The Laryngoscope

Transplantation of Bone Marrow-Derived Neurospheres Into Guinea Pig Cochlea

Hideaki Ogita, MD; Takayuki Nakagawa, MD, PhD; Tatsunori Sakamoto, MD, PhD; Takatoshi Inaoka, MD; Juichi Ito, MD, PhD

Objectives/Hypothesis. To investigate the potential of neurally induced bone marrow stromal cells (BMSCs) as transplants for replacement of spiral ganglion neurons.

Methods. BMSCs were harvested from the femurs and tibias of adult guinea pigs. BMSCs were cultured with neural induction media and formed spheres. The capacity of BMSC-derived spheres for neural differentiation was examined by immunocytochemistry in vitro. BMSC-derived spheres were injected into the modiolus of the intact cochleae or those locally damaged by ouabain, followed by histological and functional analyses.

Results. In vitro analysis revealed a high capacity of BMSC-derived spheres for neural differentiation. After transplantation into the cochlear modiolus, the survival and neural differentiation of BMSC-derived spheres was observed in both the intact and damaged cochleae. In intact cochleae, transplants settled in various portions of the cochlea, including the cochlear modiolus, whereas in damaged cochleae, transplants were predominantly observed in the internal auditory meatus. Transplantation of BMSC-derived spheres resulted in no functional recovery of the cochlea or protection of host spiral ganglion neurons.

Conclusions. The present findings indicate that BMSC-derived spheres can be a source for replacement of spiral ganglion neurons, although further manipulations are required for functional recovery.

Key Words: Allograft, cell therapy, cochlea, neurosphere, spiral ganglion neuron, regeneration.

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INTRODUCTION

Treatment options for sensorineural hearing loss are currently limited to cochlear implants and hearing aids. Hence, there is a requirement for alternative means of biological therapy, including cell-based therapy. Indeed, recent studies have indicated that cell-based therapy could be utilized as a therapeutic option for inner ear disorders.[1–3] Spiral ganglion neurons (SGNs), primary auditory neurons, are located in the modiolus of the cochlea and transmit sound stimulation to the central auditory system. The loss of SGNs, therefore, compromises auditory function. In addition, SGN loss also reduces the effectiveness of cochlear implants, which can improve impaired hearing by stimulating SGNs. SGNs are, therefore, a primary target for cell transplantation in the auditory system.

Bone marrow stromal cells (BMSCs) are a heterogeneous population of stem/progenitor cells with pluripotent capacity to differentiate toward a neuronal phenotype,[4,5] and consequently the possible use of BMSCs for the treatment of neurological diseases has acquired enormous importance. BMSCs have great potential as therapeutic agents, because they are easy to isolate and expand. Previously, the potential of BMSC transplantation for the treatment of inner ear disorders has been investigated.[6–9] These previous studies have demonstrated that undifferentiated BMSCs are able to settle in the cochlea and have a high capacity for migration. However, limited numbers of transplants differentiated into neurons after transplantation into the intact or damaged cochlea,[6–8] which indicates that neural induction of BMSCs before transplantation is required for SGN replacement by BMSC transplantation.

The aim of this study was to elucidate the neural expression profile of neurally induced BMSCs of guinea pigs and their ability to retain neural differentiation potential when transplanted into the intact or damaged cochleae of guinea pigs. In addition, we examined the capacity of neurally induced BMSCs for functional and histological replacement of SGNs.

MATERIALS AND METHODS

Experimental Animals

A total of 18 Hartley-strain guinea pigs were purchased from Japan SLC Inc. (Hamamatsu, Japan). The Animal

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Research Committee of the Graduate School of Medicine, Kyoto University, Kyoto, Japan, approved all of the experimental protocols. Animal care was carried out under the supervision of the Institute of Laboratory Animals of the Graduate School of Medicine, Kyoto University. All of the experimental procedures were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

BMSCs

Bone marrow was isolated from the femurs and tibias of 6- to 8-week-old guinea pigs (n = 4, Japan SLC Inc.). Under general anesthesia with midazolam (8 mg/kg, intramuscular injection) and xylazine (8 mg/kg, intramuscular injection), the epiphyses of the femurs and tibias were removed, and the marrow was flushed out into a 100-mm culture dish. The isolated bone marrow, composed of hematopoietic and stromal cells, was maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Gaithersburg, MD) supplemented with 10% fetal bovine serum (Thermo Trace Ltd., Noble Park, Victoria, Australia) and 1% antibiotic-antimycotic (Invitrogen) at 37°C with 5% CO_2. The medium was changed twice weekly until the cells were 80% confluent. Nonadherent cells were removed during the medium-change procedure. The BMSCs were passaged three to five times before use. BMSCs at this stage were defined as undifferentiated.

Neural Induction of BMSCs

For neural induction, cultured BMSCs were enzymatically detached from culture dishes. The BMSCs were plated into 100-mm culture dishes at a density of 2×10^6 cells/well, and cultured in serum-free DMEM/F-12 medium (Invitrogen) supplemented with B27 (Invitrogen), 20 ng/mL of basic fibroblast growth factor (bFGF, Invitrogen), and 20 ng/mL of epidermal growth factor (EGF, Invitrogen). We added the same amounts of bFGF and EGF every 3 days. After 7 days of culture, BMSC-derived spheres were collected and fixed with 4% paraformaldehyde (PFA) for 15 minutes. The characteristics of BMSC-derived spheres were examined by immunohistochemistry for nestin and Musashi-1. Anti-nestin mouse monoclonal antibody (1:500; BD Biosciences, San Jose, CA) or anti-Musashi rabbit polyclonal antibody (1:500; Chemicon, Billerica, MA) was used as the primary antibody. Alexa Fluor 488-conjugated anti-mouse donkey IgG (1:1000; Invitrogen) and Alexa Fluor 555-conjugated goat anti-rabbit IgG (1:1000; Invitrogen) were used as the secondary antibodies. We counted the numbers of spheres and the number of marker-positive spheres in five randomly selected fields (3.4 mm^2 in area), and then calculated the ratio of nestin or Musashi-1 expressing spheres to the total number of spheres. Four independent cultures were performed.

In Vitro Neural Differentiation

To investigate the ability of BMSC-derived spheres to neurally differentiate, BMSC-derived spheres were plated onto 8-well chamber slides at a density of 100 spheres/well in serum-free DMEM/F-12 medium supplemented with B27, retinoic acid (1 mM, Sigma, St. Louis, MO), and dibutyryl cyclic adenosine monophosphate (AMP) (1 mM, Sigma). After 7 days of culture, the cells were fixed with 4% PFA for 15 minutes and immunostained for beta-III tubulin. Anti–beta-III tubulin rabbit polyclonal antibody (1:500; Sigma) was used as the primary antibody, and Alexa Fluor 488-conjugated anti-rabbit donkey IgG (1:1000; Invitrogen) was used as the secondary antibody. We counted the total numbers of the cells and the number of beta-III tubulin-positive cells in five randomly selected fields (3.4 mm^2 in area), and then calculated the ratio of beta-III tubulin expressing cells to the total number of cells. Four independent cultures were performed.

Transplantation Procedure

After labeling with DiI (Invitrogen, 5 μg/mL), the cell suspension of BMSC-derived spheres (10^5 cells in 10 μL DMEM) was injected into the cochlear modiolus of guinea pigs weighing 300 to 330 g as described previously.[10] Briefly, under general anesthesia with midazolam and xylazine, a small hole was made on the left otic bulla to expose the round window niche and the basal turn of the cochlea. After cochleostomy in the basal turn of the cochlea, a glass pipette, which was connected to a microsyringe (Hamilton, Reno, NV), was inserted into the cochlear modiolus of the basal portion of the cochlea. The glass pipette was removed 1 minute after completion of the infusion. Finally, the cochleostomy site was closed with a fat graft and then covered with fibrin glue.

BMSC-Derived Sphere Transplantation

BMSC-derived spheres were transplanted into intact or damaged cochleae of guinea pigs weighing 300 to 330 g. Four weeks after transplantation, four intact guinea pigs were transcardially perfused with phosphate buffered saline (PBS) at pH 7.4, followed by 4% PFA, and sacrificed under general anesthesia with an overdose of pentobarbital. The temporal bones were immediately dissected out and immersed in the same fixative for 4 hours at 4°C.

Ten guinea pigs received local application of ouabain (5 μL at a concentration of 5 mM in saline; Sigma), which causes SGN degeneration,[9] through the round window membrane under general anesthesia with midazolam and xylazine. One week after application, the electrically evoked auditory brainstem response (eABR), which has been used for functional evaluation of SGNs, was measured before cell transplantation as previously described.[10] Eight animals that showed no eABRs were used in the following experiments. Immediately after the eABR measurements, four animals received transplantation of BMSC-derived spheres similar to intact guinea pigs, and the other four animals received an injection of the culture media and were used as controls. Four weeks later, the cochlear specimens were collected after eABR recording.

