 7 and 30 days after transplantation. As shown in Fig. 2A, Aβ injection was able to provoke the activation of microglial cells and the microglia tended to localize near the Aβ plaque (Fig. 2A, especially Aβ/PBS group). To examine whether BM-MSCs treatment increased microglial activation, we analyzed the number of Iba-1 positive cells in acutely induced AD mice in comparison to the control mice (PBS- and dead cell-treated group). Indeed, the animals treated with BM-MSCs exhibited a significant increase of microglial activity compared with control group in pathological Aβ condition (Fig. 2A and E, Aβ/BM-MSC) ($p < 0.05$). In addition, to clarify whether a proportion of Iba-1^+ cells could be derived from transplanted BM-MSCs, we labeled BM-MSC with nanoparticle (0.1 mg/ml, NFP-STEM Silanol TMSR-RITC 50, BITERIALS, Korea) and transplanted TMSR-labeled BM-MSCs into the hippocampus in an acutely induced AD model. After transplantation, we performed Iba-1 immunostaining using brain sections from BM-MSCs, labeled TMSR, transplanted to an acutely induced AD model. We found that Iba-1^+ cells were increased around the BM-MSCs in the hippocampus of mouse AD model. However, the Iba-1^+ cells have not co-localized with BM-MSCs, labeled TMSR (Fig. 2B). This finding demonstrated that BM-MSCs transplantation seems to strongly induce endogenous microglia/macrophage activation rather than differentiation of microglia/macrophages from BM-MSCs. Moreover BM-MSCs transplantation decreased the Aβ deposition in the an acutely induced AD model, throughout the DG of the hippocampus compared with the control (PBS- and dead cell-transplanted group) mice (Fig. 2A, C, D, and F) ($p < 0.05$). Statistical differences among groups were examined using analysis of variance followed by the Tukey HSD test using the SAS statistical package (release 8.1; SAS Institute Inc., Cary, NC) ($p < 0.05$ was considered). We also found that the grafting of BM-MSCs gave rise to the appearance of microglia regardless of pathological condition, i.e.: PBS/BM-MSC group (Fig. 2D and F). However, 30 days after BM-MSCs transplantation, no differences could be found between the transplanted mice and the control mice (data not shown). Thus, BM-MSCs transplantation per se accelerates the activation of microglia and in our AD acutely induced model it reduces the accumulation of Aβ plaques.

It has previously been observed that Aβ were able to lead the microglia to pathogenic sites and BM-MSCs were shown to further induce activation of microglia in Fig. 2. It is necessary to note that