BRAIN RESEARCH 1605 (2015) 49–58

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Intravenous transplantation of bone marrow-derived mononuclear cells prevents memory impairment in transgenic mouse models of Alzheimer's disease

Takuya Kanamaru[a,b], *Naomi Kamimura*[a,*], *Takashi Yokota*[a], *Kiyomi Nishimaki*[a], *Katsuya Iuchi*[a], *Hyunjin Lee*[a], *Shinya Takami*[c], *Hiroki Akashiba*[c], *Yoshitsugu Shitaka*[c], *Masayuki Ueda*[b], *Ken-ichiro Katsura*[b], *Kazumi Kimura*[b], *Shigeo Ohta*[a]

[a]*Department of Biochemistry and Cell Biology, Institute of Development and Aging Sciences, Graduate School of Medicine, Nippon Medical School, 1-396 Kosugi-cho, Nakahara-ku, Kawasaki-shi , Kanagawa 211-8533, Japan*

[b]*Department of Neurological Science, Graduate School of Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan*

[c]*Pharmacology Research Laboratories, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba-shi , Ibaraki 305-8585, Japan*

ARTICLE INFO

Article history:
Accepted 6 February 2015
Available online 16 February 2015

Keywords:
Intravenous transplantation
Mononuclear cell
Amyloid-β
Neurodegeneration
Alzheimer's disease

ABSTRACT

Stem cell transplantation therapy is currently in clinical trials for the treatment of ischemic stroke, and several beneficial aspects have been reported. Similarly, in Alzheimer's disease (AD), stem cell therapy is expected to provide an efficient therapeutic approach. Indeed, the intracerebral transplantation of stem cells reduced amyloid-β (Aβ) deposition and rescued memory deficits in AD model mice. Here, we show that intravenous transplantation of bone marrow-derived mononuclear cells (BMMCs) improves cognitive function in two different AD mouse models, DAL and APP mice, and prevents neurodegeneration. GFP-positive BMMCs were isolated from tibiae and femurs of 4-week-old mice and then transplanted intravenously into DAL and APP mice. Transplantation of BMMCs suppressed neuronal loss and restored memory impairment of DAL mice to almost the same level as in wild-type mice. Transplantation of BMMCs to APP mice reduced Aβ deposition in the brain. APP mice treated with BMMCs performed significantly better on behavioral tests than vehicle-injected mice. Moreover, the effects were observed even with transplantation after the onset of cognitive impairment in DAL mice. Together, our results indicate that intravenous transplantation of BMMCs has preventive effects against the cognitive decline in AD model mice and suggest a potential therapeutic effect of BMMC transplantation therapy.

Abbreviations: AD, Alzheimer's disease; ALDH2, aldehyde dehydrogenase 2; APP, amyloid precursor protein; BMMC, bone marrow-derived mononuclear cell

*Corresponding author. Fax: +81 44 733 9268.
E-mail address: nanakash@nms.ac.jp (N. Kamimura).

http://dx.doi.org/10.1016/j.brainres.2015.02.011
0006-8993/

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease involving senile dementia and is characterized by senile plaques consisting of extracellular amyloid β-peptide (Aβ) deposits in the brain and atrophy of the cerebral cortex or hippocampus (Duyckaerts et al., 2009). According to the amyloid hypothesis, deposition of Aβ in the brain is the primary culprit promoting AD pathogenesis (Hardy and Selkoe, 2002). Recent studies have shown that prior to plaque development, amyloid β-derived diffusible ligands (ADDLs) play a pivotal role in triggering the cognitive deficits and neurodegeneration of AD by specifically targeting synapses and disrupting synaptic signaling pathways (Pozueta et al., 2013; Krafft and Klein, 2010). Over the past decades, intense research has evaluated potential therapies, with numerous clinical trials to evaluate these putative therapies. However, most studies have reported little clinical improvement in addition to various side effects; therefore, current therapeutic strategies for AD (e.g., acetylcholine esterase inhibitors and *N*-methyl-D-aspartate receptor antagonists) typically only relieve the symptoms and do not cure AD.