Specimens (10-μm thick) were prepared using a cryostat after decalcification with 0.1 M ethylenediaminetetraacetic acid in PBS for 3 weeks at 4°C. Then, immunostaining for beta-III tubulin was performed, followed by nuclear staining with 4′,6-diamino-2-phenylindole dihydrochloride (DAPI; 2 μg/mL PBS, Invitrogen). Specimens were viewed with a Leica TCS-SPE confocal laser-scanning microscope (Leica Microsystems Inc., Wetzlar, Germany). Five midmodiolar sections (each separated by 30 μm) were provided for quantitative analyses from each tissue sample. We defined the cells that were positive for DiI with a distinct nucleus identified by DAPI as surviving transplants. The numbers of transplants were counted in the internal auditory meatus and in five cochlear compartments (the modiolus, the scala vestibuli, the scala media, the scala tympani, and the lateral wall). We also counted the numbers of beta-III tubulin-positive transplants, and calculated the ratio of beta-III tubulin expressing transplants to total surviving transplants. In addition, the densities of SGNs in the Rosenthal canals were quantified in ouabain-treated specimens as described previously.[11] All data are presented as the mean ± 1 standard deviation.

Statistical Analyses

We statistically compared the total numbers of surviving transplants and the ratios for beta-III tubulin expression between transplants in damaged cochleae and those in intact cochleae, using unpaired *t* tests. The difference in the locations of surviving transplants between damaged and intact cochleae

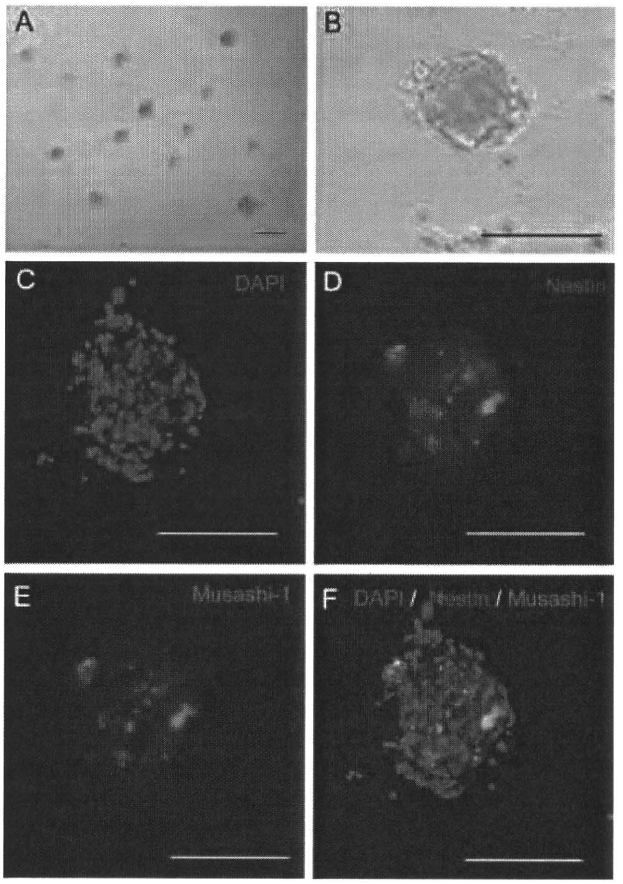

Fig. 1. BMSC-derived spheres. (A, B) Phase contrast images. (C) 4′,6-diamino-2-phenylindole dihydrochloride (DAPI) staining. (D) Immunostaining for nestin. (E) Immunostaining for Musashi-1. (E) Merged image. Scale bars = 500 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

was examined by two-way analysis of variance. In damaged models, the difference in the density of remaining SGNs between control and transplanted cochleae were compared using unpaired t tests. P values of $< .05$ were considered to be statistically significant.

RESULTS

Neural Induction of BMSCs

After 2 to 3 days in vitro, BMSCs began to form spheres. On day 7, 1.28 ± 0.71 × 10^4 spheres were identified in each culture dish (Fig. 1A, 1B). Immunohistochemistry revealed the expression of nestin and Musashi-1 in the BMSC-derived spheres (Fig. 1C–1E). The expression of nestin was found in 91.9 ± 4.7% of total BMSC-derived spheres, and that of Musashi-1 was found in 93.6 ± 2.9%, suggesting that neurospheres were generated from guinea pig BMSCs.

In Vitro Neural Differentiation

We transferred the BMSC-derived spheres into differentiation medium containing retinoic acid and dibutyryl cyclic AMP to examine their capacity for neural differentiation. Sphere-forming cells attached to culture dishes and the cells migrated from the sphere (Fig. 2A). Then, some of the cells extended processes (Fig. 2B). On day 7, 89.2 ± 2.8% of the cells expressed beta-III tubulin (Fig. 2C, 2D), indicating that BMSC-derived spheres have the capacity to differentiate into neurons.

Transplantation Into Intact Cochleae

DiI-positive transplants were found in all intact cochleae following transplantation of BMSC-derived spheres. In each midmodiolar section, 74.1 ± 44.4 transplants were found. Transplants were located in multiple regions of the cochlea, predominantly in the scala tympani and the modiolus (Fig. 3A, 3C). Transplants were rarely found in the internal auditory meatus. The expression of beta-III tubulin was observed in 18.6% ± 6.4% of transplants (Fig. 3B).

Transplantation Into Damaged Cochleae

DiI-positive transplants were also identified in all transplanted cochleae that had been damaged by ouabain. The number of surviving transplants in each midmodiolar section was 72.1 ± 53.1. There was no significant difference in the number of surviving transplants between intact and damaged cochleae, whereas the locations of surviving transplants in the damaged cochleae significantly differed from those in the intact cochleae ($P = .007$). In the damaged cochleae, the settlement of transplants was observed in the modiolus, similar to observations in the intact cochleae; however, the most prominent region for settlement of transplants

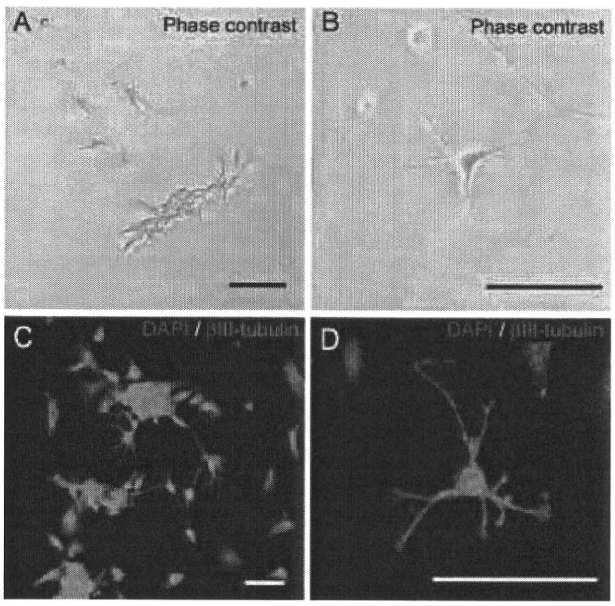

Fig. 2. Neural induction of BMSC-derived spheres in vitro. (A) Phase contrast image on day 3 in vitro. (B) Phase contrast image on day 7 in vitro. (C, D) Immunostaining for beta-III tubulin and nuclear staining with 4′,6-diamino-2-phenylindole dihydrochloride (DAPI). Scale bars = 20 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

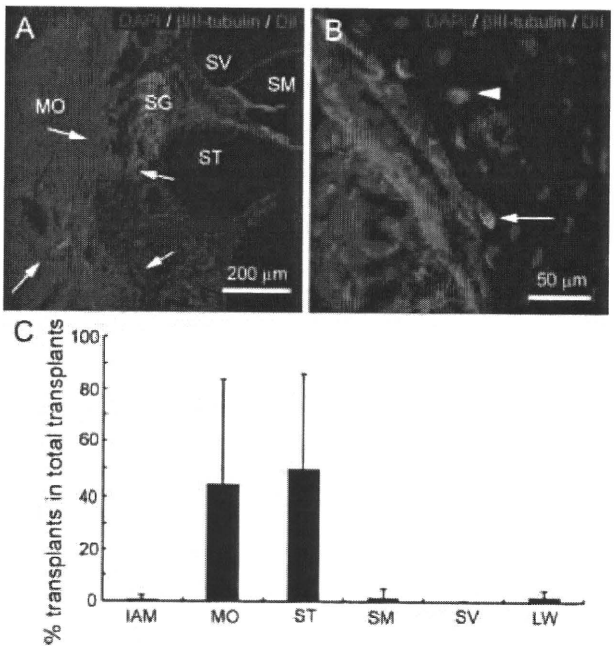

Fig. 3. Transplantation into the intact cochleae. (A) Transplants labeled by DiI (red) are located in the modiolus (MO) of the cochlear basal portion (arrows). (B) Transplant labeled by DiI (arrow) is positive for beta-III tubulin and another (arrowhead) is negative. (C) The locations of transplants in the cochlea and in the internal auditory meatus (IAM). SV = scala vestibule; SG = spiral ganglion; SM = scala media; ST = scala tympani; LW = lateral wall. Bars represent a standard deviation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

was the internal auditory meatus (Fig. 4A, 4C). The expression of beta-III tubulin was observed in 24.1 ± 5.3% of transplants in the damaged cochleae (Fig. 4B). There was no significant difference in the ratio of beta-III tubulin expressing transplants to total number of transplants between intact and damaged cochleae. These findings indicated that SGN degeneration prior to transplantation caused the migration of BMSC-derived spheres into the internal auditory meatus, and had no effects on the survival and neural differentiation of BMSC-derived spheres after transplantation.

