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Stem Cells Injected into the Deaf Rat Cochlea via Round Window Could Survive for at Least One Week in the Endolymph

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Abstract

Background: Noise-induced hearing loss (NIHL) is caused by chronic exposure to high-intensity sound. Although noise hazard has been extensively studied in the past decades, cochlear implants and hearing aids are only safe and clinically effective intervention in hearing-impaired person. In recent years, stem cell therapy has been studied to repair damaged inner ear tissue including hair cells and spiral ganglion neurons (SGNs). Because of small size and complex structure of the cochlea, it is difficult to transplant stem cells. It is necessary; stem cell injection procedure has a minimal adverse effect on target tissue. Cochleostomy are currently used to inject stem cells that can impair hearing. In addition, cell viability is an essential factor in the regeneration of damaged hair cells.

Aim: The aim of this study was to examine the survival of bone marrow mesenchymal stem cells (BMSCs) injected through the round window of the NIHL rat model.

Methods: Adult male rats were exposed to the 110 dB white noise paradigm for 6 hours in 5 days to induce deafness. Distortion-product otoacoustic emission (DPOAE) was recorded before and after noise exposure. Hoechst labeled-cells were transplanted into the rat cochlea through the round window. Hoechst-labelled MSCs were assayed in the endolymph for 24h, 72h, seven and 10 days after injection.

Results: Our findings revealed that stem cell transplantation into the cochlea via round window niche is a safe surgical approach. As well, cells transplanted into the endolymph survived for a postoperative period of at least 1 week.

Conclusion: BMSC can survive at least one week in adult rat cochlea after injection through the round window niche.

Conflicts of Interest: The Authors declare no conflicts of interest.
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Introduction

Noise-induced hearing loss (NIHL) is a type of hearing loss that results from prolonged exposure to high-intensity sounds or exposure to maximum decibel sounds. If the sound is too loud, stronger stimuli can damage hair cells (1). Mammalian hair cells are irreparable.

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As a result, damaged hair cells cannot send electrical messages to the brain. To date, noise-induced injury has been extensively studied over the past few decades, but hearing protection devices are clinically effective in preventing NIHL , and hearing aids and cochlear implant are the only cure (2). Currently, there is no definitive cure for regeneration of damaged hair cells caused by noise exposure. Stem cell transplantation has been investigated in recent years to repair damaged inner ear tissues (hair cells and auditory neurons). Stem cells have the ability to regenerate, differentiate and produce other cells types (3, 4). Preliminary findings from stem cells utilization for numerous disease displays that stem cells can become highly specialized cell types, and these new cells can function in animal models for repairing (5-7). Mesenchymal stem cells (MSCs) are mature stem cells that obtained from variety of tissues, especially the bone marrow. These cells have the ability to differentiate into mesoderm and non-mesoderm cells (8).

Because of the small size and complex structure of the cochlea, stem cell transplantation in the inner ear is difficult, and it is essential that the stem cell injection procedure has the least negative effect on the target tissue. The cochlea consists of Scala Vestibuli and the Scala Tympani containing the perilymph and Scala Media containing the endolymph fluid. The organ of Corti is located in the Scala Media of the inner ear (9).

There are two common methods of transplanting cells into the pre-lymphatic duct of the inner ear, which include cochleostomy and injection through the round window membrane (10, 11). Although the endolymphatic space is closed by tight junctions, studies have shown that cells injected through the lymphatic space were detected in the middle space, indicating that grafted cells can migrate from the perilymph space to the endolymphatic environment (12). Round window niche was used as a place to diffuse drugs (13). However, the viability of bone marrow-derived mesenchymal stem cells (BMSCs) transplanted through the round window has not been studied. Transplanted MSCs in this way may regenerate damaged hair cells and improve auditory function. In the present study, we investigated the survival rate of BMSCs transplanted through the round window niche to the cochlea in deaf male rats.

Methods

Animals

Sixteen male Wistar rats (3 months, 200–250 g) were purchased from the Laboratory Animal Center of Shahid Beheshti University of Medical Sciences. Animals were maintained at a constant temperature (22 ± 2°C) and regular 12 h light/dark cycle (14). These rats were randomly assigned to two groups (n = 8): sham (control) and noise exposure groups. This study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences based on National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

Noise exposure

The cochlear auditory function was assessed by the DPOAE test prior to exposure to noise. The DPOAE test was performed on day 0 (baseline) and day 6 (no noise exposure) in the control group (n = 8). DPOAE was also evaluated in the noise exposure groups on day 0 (before) and day 6 (after) of the noise exposure paradigm. DPOAE measurements (dB SPL) were performed for various stimulation frequencies (2-10 kHz). Animals with abnormal DPOAE were excluded from this experiment. Hearing-normal rats were exposed to 110 dB white noise for 6 hours a day for 5 consecutive days. Sound level meters was used to measure sound intensity (15).

