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Research report

Conditioned medium from the stem cells of human dental pulp improves cognitive function in a mouse model of Alzheimer's disease

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HIGHLIGHTS

- Intranasal administration of SHED-CM improves cognition in a mouse model of AD.
- SHED-CM converts the pro-inflammatory AD environment to an anti-inflammatory one.
- SHED-CM induces the accumulation of M2 microglia in the mouse AD brain.
- SHEDs secrete multiple factors beneficial for the treatment of AD.

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ABSTRACT

Alzheimer's disease (AD) is a progressive, neurodegenerative disease characterized by a decline in cognitive abilities and the appearance of β-amyloid plaques in the brain. Although the pathogenic mechanisms associated with AD are not fully understood, activated microglia releasing various neurotoxic factors, including pro-inflammatory cytokines and oxidative stress mediators, appear to play major roles. Here, we investigated the therapeutic benefits of a serum-free conditioned medium (CM) derived from the stem cells of human exfoliated deciduous teeth (SHEDs) in a mouse model of AD. The intranasal administration of SHEDs in these mice resulted in substantially improved cognitive function. SHED-CM contained factors involved in multiple neuroregenerative mechanisms, such as neuroprotection, axonal elongation, neurotransmission, the suppression of inflammation, and microglial regulation. Notably, SHED-CM attenuated the pro-inflammatory responses induced by β-amyloid plaques, and generated an anti-inflammatory/tissue-regenerating environment, which was accompanied by the induction of anti-inflammatory M2-like microglia. Our data suggest that SHED-CM may provide significant therapeutic benefits for AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease, characterized by the deterioration of cognitive function associated with the deposition of β-amyloid (Aβ) peptides in the brain [1–4]. Aβ peptides are liberated from larger transmembrane amyloid precursor proteins [5] and generate Aβ_{1-42}/Aβ_{1-40} peptides [2–4]. However, the accurate mechanisms of Aβ-induced neurotoxicity and neuroinflammation are not obviously understood. Previous reports showed that a mouse model of an intracerebroventricular (i.c.v.) injection of Aβ1 – 40 peptide significantly impaired memory acquisition, but not memory retrieval, that resembled the episodic anterograde memory deficit observed in the early phases of AD [6,7]. Thus, the single injection of Aβ1 – 40 peptide into the mouse can be an usefulness for the investigation of molecular mechanisms underlying Aβ peptide toxicity, including the microglial activation and oxidative stress, neuroinflammation and synaptic deficits that lead to cognitive impairments [6].

In AD, elevated levels of Aβ peptides form amyloid plaques that induce the differentiation of M1-type pro-inflammatory microglia, which release high levels of cytokines, glutamate, reactive oxygen species [8] and nitric oxide (NO) [2,9–11]. The resulting oxidative

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stress accelerates the generation of 3-nitrotyrosine (3-NT) in neurons, which suppresses catecholamine synthesis and depresses neuro-synaptic transmission [2,9,12,13]. Recent studies indicate that distinct subpopulations of microglia, including the alternatively activated anti-inflammatory M2 microglia, play pivotal roles in the pathophysiology of AD. M2 microglia counteract the M1-mediated pro-inflammatory environment associated with AD, and promote tissue regeneration by secreting anti-inflammatory cytokines [2,5,10,14], scavenging cellular debris, enhancing axonal elongation and promoting neo-vascularization [15]. Thus, treatments that induce M2 differentiation could provide significant therapeutic benefits for AD.

AD mice have been treated by transplanting various types of stem cells and their derivatives, including human neural stem cells [16], embryonic stem-cell derivatives [17], adult bone-marrow stromal cells (BMSCs) [18], adipogenic stem cells [19] and umbilical cord blood-derived stem cells [20], which have elicited substantial functional recovery through cell-replacement and/or paracrine mechanisms. However, most studies indicate that the cell grafts exhibit poor differentiation and survival in mouse models of AD, suggesting that the functional recovery from AD may be mediated by paracrine mechanisms [16,21,22]. Stem cells are known to secrete a broad repertoire of trophic and immunomodulatory factors, which can be harvested in serum-free conditioned media [23,24]. Recent studies have shown that the factors derived from various types of stem cells have the potential for treating a myriad of intractable diseases [16,21,25]. However, the therapeutic effects of these stem cell-derived factors for AD are largely unknown.

Human adult dental pulp stem cells (DPSCs) and stem cells from human exfoliated deciduous teeth (SHEDs) are self-renewing mesenchymal stem cells (MSCs) residing within the perivascular niche of the dental pulp [26,27]. These cells are thought to originate from the cranial neural crest, which expresses early markers for both MSCs and neural stem cells [26–28], and can differentiate into functional neurons and oligodendrocytes under the appropriate conditions [28–30]. The engraftment of these dental pulp stem cells promotes the functional recovery from various acute and chronic CNS insults through paracrine mechanisms that activate endogenous tissue-repairing activities [28,31–36]. Here, we examined the therapeutic benefits of SHED-CM for mouse AD-like model.