Effects of Transplantation on Cochlear Function

We used eABR recording to monitor SGN function. All the animals receiving eABR evaluation showed no responses before an injection of a cell suspension or a culture medium. Four weeks postoperation, positive eABRs were identified in two of four animals in each group. Thresholds of eABRs in the two animals that showed positive responses in the transplanted group were 300 and 400 μA, and those in the sham-operated group were 250 and 650 μA. These findings indicated that transplantation of BMSC-derived spheres into the cochlear modiolus induced no significantly functional recovery of the cochlea.

We quantified SGN densities after cell transplantation or sham operation to evaluate the effects of BMSC-derived spheres on enhancement of the survival of remaining host SGNs. Local ouabain application caused severe degeneration of SGNs, especially in the basal turn of cochleae. No significant differences in the SGN density of the basal, second, or third turn of cochleae were found between transplanted and sham-operated specimens (Fig. 5), indicating that transplantation of BMSC-derived spheres did not promote the survival of remaining host SGNs, which is consistent with eABR results.

DISCUSSION

The present findings demonstrate that guinea pig BMSCs are able to form spheres that have the capacity to differentiate into neurons in vitro. We aimed to replace SGNs, which are located in the cochlear modiolus, with BMSC-derived neurons. We thus directly injected BMSC-derived spheres into the modiolus of intact or damaged cochleae of guinea pigs. For accurate introduction of the cells into the cochlear modiolus, the size of the cochlea is a critical issue. Previously, we tried to introduce transplants into the cochlear modiolus of mice,[12] in which the success rate for the settlement of the transplants was not satisfactory. In addition, functional evaluation following cell transplantation is virtually impossible. On the other hand, guinea pig[10,13] or chinchilla[6] model systems exhibited better settlement

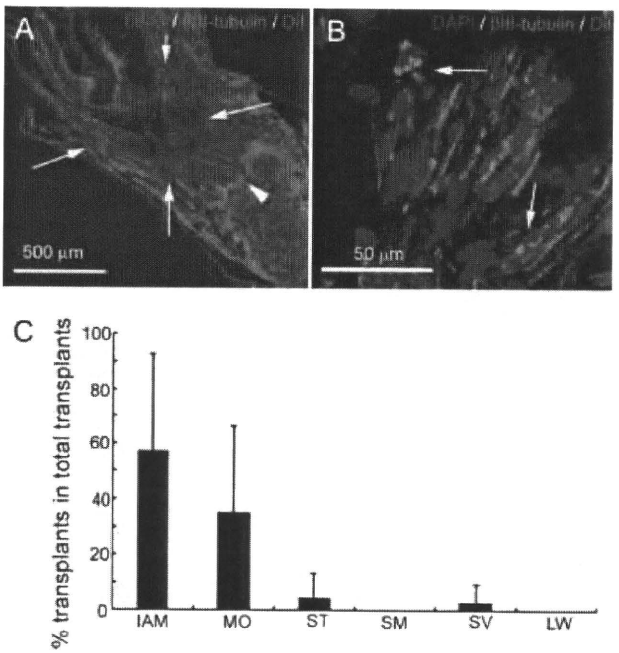

Fig. 4. Transplantation into the damaged cochleae. (A) In the internal auditory meatus (IAM), transplants labeled by DiI (red) are observed (arrows). An arrowhead indicates the location of the glial-schwann junction. (B) A transplant labeled by DiI (arrow) is positive for beta-III tubulin and another (arrowhead) is negative. (C) The locations of transplants in the cochlea and in the internal auditory meatus (IAM). MO = modiolus; ST = scala tympani; SM = scala media; SG = spiral ganglion; SV = scala vestibule; LW = lateral wall. Bars represent a standard deviation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

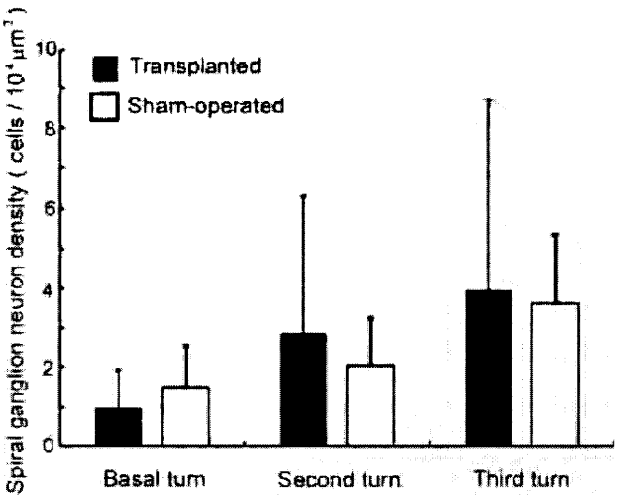

Fig. 5. Densities of remaining spiral ganglion neurons in the basal, second, or third turn of transplanted or sham-operated cochleae. There are no significant differences in the density of spiral ganglion neurons between transplanted and sham-operated cochleae.

of transplants in the cochlear modiolus and enabled functional evaluation using eABRs.[10,13] Moreover, our refined technique for cell introduction into the cochlear modiolus of guinea pigs caused no significant elevation of eABR thresholds.[10] Based on these previous findings, we used guinea pigs as experimental animals in the present study.

After transplantation of BMSC-derived spheres into the intact or damaged cochleae, BMSC-derived neurons were found in various portions of cochleae, including the cochlear modiolus. These findings indicate that BMSCs can be an alternative source of transplants for replacing SGNs. However, measurements of eABRs in the present study revealed no significant improvements of eABR thresholds after transplantation of BMSC-derived spheres. Previously, we demonstrated the recovery of eABR thresholds after transplantation of embryonic stem (ES) cell-derived neural progenitors in a different injury model.[13] There are several possible explanations for this lack of functional recovery following transplantation of BMSC-derived spheres. One is insufficient neurite elongation from BMSC-derived neurons to the central nervous system. Another possibility relates to different subtypes of neurons that are generated from BMSC-derived spheres. Previous studies have demonstrated that glutamatergic neurons are generated from both ES cells[14] and BMSCs,[15] meaning that both cell types have the capacity for differentiation into glutamatergic neurons. To achieve functional SGN restoration by transplantation of BMSC-derived spheres, additional treatments are required to enhance elongation of neurites from BMSC-derived neurons or to induce differentiation of BMSC-derived spheres into glutamatergic neurons.

Interestingly, the localization of transplants was different between the intact and damaged cochleae. In the intact cochleae, a number of transplants were found in the scala tympani. We injected BMSC-derived spheres through the scala tympani.[10] Therefore, transplants that were found in the scala tympani may have originated from the leakage of injected cell suspensions. In the intact cochlea, there are limited spaces in the cochlear modiolus, because host SGNs and auditory nerves are present, which may cause the leakage of injected cell suspensions into the scala tympani. On the other hand, the loss of host SGNs may result in an increase of spaces for transplants in the cochlear modiolus. Hence, in the damaged cochleae limited numbers of transplants were observed in the perilymphatic spaces including the scala tympani. In the damaged cochleae a number of transplants were found not only in the cochlear modiolus but also in the internal auditory meatus. Transplants in the internal auditory meatus may migrate from the cochlear modiolus, which is an injected site. The degeneration of SGNs could make a path from the cochlear modiolus of the basal portion to the internal auditory meatus, or stimulate production of chemotactic factors that promote the migration activity of BMSC-derived spheres. Future studies should be performed to determine the mechanisms of migration of BMSC-derived spheres into the internal auditory meatus.

CONCLUSION

The present findings demonstrate that BMSCs are a preferable source of neurospheres and that BMSC-derived spheres retain the ability for neural differentiation after transplantation into the cochlea. Functional restoration of damaged cochleae was not achieved by transplantation of BMSC-derived spheres, although a number of transplant-derived neurons settled in the cochlea and in the internal auditory meatus. To achieve functional restoration of SGNs by transplantation of BMSC-derived spheres, additional treatments including local application of neurotrophic or growth factors may be required.

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ORIGINAL ARTICLE

Inner ear drug delivery system from the clinical point of view

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Abstract

Conclusion: Three types of inner ear drug delivery systems (DDS) that were ready to be applied in clinics were developed. *Objectives:* To develop clinically applicable inner ear DDS for the treatment of inner ear disorders. *Methods:* Inner ear DDS using clinically applicable materials were developed and evaluated. *Results:* The systemic application of stealth-type nanoparticles encapsulating betamethasone provided superior therapeutic results for the treatment of noise-induced hearing loss compared with the systemic application of betamethasone in mice. Microparticles made of biodegradable polymer (poly (lactic/glycolic) acid, PLGA) encapsulating lidocaine were placed on the round window membrane of guinea pigs, and resulted in reasonable concentrations of lidocaine in the cochlea without serious adverse effects. The phase I/IIa clinical trial of the application of insulin-like growth factor-1 (IGF-1) in combination with gelatin hydrogel on the round window membrane was conducted, recruiting patients with acute sensorineural hearing loss after the failure of systemic application of steroids.