Auditory assay

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DPOAE testing was conducted in a sound-attenuated chamber covered with acoustic foam. Prior to each session, animals were anesthetized by intraperitoneal injection of a mixture of ketamine/xylazine (90:10 mg/kg). In all rats, the DPOAE response from the left ear was recorded with the NeuroAudio system (NEUROSOFT, Russia).

Histological assay

Following anesthesia and decapitation of rat, left cochlea was quickly removed and placed in ice-cold 10 mM phosphate buffered saline (PBS). 4% paraformaldehyde (PFA) was perfused through the oval and round windows of the cochlea. The cochlea was then fixed overnight with 4% PFA. The fixed cochlea was decalcified with 5% ethylenediaminetetraacetic acid (EDTA, pH = 7.4) for 2 weeks. Next, paraffin-embedded tissue was cut to 5 µm section in thickness at microtomy. Sections were deparaffinized, rehydrated and later stained with hematoxylin-eosin (H&E) staining (Sigma-Aldrich, USA).

Isolation of BMSCs

Rats were anesthetized with combination of ketamin (90 mg/kg) and xylazine (10 mg/kg). BMSCs were extracted from bone marrow of femur and tibia and rinsed with PBS buffer. Then cells were cultured in DMEM (Gibco, USA) with 10% fetal bovine serum (FBS, Gibco). Cell cultures were maintained in culture flasks in a humidified incubator (37 °C, 5% CO2). Then fourth passage cells were observed under phase-contrast microscopy.

Hoechst labeling

In order to determination of BMSCs migration to the cochlear endolymph, Hoechst 33342 (Hoechst 33342; Thermo Fisher Scientific, USA) -labled BMSCs were transplanted into the perilymph through the round window niche. Deaf rats were transplanted with 1×10^5 Hoechst -labeled BMSCs. 24h, 72h, 7 and 10 days after BMSCs injection, animals were sacrificed and the cochleae were extracted. The cochleae were fixed through intrascalar perfusion of 4% PFA and then decalcified in 5% EDTA for 2 weeks. Then cut the block embedded in paraffin to a thickness of 5 µm. The number of Hoechst-labeled BMSCs in cochlear endolymph was assessed by fluorescence microscopy (Nikon Inc., USA). Data analysis was performed using ImageJ software.

Statistical analysis

Data are presented as the mean ± SEM. One-way ANOVA was performed using GraphPad Prism version (GraphPad Software, USA). P-value less than 0.05 is statistically significant.

Results

Characterization of BMSCs

MSCs obtained from rat bone marrow (passage 4) showed a typical fibroblast-like shape under phase-contrast microscopy (Figure 1).

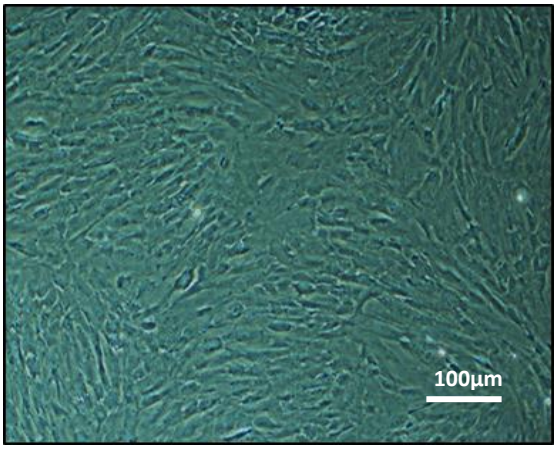

Figure 1. Morphology and phenotypic characterization of cultured BMSCs at passage 4, (Scale bar: 100 µm).

Hearing function assay

Cochlear function was assessed by the DPOAE test. According to the DPOAE results, no significant difference was observed in the DPOAE response between the sham groups on day 0 and day 6 at all frequencies (Figure 2). In the noise group, the response of DPOAE was significantly lower on day 6 than on day 0 (Figure 2).