2. Materials and methods

2.1. Animals

Male, 9-week-old imprinting control region (ICR) mice (35–37 g) (Nihon SLC Co., Shizuoka, Japan) were used throughout the study. They were housed in a controlled environment (23 ± 1 °C, 50 ± 5% humidity), maintained on a 9:00 a.m.– 9:00 p.m. light cycle, and allowed access to food and water. All experiments were performed in accordance with the Guidelines for Animal Experiments of Nagoya University Graduate School of Medicine. All animal procedures and care conformed to the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

2.2. Treatment and experimental design

The $A\beta_{1-40}$ peptide (obtained from Bachem, Bubendorf, Switzerland) was dissolved in saline as a 1.0 mg/ml stock solution and stored at −20 °C before use. The reverse peptide, $A\beta_{40-1}$ (Bachem) was prepared in the same way and used as a control. Both peptide stock solutions were incubated at 37 °C for 4 days, to allow aggregation prior to administration [37]. The peptides were then administered by intracerebroventricular (i.c.v.) injection as described previously [37–41]. Briefly, a microsyringe with a 28 -gauge stainless-steel needle, which was 3.0-mm long, was used for all peptide injections. The mice were anesthetized lightly with ether, and the needle was inserted unilaterally 1 mm to the right of the midline point, at an equal distance between the eyes and the ears and perpendicular to the plane of the skull. Thereafter, an i.c.v. injection of 5 μl peptide (5 μg) or vehicle was delivered gradually over 3 min. The mice recovered rapidly and within 1 min of the injection exhibited normal behavior. The administration site was confirmed in preliminary experiments, and neither the insertion of the needle nor the volume of injection significantly influenced the survival, behavioral responses, or cognitive functioning of the mice.

2.3. Isolation of SHEDs from deciduous teeth

Human dental pulp tissues were obtained from clinically healthy, deciduous teeth extracted from patients. The ethics committee of the Nagoya University approved the experimental protocols. The SHEDs were isolated and cultured as previously described [16]. Briefly, the pulp was removed gently and digested for 1 h at 37 °C in a solution containing 3 mg/mL collagenase type I and 4 mg/mL dispase. After filtration through 70-mm cell strainers (Falcon; BD Labware), the cells were cultured at 37 °C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 20% mesenchymal cell growth supplement (Lonza, Inc.) and antibiotics (100 U/mL penicillin, 100 mg/mL streptomycin, and 0.25 mg/mL amphotericin B; Gibco). After primary culture, the cells were sub-cultured at 1×10^4 cells/cm^2 and used in experiments after three to five passages.

2.4. Preparation of SHED-CM, BMSC-CM and Fibro-CM

SHEDs, human bone marrow mesenchymal stem cells (BMSCs) (Lonza, Inc.) and human skin fibroblasts (Fibros) (Health Science Research Resources Bank) were cultured in serum-free DMEM. The conditioned media from SHEDs, BMSCs and Fibros were collected after 48 h of culture and centrifuged at 1500 rpm for 5 min. The supernatants were re-centrifuged at 3000 rpm for 3 min, followed by collection of the second supernatants, which were designated as SHED-CM, BMSC-CM and Fibro-CM, respectively. The protein concentration of each CM supernatant was measured using the BCA protein assay kit (Pierce). The average protein concentration of each CMs was 3 μg/ml. We used most of CM without enrichment or dilution. We found the strong therapeutic effects of this native SHED-CM for the treatment of mouse AD-like model. Furthermore the untreated mouse receiving SHED-CM exhibited no or little adverse events (data not shown).

2.5. Intranasal administration of the CM samples

Twenty-four hours after *i.c.v.* injection of the $A\beta_{1-40}$ peptide (day 1), the mice were anesthetized again with 1.5% isoflurane in O_2. The animals were divided randomly into four groups: the SHED-CM, BMSC-CM, Fibro-CM and DMEM (control) groups ($n = 10$ per group). A total of 50 μL of each of the CM and control samples were administered to the mice intranasally with a Hamilton microsyringe over the course of 10 min, at 2-min intervals. During these procedures, the mouth and opposite nostril were closed. Intranasal administration was performed twice a day for 4 days from days 1 to 4.

2.6. Novel object recognition test

Novel object recognition analysis was performed on days 3 to 5 after the *i.c.v.* injection of $A\beta_{1-40}$ (day 0) [39,41,42]. This method is used to measure of cognitive dysfunction in mouse models of natural aging and AD [43]. A plastic chamber (35 × 35 × 35 cm) was used