Keywords: *Sensorineural hearing loss, tinnitus, biodegradable polymer, gelatin hydrogel, betamethasone, insulin-like growth factor 1, lidocaine*

Introduction

Sensitive sensors in the inner ear, hair cells, are mechanically protected in the bony capsule. The unique high potassium environment required for hair cells to work is actively maintained in the endolymph, which is sealed by tight junctions. The blood–labyrinthine barrier [1] is partly composed of tight junctions and also a system to protect these delicate cells from agents that may cause damage. However, these isolation systems make inner ear diseases difficult to be treated. Direct access into the inner ear is difficult because of the bony capsule. The blood flow of the inner ear is accordingly limited; 1/10 000 of cardiac output in rodents and 1/1 000 000 in humans [2]. It is difficult to deliver systemically applied therapeutic agents into the inner ear because of this limited blood flow and the existence of the blood–labyrinthine barrier [3]. Specific strategies to deliver therapeutic agents into the inner ear are required to overcome this difficulty.

The purpose of a drug delivery system (DDS) is to deliver a drug to a specific site in a specific time and release pattern [4]. Several types of inner ear-specific DDS have been developed, most of which use the round window (RW) as a route to deliver the agent into the inner ear, because the RW is a unique structure in that the inner ear is not covered with bone but sealed with a RW membrane (RWM). One well studied example of inner ear DDS is RWμCath™ (DURECT™ Co., Cupertino, USA), which utilizes the catheter tip placed on the RWM to deliver the therapeutic agent. Plontke et al. [5] conducted a clinical trial using this device. Patients with acute

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sensorineural hearing loss and insufficient recovery after systemic glucocorticoid treatment were included in that study, and significantly better improvement of hearing after a continuous intratympanic delivery of glucocorticoid via RWμCath was observed. This device would be applicable to other therapeutic agents; however, major surgery is required before and after the drug application, and more seriously, it is impossible to use this device because this has been commercially discontinued.

It is obviously mandatory to develop clinically applicable and available inner ear DDS. Here we show our approaches to realize this aim. The first is the systemic approach to deliver drugs to the inner ear more effectively. The other two involve local drug delivery via the RWM.

Material and methods

Inner ear DDS via systemic application – stealth-type nanoparticles

It would be more useful and its clinical application would be wider if a systemically applied therapeutic agent could be delivered selectively into the inner ear; however, to date, there is no reported system available to achieve this aim. Instead, we tried to improve the utilization of drugs in the inner ear. We used stealth-type nanoparticles for this purpose, which are made of biodegradable polymer, poly lactic acid (PLA), with polyethylene glycol coating (Figure 1A). Stealth-Nano-Steroid, stealth-type nanoparticles containing betamethasone, have been shown to accumulate preferentially in artificially inflamed joints as a model of rheumatoid arthritis and to reduce inflammation [6]. We first tested the distribution of stealth-type nanoparticles in the inner ear. In terms of clinical application, PLA is widely used as absorbable surgical threads, pins, screws and facial injectables (Sculptra®). Also, polyethylene glycol (PEG) is frequently used to modify the molecular weight, size and solubility of therapeutic drugs. These factors support the clinical safety of Stealth-Nano-Steroid.

Inner ear drug delivery via the round window

Intratympanic injection has been used as a method to realize inner ear treatment to deliver aminoglycosides, steroids and other therapeutic drugs [7]. However, the pharmacokinetics of intratympanically applied drugs are not stable because of the dynamic environment of the tympanic cavity; e.g. liquid in the tympanic cavity is easily ejected into pharynx by swallowing. To stabilize drug delivery via the RWM into the inner ear, we used microparticles made of biodegradable polymer and gelatin hydrogel. These slow releasing materials are placed on the RWM, and as these degrade, encapsulated therapeutic molecules diffuse into the inner ear.

Local application using PLGA microparticles

While tinnitus is a common symptom among patients with hearing impairment, no specific therapeutic strategy has been established. Lidocaine is known to be effective via intratympanic application [8,9]. However, it has been an unacceptable option because of its short effective duration (up to several hours) and serious vertigo after the application due to inner ear anesthesia [10]. We designed the inner ear DDS to reduce the concentration in the inner ear and elongate the release of lidocaine [11]. Poly (lactic/glycolic) acid (PLGA) is another commonly used biodegradable polymer. PLGA microparticles encapsulating lidocaine (Figure 1B) were applied on the RWM of guinea pigs and the lidocaine concentrations in the cochlea were measured at various time points.

Local application using gelatin hydrogels

Gelatin is a natural polymer composed mainly of collagen. By crosslinking with glutaraldehyde, gelatin forms hydrogel. The isoelectric point of gelatin can be modified to yield either a negatively charged acidic gelatin or a positively charged basic gelatin at physiological pH. This allows specific design so that electrostatic interaction takes place between a charged bioactive molecule (e.g. proteins and plasmid DNAs) and gelatin. The crosslinking density of gelatin hydrogels affects their degradation rate. Accordingly, gelatin hydrogels can be used as a delivery vehicle for the controlled release of bioactive molecules [12] (Figure 1C).

Various growth factors including brain-derived neurotrophic factor (BDNF) [13], hepatocyte growth factor (HGF) [14] and insulin-like growth factor 1 (IGF-1) have been placed on the RWM of the cochlea in combination with gelatin hydrogel to test the possibility of their use as therapeutic agents for the treatment of hearing impairment in rodents. Among them, IGF-1 has been shown to be protective [15] and therapeutic [16] against noise-induced inner ear damage, and therapeutic against ischaemic inner ear damage [17]. In addition, recombinant human IGF-1 (rhIGF-1, Mecasermin®, Astellas Pharma Inc., Tokyo, Japan) is commercially available as an orphan drug for

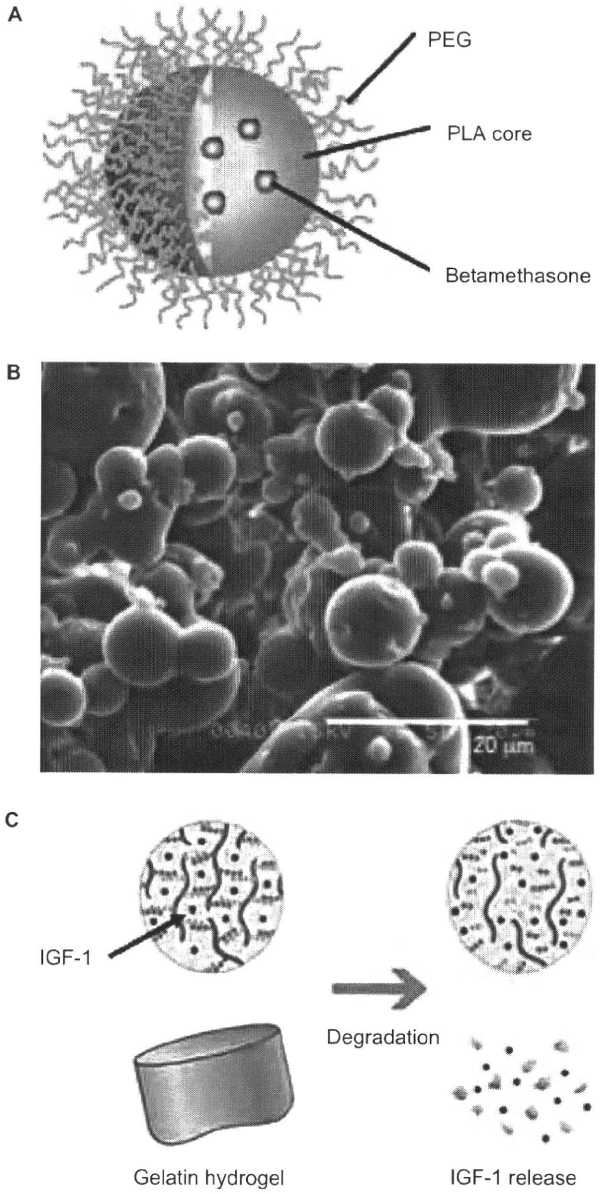

Figure 1. (A) Schematic illustration of a stealth-type poly lactic acid (PLA) nanoparticle with polyethylene glycol (PEG) coating and encapsulated betamethasone. (B) Scanning electron microscopic view of poly (lactic/glycolic) acid (PLGA) microparticles encapsulating lidocaine. (C) Schematic illustration of a gelatin hydrogel drug delivery system (DDS). Target molecules (IGF-1) entrapped in the crosslinked gelatin polymer are gradually released from the polymer matrix as gelatin hydrogel degrades.

the treatment of a type of juvenile growth failure, a certain type of diabetes mellitus and dwarfness.

Against this background, we conducted and have finished a phase I/IIa clinical trial to examine the safety and efficacy of local IGF-1 application via the RWM using gelatin hydrogel for patients with acute sensorineural hearing loss (UMIN000000936). Subjects are patients with acute sensorineural hearing loss, (1) with abnormality in evoked otoacoustic emission, (2) within 29 days after the onset of hearing loss, (3) determined as non-responders to systemic steroid application, and (4) age over 20 years. Major exclusion criteria are (1) presenting active middle ear abnormality, (2) history of previous other treatments including systemic application of batroxobin, prostaglandin I, and hyperbaric oxygen therapy, except for systemic steroid application. Each registered patient received a tympanotomy and the RW niche was inspected with a thin endoscope. Gelatin hydrogel combined with recombinant human IGF-1 (rhIGF-1) was placed on the RWM. Average hearing levels and adverse events were followed up for 24 weeks.