Stem cell therapies have the potential to be effective because they regenerate and replace cells and tissues themselves. Indeed, clinical trials of stem cell transplantation are presently under way for treatment of ischemic stroke (Honmou et al., 2012; Moniche et al., 2012). It has already been reported that AD symptoms can be mitigated by transplanting stem cells derived from human umbilical cords, amniotic membrane-derived epithelial cells, and mesenchymal stem cells (MSCs) into the brains of AD model animals (Bae et al., 2013; Kim et al., 2012; Lee et al., 2010; Ma et al., 2013; Magga et al., 2012; Njie et al., 2012; Yang et al., 2013b; Zilka et al., 2011). The stem cells were directly transplanted intracranially in these reports. Although direct (intracerebral) injection provides the most efficient delivery of stem cells to the brain, a less invasive and safer technique will be required for clinical application in humans. In addition, MSCs require a long culture duration, thus adding to the difficulty of preparing cells as needed. Considering clinical applicability, in the present study we decided to intravenously transplant bone marrow-derived mononuclear cells (BMMCs) in mouse models of AD. Intravenous transplantation is easier and less invasive than intracranial transplantation, and BMMCs do not require culturing and are easily purified by centrifugal separation (Kamiya et al., 2008). Moreover, BMMCs contain MSCs and other several progenitor white cells, including hematopoietic stem cells and endothelial progenitor cells (Orkin, 2000; Weissman et al., 2001). Another advantage of BMMCs in comparison to MSCs is the heterogeneity of the cell population, which could provide bone marrow-derived growth factors and cytokines that may also regulate cellular growth and regeneration via secreted molecules (Strauer and Ran, 2003).

We previously constructed transgenic (DAL) mice expressing a dominant-negative mutant form of mitochondrial aldehyde dehydrogenase 2 (ALDH2) (Ohsawa et al., 2008), which is an enzyme that protects against oxidative stress by detoxifying toxic aldehydes, such as 4-hydroxy-2-nonenal (HNE), an end product of lipid peroxides. ALDH2 dysfunction is proposed to contribute to AD (Chen et al., 2014; Ohta and Ohsawa, 2006). DAL mice exhibit several AD-like phenotypes including neurodegeneration, cognitive decline, tau phosphorylation, and acceleration of onset with apolipoprotein E (ApoE) accumulation. The phenotype emerges in an age-dependent manner (Ohsawa et al., 2008). Additionally, ALDH2-deficient cells have an increased level of oxidative stress, as shown by oxidation–reduction-sensitive green fluorescent protein (GFP) (Endo et al., 2009). To investigate the preventive effect against neuronal degeneration, we transplanted BMMCs in DAL101 (DAL) mice. Furthermore, to investigate the preventive effect on aggregation of Aβ, we transplanted BMMCs in Tg2576 (APP) mice, where mutated APP leads to the overproduction of Aβ (Hsiao et al., 1996).

2. Results

2.1. *BMMCs are detected in the brains of DAL mice after transplantation*

We first examined whether intravenously transplanted BMMCs can reach the brain. To examine whether BMMCs can immediately arrive at the cerebral parenchyma and cross the blood–brain barrier, we collected the brains of 18-month-old DAL mice 5 min after transplantation and then prepared brain sections or isolated DNA from whole brains. To detect the transplanted BMMCs in the brain, we used BMMCs harvested from transgenic mice that express enhanced GFP (EGFP); many BMMCs (EGFP-positive cells) were observed in the brain sections of BMMC-treated mice (Fig. 1A), and the EGFP sequence was detected by PCR in DNA isolated from the brains of BMMC-treated mice (Fig. 1B). We estimated from the content of the *EGFP* gene relative to the genomic DNA content that the transplanted cells comprised 0.008% of the total host cells. These results show that transplanted BMMCs can cross the blood–brain barrier and reach the brain.

2.2. *BMMCs prevent learning and memory impairment in DAL mice*

To examine whether transplantation of BMMCs has preventive effects against spatial learning and memory impairment in mouse model of AD, we performed a behavioral analysis using a spontaneous Y-maze alternation test (Y-maze). DAL mice present age-dependent cognitive decline and show learning and memory impairment at the age of 12 months Thus, at the age of 9 months, DAL mice received BMMCs intravenously, and the Y-maze test was performed at 12 months of age (Fig. 2B). The alternation rate of BMMC-treated DAL mice was significantly higher than that of vehicle-treated mice 3 months after transplantation (total age of 12 months) (65.9±4.9% versus 59.3±6.5%, $P<0.01$). The performance of BMMC-treated DAL mice was similar to that of wild-type mice (65.9±4.9% versus 66.3±9.7%, $P=0.91$). Next, we examined continuous transplantation because we previously reported that repeated transplantation is more effective than single transplantation in a rat model of ischemic stroke (Kamiya et al., 2014). To examine the effect of continuous transplantation, DAL mice were transplanted with BMMCs again after the first behavioral test and the second Y-maze test was performed at 15 months of age