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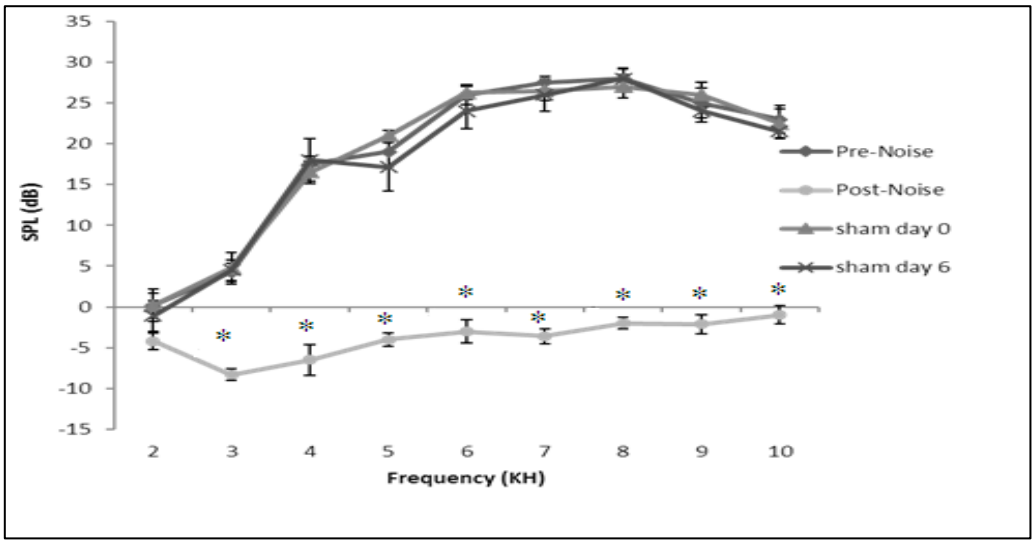

Figure 2. Auditory function of rats assessed by DPOAE assay. Mean ± SEM of DPOAE (in dB SPL) in the sham group at day 0 and day 6 and in the noise group before (day 0) and after (days 6) noise exposure. SPL; sound pressure level. *P <0.001 compared to day 0 (one-way ANOVA).

Histological assay

H&E staining showed the abnormal arrangement of the cochlear hair cells and degeneration of SGNs in deaf rat (B) when compared to control rats (A) (n = 3) (Figure 3).

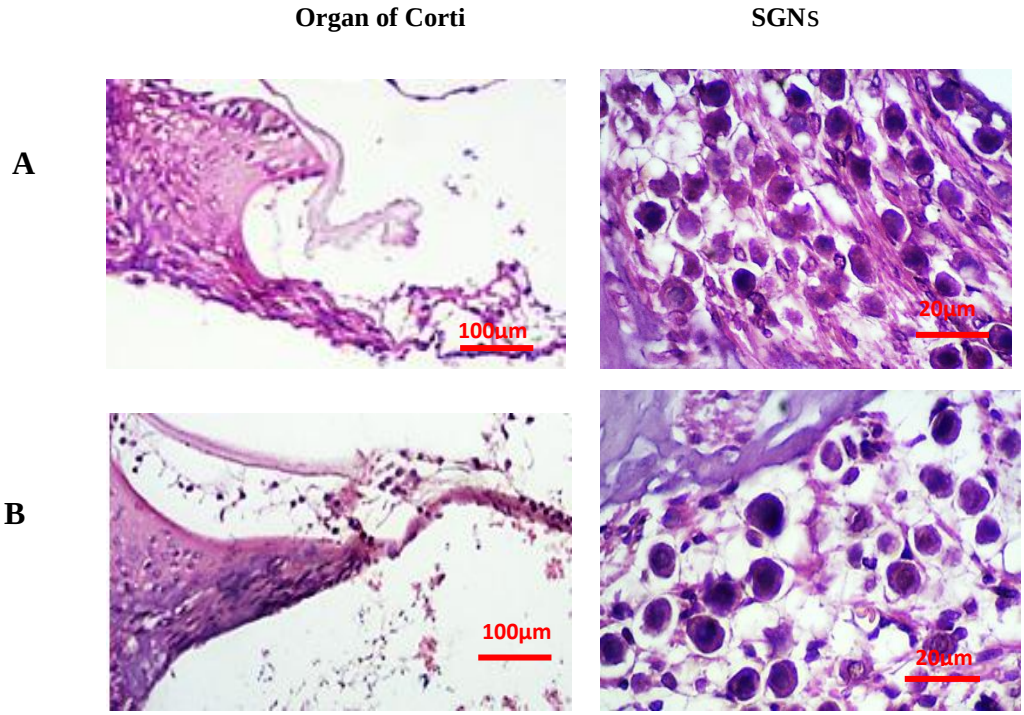

Figure 3. Effect of noise exposure on the structure of rat cochlea. Comparison of abnormal morphology of auditory hair cells and degeneration of SGNs in deaf rats (B) with normal organ of Corti and SGNs structure in the control group (A), spiral ganglion neurons (SGNs), (Scale bar: 20 &100 µm).

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BMSCs migration assay

To evaluate the migration of BMSCs to the cochlear endolymph, Hoechst 33342-stained BMSCs were injected through the round window into noise-induced deaf rats. Hoechst-labeled MSCs was assayed in the endolymph for 24h, 72h, 7 and 10 days following transplantation. Implanted cells was survived in the endolymph 1 week following engraftment (Figure 4).