Results and Discussion

Inner ear DDS via systemic application – stealth-type nanoparticles

The systemic application of conventional nanoparticles made of PLGA without PEG coating did not lead to distribution in the inner ear [18]. On the other hand, stealth nanoparticles encapsulating rhodamine B distributed to the inner ear. Systemic application of Stealth-Nano-Steroid after the noise-induced hearing loss showed higher concentrations of betamethasone in the inner ear, and better recovery of hearing compared with the simple systemic application of betamethasone (in print).

Local application using PLGA microparticles

When PLGA microparticles encapsulating lidocaine (Figure 1B) were applied on the RWM of guinea pigs and the lidocaine concentrations in the cochlea were measured at various time points, the highest concentrations were observed on day 3. Nystagmus was not induced by this procedure. Hearing thresholds determined by auditory brainstem responses showed only temporal elevation on day 7. Inflammatory responses in the middle and inner ear were not observed except for minor mucosal thickening and lymphatic cell infiltrations. These results suggest a high possibility for the clinical application of these particles for the treatment of tinnitus without causing serious adverse effects [11]. Animal experiments to show the effectiveness of these particles are difficult because tinnitus is a subjective symptom; however, there are a number of animal models to evaluate tinnitus in rodents [19,20]. We are investigating the effects on the reduction of tinnitus in rodents, and at the same time, a clinical trial is planned.

Local application using gelatin hydrogels

With this method, average hearing levels were comparable to hyperbaric oxygen therapy, which we usually use as a rescue after the failure of systemic steroid therapy. No serious related adverse events were observed. Details of the results will be published separately.

Conclusions

We have developed several DDS that can be used for the treatment of inner ear diseases. All the materials described above were selected from those that are already used in clinics to facilitate clinical applications. These strategies will become templates to realize clinical application of other candidate agents for the treatment of inner ear diseases. We would like to focus more on the demonstration of clinical usefulness of these inner ear DDS.

Acknowledgments

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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ORIGINAL ARTICLE

Cochlear implantation in patients with prelingual hearing loss

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Abstract

Conclusion: The average age at the time of cochlear implantation is progressively being reduced. While cochlear obstruction and perilymph/cerebello-spinal fluid gusher were found in some cases, preoperative MRI and CT scans were predictive of such occurrences. The preoperative developmental quotient in the Cognitive-Adaptive Area was strongly correlated to the postoperative development in the Language-Social Area. *Objective:* To summarize the background, implant devices, intraoperative findings, and postoperative developmental quotients of prelingually deafened patients who underwent cochlear implantation. *Methods:* We conducted a retrospective chart review of 134 prelingually deafened cochlear implant recipients. *Results:* The median age at implantation was 3 years and 5 months. Most patients were born deaf without any known etiologies. In most cases, the transmastoid facial recess approach was utilized. Cochlear obstruction was identified in four patients, all of whom lost their hearing as a result of meningitis. Perilymph/cerebello-spinal fluid gusher was observed in six patients with inner ear anomalies. The preoperative developmental quotient for the Cognitive-Adaptive Area showed significant correlation with the postoperative developmental quotient in the Language-Social Area with a correlation coefficient of 0.71.

Keywords: *Cochlear implant, deaf, children*

Introduction

Cochlear implantation is now a standard treatment for patients with profound sensorineural hearing loss. While the cochlear implant was initially designated for use in patients with post-lingual hearing loss, after encouraging early reports [1], many studies revealed that cochlear implantation is also effective for patients with prelingual hearing loss. Now cochlear implantation is widely accepted for patients with prelingual hearing loss.

At the Kyoto University Hospital Department of Otolaryngology-Head and Neck Surgery, the first cochlear implantation was performed in 1987. We started cochlear implantation in children in July 1991. In this paper we report the background, implant devices, intraoperative findings, and postoperative development of prelingually deafened patients who underwent cochlear implantation.

Material and methods

Between April 1987 and December 2009, 287 cochlear implantations were performed at the Kyoto University Hospital Department of Otolaryngology-Head and Neck Surgery. Since the first cochlear implantation surgery in a child in July 1991, 134 prelingually deaf patients have received cochlear implantation (73 males and 61 females). We analyzed patient demographics, implant devices, intraoperative findings, and postoperative developmental quotients for these patients.

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Patient demographics

Patient demographics included the age at operation, etiology of the deafness, and preoperative developmental quotient. The preoperative developmental quotient (DQ) was evaluated using the Kyoto Scale for Psychological Development. The Kyoto Scale of Psychological Development is a widely used developmental test with satisfactory reliability and validity in Japan. This scale consists of three subtests: a test for the Postural-Motor Area (PM) of development, a test for the Language-Social Area (LS) of development, and a test for the Cognitive-Adaptive Area (CA) of development. In most patients, the DQ for the PM was above the upper limit, so we focused analysis on the DQ for the LS and the CA.

Implant devices

All of the implanted devices were multichannel devices. Patients were implanted with the Nucleus 22 between 1987 and 1999, and the Nucleus 24 between 2000 and 2007. After the approval of the HiRes90K and Combi40+/Pulsar devices in April 2008, the patients' families were allowed to choose the implant device for themselves.

Intraoperative findings

The surgical approaches utilized were collected from the operation records. The incidence of obstruction of the cochlea and perilymph/cerebello-spinal fluid gusher was also reviewed.

Postoperative development

In 36 patients, the DQ was obtained more than 6 months after the cochlear implantation surgery. In these patients, the DQ for LS and CA before and after the operation was compared using a paired *t* test. The correlation of preoperative DQ and the postoperative DQ for LS was evaluated by calculating Pearson's correlation coefficient.

Results

Patient demographics

The age at implantation ranged from 1 year and 2 months to 41 years and 11 months. The median age at implantation was 3 years and 5 months (Figure 1). The median age at implantation in the most recent 4-year interval was lower than in previous years (Figure 2).

In all, 101 patients were born deaf without any known etiologies. The other etiologies of deafness included: inner ear and/or internal auditory canal anomaly in 17 patients; meningitis in 8 patients; cytomegalovirus infection in 3 patients; rubella infection in 2 patients; drug-induced in 1 patient; bilateral sudden hearing loss in 1 patient; and Goldenhar syndrome in 1 patient.

The preoperative DQ for LS ranged from 13 to 117, with an average of 61. Only 20 patients (15%) were within the normal range (≥ 80). The preoperative DQ for CA ranged from 39 to 121, with an average of 88. Seventy-nine patients (59%) were scored in the normal range (≥ 80).

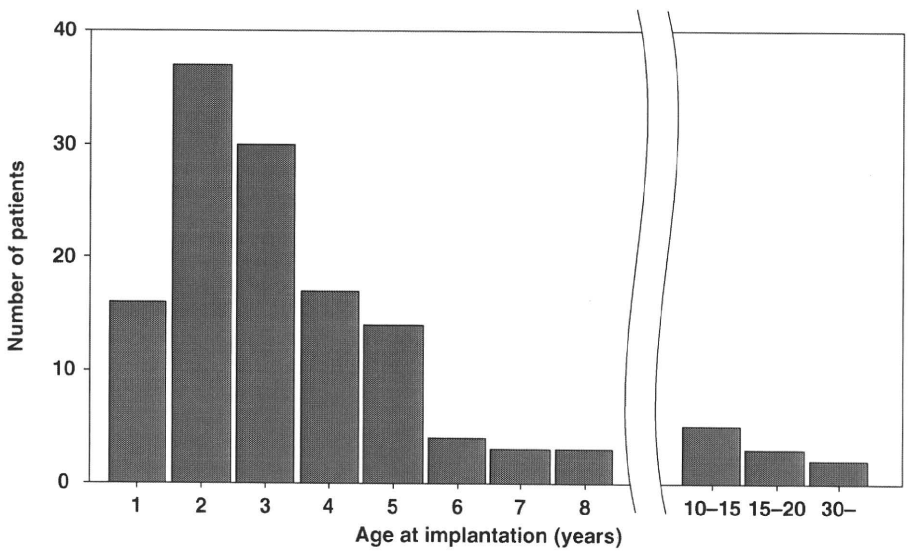

Figure 1. Age distribution at the time of cochlear implantation. The age at implantation ranged from 1 year and 2 months to 41 years and 11 months. The median age at implantation was 3 years and 5 months. Note that most patients underwent surgery at or before the age of 3 years.

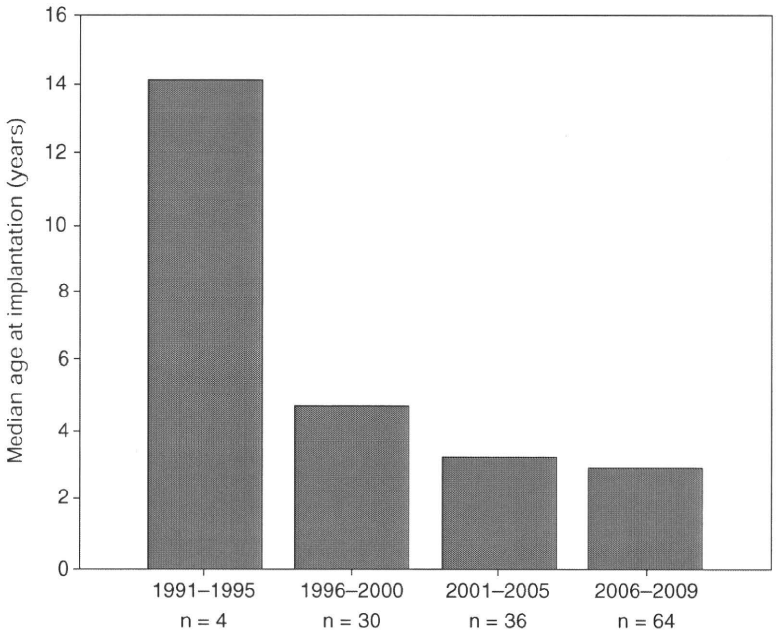

Figure 2. Change in the median age at the time of cochlear implantation over time. The age of cochlear implantation recipients is continually getting lower and the number of patients is getting larger. The median age at implantation in the most recent 4-year interval was lower than in previous years.

Implant devices

Twenty-five patients were implanted with the Nucleus 22 device, 105 patients with the Nucleus 24, and the other 4 patients with the HiRes90K. No patients were implanted with MEDEL devices.

Intraoperative findings

In 123 patients, the electrodes were inserted using the transmastoid facial recess approach. In four patients, partial removal of the posterior canal wall with subsequent reconstruction was needed because of poor mastoid development. In two patients, the posterior canal wall was totally removed and the external auditory canal was closed to control middle ear inflammation. In four patients, the electrodes were inserted through the external auditory canal. One patient had atresia, and the electrode was inserted after opening the tympanic cavity.

In four patients (3.0%), cochlear obstruction was identified during the operation. All had lost their hearing as a result of meningitis. In one of the four patients, the inferior portion of the basal turn of the cochlea was ossified, although the preoperative MRI showed a narrow but patent cochlea (Figures 3 and 4). Perilymph/cerebello-spinal fluid gusher was observed in six patients (4.5%). All six patients had inner ear anomalies. In four patients, the cribriform plate was absent (Figure 5). In the other two patients, the vestibular aqueduct was enlarged. In all six cases, the perilymph/cerebello-spinal fluid gusher was controlled with reverse Trendelenburg positioning, administration of mannitol, and subsequent electrode insertion and packing of the cochleostomy with the temporal muscle fascia.

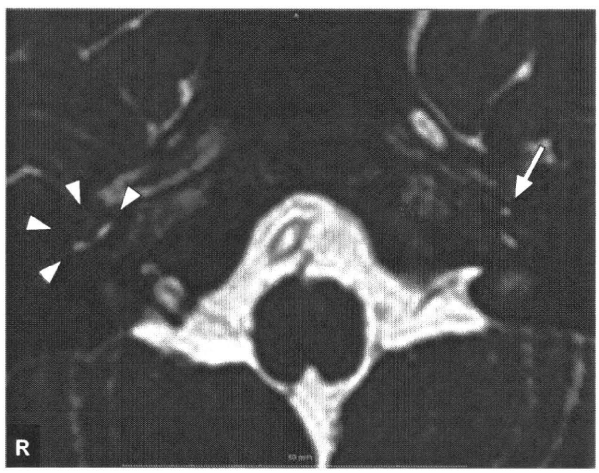

Figure 3. Preoperative high resolution T2-weighted MRI of a patient with an obstructed cochlea. The inferior portion of the basal turn of the cochlea was narrow but patent on the right side (arrowhead). Only the ascending portion of the basal turn of the cochlea was visible on the left side (arrow). In four patients, cochlear obstruction was identified during the operation. All had lost their hearing as a result of meningitis.

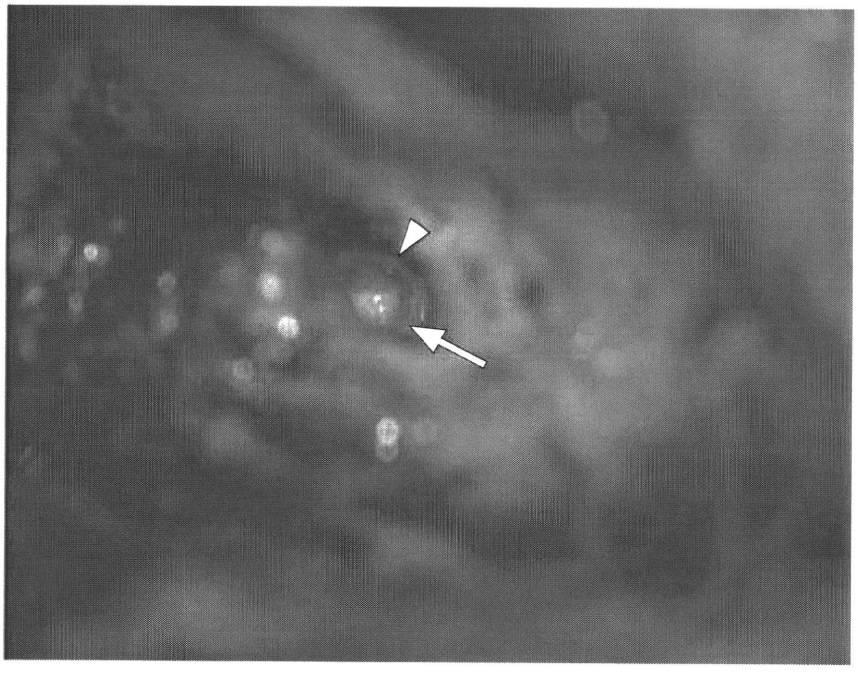

Figure 4. Intraoperative findings of a cochlear implantation surgery in the right ear. The inferior portion of the basal turn of the cochlea was totally ossified, although the preoperative MRI showed a narrow but patent cochlea. Note that the color of the scala tympani (arrow) and scala vestibuli (arrowhead) is different as a result of the different degrees of ossification.

Postoperative developmental quotient

In the 36 patients in whom postoperative DQ was measured, the average preoperative DQ was 60 for LS and 86 for CA, which was similar to the preoperative average of all 134 patients. The postoperative DQ for LS was 77, which was significantly higher than the preoperative value ($p < 0.01$, paired t test) (Figure 6), but still lower than the normal value (≥ 80). The correlation coefficient between pre- and postoperative

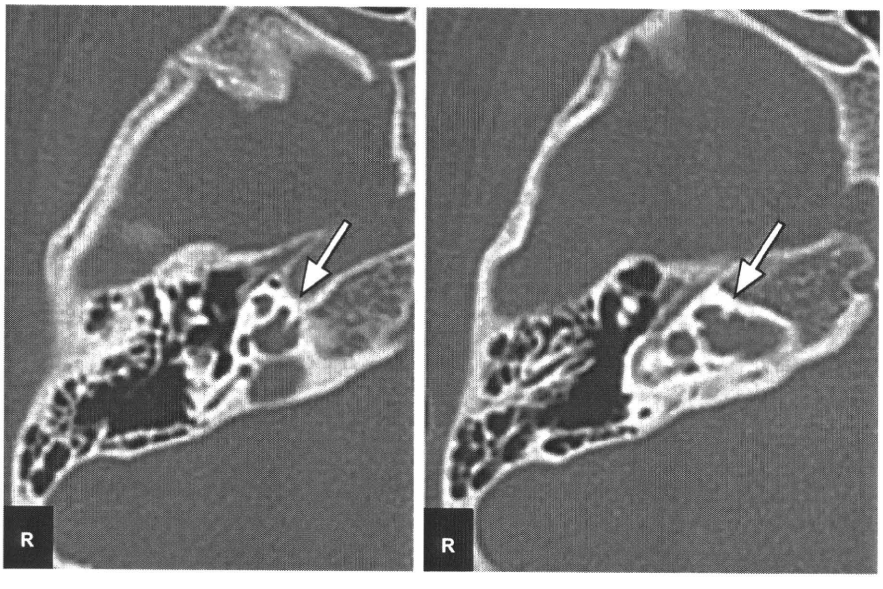

Figure 5. Preoperative high resolution CT scan of a patient. In this patient, two serial slices of CT images showed that the cribriform plate was absent (arrow). During the operation, perilymph/cerebello-spinal fluid gusher occurred after cochleostomy. All six patients showing perilymph/cerebello-spinal fluid gusher had inner ear anomalies.