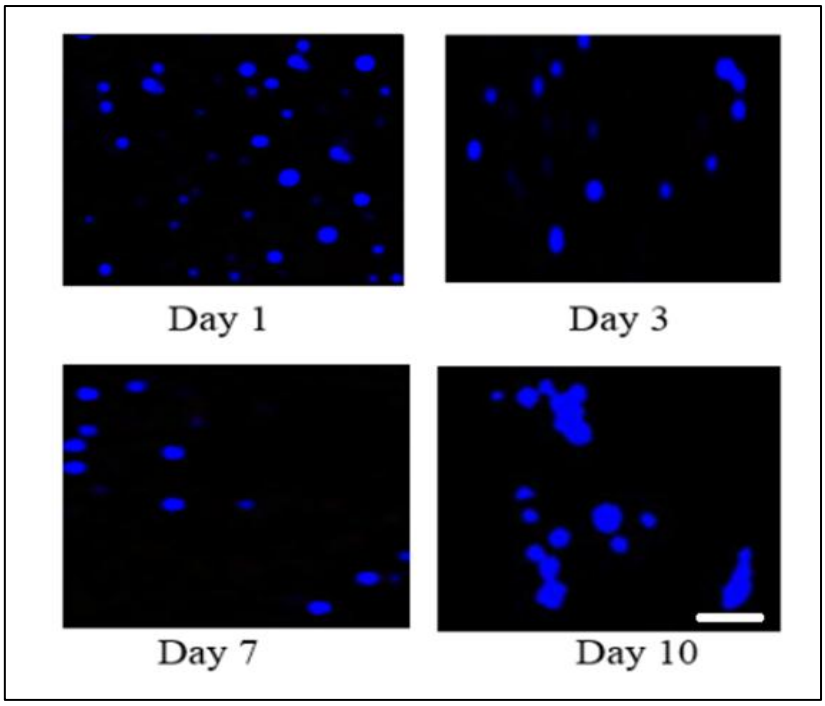

Figure 4. Shape and viability of implanted BMSCs in the endolymph. Hoechst-labeled cells were able to survive in endolymph for at least one week. On day 10 the cells diminished and appeared as aggregates (Scale bar = 100 μm).

Discussion

In this study, we examined the survival rate of transplanted BMSCs through the round window in a rat model of NIHL. Our findings revealed that stem cell transplantation into the cochlea through the round window niche is a safe surgical approach. As well, implanted cells survived in the endolymph for a postoperative period of at least 1 week.

Previous studies have shown exogenous stem cells survive short-term in the mammalian cochlea (16-19). Several studies have considered transplantation of MSCs into the rat cochlea. In a study by Matsuoka et al., MSCs were transplanted into the pre-lymphatic space. They have shown that seven days following injection, stem cells were transferred to the tympanic and vestibular scala, and only slightly located in the media scala. GFP-positive cells were not found in the cochlear modiolus (20). In another experiment, various routes of transplantation were compared by intraperilymphatic and cochlear modiolar injection. No cells were detected in the Rosenthal canal at the pre-lymphatic adminstration. However, on modiolar injection, undifferentiated MSCs were able to survive in the cochlea of both control and deaf group, and were larger in the deaf group than in the control group (21). Research has shown that the use of mitochondrial 3-nitropropionic acid (3NP)

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induces acute SNHL and selective degeneration of fibrocytes in the lateral wall of the cochlea, without significant damage to hair cells and SGNs (22). In this model, allogeneic BMSCs were transported through the lateral semicircular canal. MSCs were observed in the ampullary area near the graft site and in the perymphatic ducts. Sstem cells transplantation into cochlear lateral wall fibrocytes was confirmed by the presence of BrdU marker and connexin 26. In addition, hearing improvement was approved using ABR (23). Previous study has evaluated the survival of mouse embryonic stem cells in repair of damaged SGNs in the cochlea of animal model of SNHL (24).

Yong Fu et al. examined the transplantation of neural stem cells (NSCs) through the round window into the cochlea of normal mice and evaluated their effect on auditory function and structure (25). Iguchi and colleagues (2003) report that approximately 10% of NSCs injected into the cochlea of mice were observed 4 weeks after transplantation (26). Other studies investigated the delivery of dorsal root ganglion neurons (DRGN) to the base of the cochlea via the scala tympani (18) (27). In addition, cell engraftment into the auditory nerve was reported by Naito *et al* (28) and Regala *et al* (19). In all studies, viability and migration of cells delivered to the cochlea was up to 4 weeks.

Conclusion

This study demonstrated that delivering BMSC to the cochlea of hearing-impaired rats through the round window niche is a safe surgical approach. In addition, the cells transplanted into the endolymph survived for at least one week after surgery. This will be the time frame for additional steps to induce the differentiation of transplanted cells into new hair cells and auditory neurons.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Ethics

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