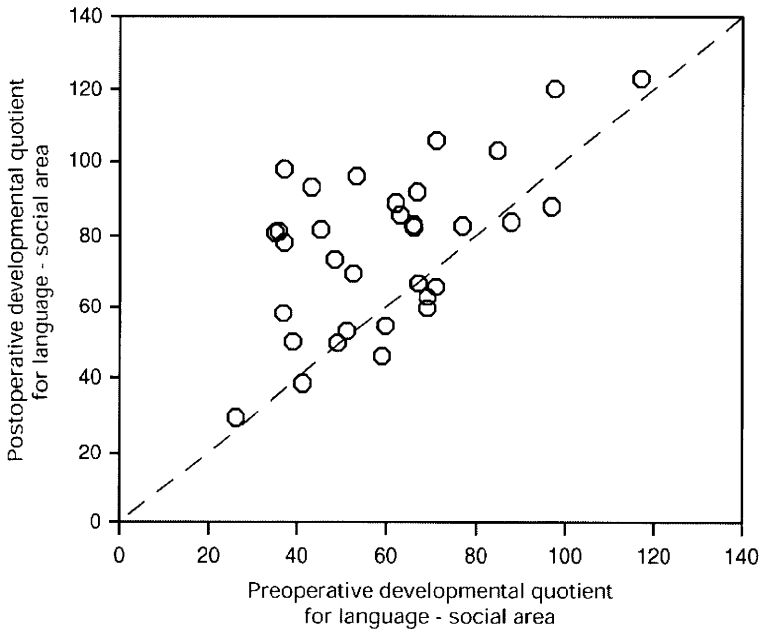

Figure 6. The pre- and postoperative developmental quotient (DQ) for the Language-Social Area (LS). The dashed line indicates where the pre- and postoperative DQ shows the same value. The average pre- and postoperative DQ for the LS were 60 and 77, respectively. The improvement in this area following cochlear implantation was significant ($p < 0.01$, paired t test).

DQ for LS was 0.57 (Pearson's correlation coefficient, $p < 0.01$). The postoperative DQ for CA was 90, and was not significantly changed from the preoperative value ($p = 0.11$, paired t test). However, the correlation coefficient between preoperative DQ for CA and postoperative DQ for LS was 0.71 (Pearson's correlation coefficient, $p < 0.01$) (Figure 7), meaning that the preoperative DQ for CA was strongly correlated with postoperative development in the linguistic area.

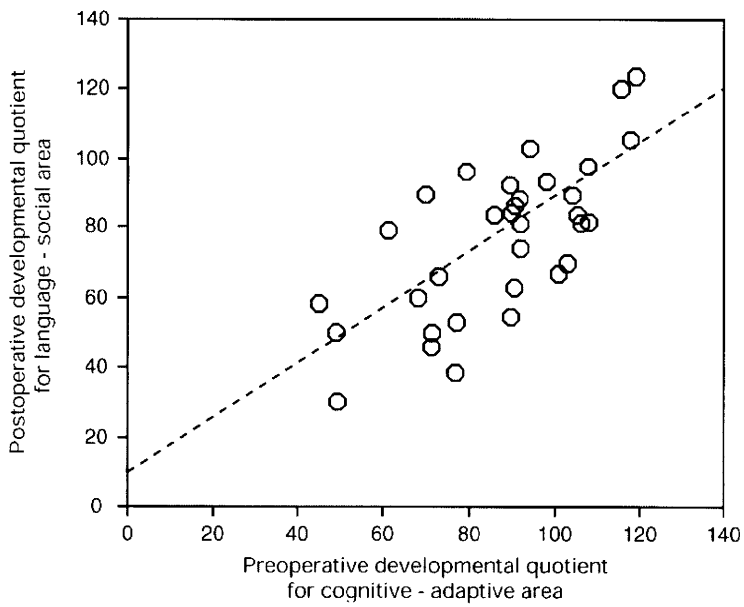

Figure 7. The preoperative developmental quotient (DQ) for the Cognitive-Adaptive Area (CA) and postoperative DQ for the Language-Social Area (LS). A strong correlation was observed ($r = 0.71$, $p < 0.01$, Pearson's correlation coefficient). The dotted line indicates the estimated regression line (regression coefficient = 0.79, intercept = 8.00). This means that the preoperative DQ for the CA was strongly correlated with postoperative development in the linguistic area.

Discussion

Cochlear implantation in patients with prelingual hearing loss is widely accepted [2,3]. Numerous studies have reported that the age at implantation is an important prognostic factor for linguistic development [4,5]. Excluding those patients who received operations during the first 5 years in which cochlear implantation was being performed at Kyoto University Hospital, most of the patients in our institute underwent operation at or before the age of 3 years. With the improvement in devices and coding strategies, old age is no longer a contraindication for cochlear implantation [6]. In such cases, however, the benefit is limited and the indications for cochlear implantation in prelingually deafened adults must be determined carefully.

In most patients, the standard transmastoid facial recess approach was utilized. In some cases, however, alternative procedures were needed. Previous studies reported the effectiveness of total or partial removal of the posterior canal wall and subsequent reconstruction with hard tissue [7] or soft tissue [8]. This procedure facilitates the approach to the round window and is useful in cases with poor mastoid development; four of our patients required this procedure. Radical mastoidectomy and closure of the external auditory canal is effective in controlling middle ear inflammation [9]. We adopted this technique in two cases with accompanying inflammation. The transmeatal approach was proposed as a minimally invasive surgery [10,11]. In addition to the minimal invasiveness, this approach is very useful in cases without mastoid development. We experienced four cases with little or no mastoid development; in these cases, the electrodes were inserted into the cochlea through the transmeatal approach.

Cochlear obstructions were found in four patients, and were apparent in all of the preoperative MRIs. In three cases, the degree of the obstruction was correctly predicted and the electrode was fully inserted after the removal of the lesions inside the cochlea. In the other case, shown in Figures 3 and 4, the preoperative MRI findings indicated that the basal turn was narrow but patent. The intraoperative findings, however, revealed that the inferior portion of the basal turn was totally ossified. The ossification was drilled out and the electrodes were fully inserted. Although the preoperative MRI predicts cochlear obstruction accurately in about 90% of patients [12], it is not perfect. Cochlear implant recipients with obstructed cochleae usually have successful outcomes. However, in patients with severe obstruction the number of active electrodes tends to be small, resulting in poor performance [13]. In patients deafened by meningitis, it is important to obtain the MRI just before cochlear implantation surgery.

In our series, perilymph/cerebello-spinal fluid gusher was observed in six patients. Although perilymph/cerebello-spinal fluid gusher is not always predictable [14], some CT findings strongly predict the incidence of the gusher; namely, an absent cribriform plate and/or an enlarged vestibular aqueduct. In all of our cases, the preoperative CT showed these findings. Perilymph/cerebello-spinal fluid gusher was alleviated by reverse Trendelenburg positioning, administration of mannitol, or just waiting. Once the perilymph/cerebello-spinal fluid gusher was alleviated, we inserted the electrode and sealed the cochleostomy with temporal muscle fascia. We did not encounter cases requiring spinal drain placement or middle ear packing.

The DQ for LS improved after cochlear implantation, as reported previously [2,3]. Developmental delay, especially in the area of cognition [15], adversely affects postoperative speech development [16]. In our study, the preoperative DQ for CA was strongly correlated with the postoperative DQ for LS. However, it is possible that the test to evaluate higher level language and social skills was difficult for them, even though they were achieving good speech development. Otherwise, patients with cognitive delay may eventually catch up with patients without such delay, since we examined the development only in the early stage. The effect of preoperative cognitive development on postoperative language improvement has yet to be explored.

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ORIGINAL ARTICLE

Multivariate analysis of hearing outcomes in patients with idiopathic sudden sensorineural hearing loss

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Abstract

Conclusions: Contralateral hearing loss is significantly correlated with poor hearing outcomes in patients with idiopathic sudden sensorineural hearing loss (ISSNHL). *Background:* The hearing outcome in patients with ISSNHL was analyzed using multiple variables. *Methods:* A retrospective chart review was conducted using 89 patients with ISSNHL. Patients within 40 dB HL of average hearing levels and/or patients whose hearing loss was restricted to low frequencies were excluded. The influence of pre-existing conditions on hearing outcome was analyzed using a polytomous universal model. Pre-existing conditions analyzed included hyperglycemia, hypercholesterolemia, hypertension, and contralateral hearing loss. In addition, the severity of hearing loss, age group, and the existence of vertigo were analyzed concomitantly. *Results:* Hearing recovery was significantly reduced in patients with a past history of contralateral hearing loss.

Keywords: *Contralateral hearing loss, pre-existing conditions, polytomous universal model*

Introduction

Idiopathic sudden sensorineural hearing loss (ISSNHL) is defined as inner ear hearing loss that develops abruptly without definitive causes. Although ISSNHL is one of the few sensorineural hearing disorders that can be cured, the hearing outcome differs greatly among cases. This diverse outcome is usually attributed to the heterogeneity of ISSNHL. No single pathophysiology can fully explain this diversity and various pathogeneses have been hypothesized to explain this disorder, including viral infections [1,2], genetic factors [3,4], and microvascular disturbance [1]. Given that different pathogeneses often result in different outcomes, prior studies have tried to correlate pre-existing pathological conditions with the hearing outcome of ISSNHL. For example, metabolic (e.g. hyperglycemia and hypercholesterolemia) and circulatory disorders (e.g. hypertension) may suggest underlying microvascular disorders as a cause of ISSNHL [5,6], while prior hearing disturbance in the contralateral ear may be suggestive of genetic factors [3]. However, the prognostic value of these factors differed from study to study. One reason for the inconsistencies may be the analytic procedures used. In previous studies aimed at evaluating prognostic values, the effects of single factors were analyzed. However, the results obtained from analyzing a single factor can be distorted by the existence of multiple background factors. Hearing level before treatment, age of the patient, and the existence of vertigo have all been reported to affect the prognosis of ISSNHL [7]; therefore, these factors should be analyzed concomitantly to more accurately assess the effect of pre-existing conditions on the hearing outcomes of ISSNHL. Since some of these factors are ordinal and the others are nominal, multifactorial analyses that can handle ordinal or nominal factors are needed. An analysis with a polytomous universal model is used to test for the effects of multiple independent factors on an ordinal dependent variable, which, in this case, is hearing outcome. These independent factors can be ordinal or nominal. Using this model it is possible to determine the significance of

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these independent variables. Thus, in this study we analyzed the influence of pre-existing pathological conditions on the post-treatment hearing results of ISSNHL using a polytomous universal model.

Material and methods

Patients

We conducted a retrospective chart review of patients who received initial treatment between January 2002 and December 2009 from the Department of Otolaryngology-Head & Neck Surgery, Kyoto University Graduate School of Medicine. One hundred and five ISSNHL patients who were more than 18 years of age visited the hospital within 2 weeks after the onset of hearing loss. Patients within 40 dB HL of average hearing levels at five frequencies between 250 and 4000 Hz and/or patients whose hearing loss was restricted to low frequencies were excluded from this study, because such patients were reported to have better hearing prognoses [8]. Some patients declined to receive the standard treatment provided by our hospital and they were also excluded from the analysis. In total, 89 patients with ISSNHL were included in the analysis. There were 50 men and 39 women ranging from 19 to 83 years of age (mean 53.0 years). All patients were hospitalized and administered corticosteroid intravenously (starting with 200 mg of prednisolone, tapered over 9 days), vitamin B12 perorally (1500 μg per day, during the follow-up) and vasodilators perorally (kallidinogenase 150 IU/mg per day, during the follow-up). Some patients were administered hyperbaric oxygen. Patient follow-ups were conducted until they showed complete recovery or their hearing level was stabilized for more than 1 month (fixed stage).

Analytic procedure

Auditory function was determined by pure-tone audiometry and was expressed by the pure-tone average (PTA in decibels) hearing level at five frequencies (250, 500, 1000, 2000, and 4000 Hz). The PTA before treatment was obtained at the first visit and the post-treatment PTA was obtained at the fixed stage.

Hearing outcome was analyzed based on the criteria prepared by the Acute Severe Hearing Loss Study Group [9]. Using these criteria, the outcome was graded into four classes: (1) complete recovery, recovery to a hearing level within 20 dB HL at all five frequencies between 250 and 4000 Hz, and/or recovery to the same hearing level as the 'good' side; (2) marked recovery, more than 30 dB recovery in the mean hearing level at the five frequencies tested; (3) slight recovery, recovery of 10–29 dB in the mean hearing level at the five frequencies tested; and (4) no response, recovery < 10 dB in the mean hearing level at the five frequencies tested. The hearing outcomes based on these criteria were analyzed using a polytomous universal model.

We recorded the following conditions for this analysis: hyperglycemia, hypercholesterolemia, hypertension, and a past history of contralateral hearing loss. In addition, severity of hearing loss, age group, and the existence of vertigo were also included in the analysis [7]. Hyperglycemic patients were defined as those who had been treated for hyperglycemia and/or whose fasting glucose at the first visit exceeded 100 mg/dl. The hypercholesterolemic patients included those previously treated for this condition and those with total serum cholesterol exceeding 240 mg/dl at the initial visit. Hypertensive patients were defined as those who had been treated for hypertension and/or whose blood pressure continued to measure 140/90 mmHg or higher before the administration of steroids. Patients with a past history of contralateral hearing loss were defined as those who had displayed distinct symptoms in the non-affected ear including hearing loss, tinnitus, and ear fullness before the onset of ISSNHL and whose PTA was above 40 dBHL. Sensorineural hearing loss compatible with normal age-related changes and/or hearing loss without subjective symptoms were not included.

In accordance with the criteria prepared by the Acute Severe Hearing Loss Study Group [9] the severity of hearing loss was described using the PTA at five frequencies (250, 500, 1000, 2000, and 4000 Hz): (1) moderate, PTA of 40–59 dB HL; (2) severe, PTA of 60–89 dB HL; (3) profound, PTA of > 90 dB HL. The patients were classified into three age groups: the young group consisted of those who were younger than 30, the middle-aged group was between 30 and 60, and those in the old group were over 60 years of age. The presence of vertigo was defined as rotatory sensation with nystagmus. Statistical analysis was conducted using SPSS software.

Results

The grand-averaged pretreatment hearing level was 79.1 dB HL, while the post-treatment hearing level was 44.6 dB HL. The averaged absolute hearing gain was 34.5 dB. Based on the criteria set by the Acute Severe Hearing Loss Study Group, 23 patients were evaluated as showing complete recovery, 35 patients

as marked recovery, 16 patients as slight recovery, and 15 patients as showing no response. The treatment results are shown in Figure 1.

The severity of the hearing loss before treatment was moderate in 18 patients, severe in 40 patients, and profound in 31 patients. Sixteen patients were classified as young, 37 patients were classified as middle-aged, and the remaining 36 patients were classified as old. The hearing outcomes according to pretreatment severity and age group are shown in Tables I and II, respectively. Among the 89 patients, 10 patients had hyperglycemia, 27 patients had hypercholesterolemia, 20 patients had hypertension, and 12 patients had contralateral hearing loss. Twenty-eight patients had accompanying vertigo.

The analysis of these treatment outcomes using the criteria prepared by the Acute Severe Hearing Loss Study Group showed that a past history of

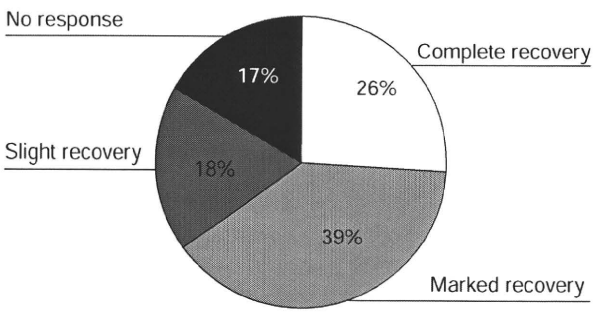

Figure 1. Overall treatment results for patients with idiopathic sudden sensorineural hearing loss. The hearing outcome was graded into four classes: complete recovery, recovery to a hearing level within 20 dB HL at all five frequencies between 250 and 4000 Hz, and/or recovery to the same hearing level as the 'good' side; marked recovery, more than 30 dB recovery in the mean hearing level at the five frequencies tested; slight recovery, recovery of 10–29 dB in the mean hearing level at the five frequencies tested; and no response, recovery < 10 dB in the mean hearing level at the five frequencies tested.

Table I. Summary of hearing outcome according to the pretreatment severity of hearing loss.

	Outcome				
Severity	Complete recovery	Marked recovery	Slight recovery	No response	Total
Moderate	7	2	5	4	18
Severe	13	13	7	7	40
Profound	3	20	4	4	31
Total	23	35	16	15	89

The severity was graded into three classes: moderate, pure-tone average (PTA) of 40–59 dB HL; severe, PTA of 60–89 dB HL; profound, PTA of greater than 90 dB HL.

Table II. Summary of hearing outcome by age group.

	Outcome				
Age group	Complete recovery	Marked recovery	Slight recovery	No response	Total
Young	5	9	2	0	16
Middle aged	7	12	11	7	37
Old	11	14	3	8	36
Total	23	35	16	15	89

The patients were classified into three age groups: the young group consisted of those who were younger than 30, the middle-aged group was between 30 and 60, and those in the old group were over 60 years of age.

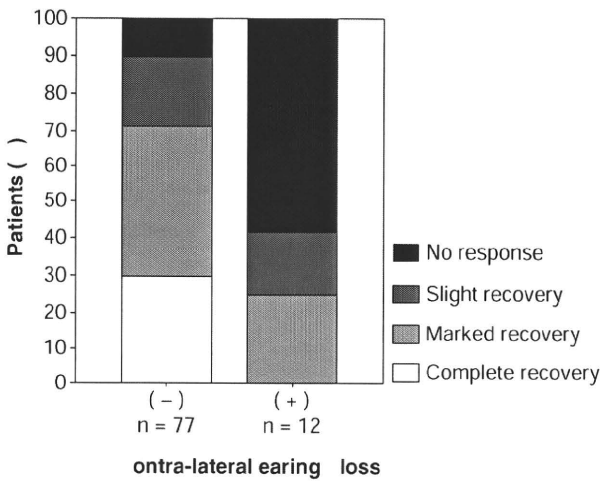

Figure 2. Hearing treatment results for patients with and without a past history of contralateral hearing loss. The treatment results from the groups with a past history of contralateral hearing loss were significantly worse than those without a past history of contralateral hearing loss.

contralateral hearing loss is significantly correlated ($p < 0.01$) with reduced recovery from ISSNHL. Figure 2 shows a statistically significant difference ($p < 0.01$, Mann-Whitney test) between the treatment results from the groups with and without a past history of contralateral hearing loss. The overall results of the analysis are shown in Table III.

Discussion

The effect of a past history of contralateral hearing loss on hearing outcomes following ISSNHL remains controversial. Stahl and Cohen have reported that the hearing outcome of ISSNHL is the same in patients with or without good hearing in the opposite ear [10]. In contrast, when Cvorović et al. analyzed 541 cases