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ORIGINAL ARTICLE

Morphologic Change and Hearing Recovery After Intratympanic Application of Insulin-Like Growth Factor-1 in Guinea Pig

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Objective: This study investigated effectiveness of intratympanic insulin-like growth factor-1 (IGF-1) application on deaf guinea pigs.

Materials and Methods: The coadministration of kanamycin and ethacrynic acid was used to deafen the guinea pigs. An absorbable gelatin sponge immersed in recombinant human insulin-like growth factor-1 (rhIGF-1) was placed on the round window membrane of the experimental ear while a sponge containing saline was applied to control ear. Hearing function was assessed by auditory brain stem responses (ABRs). The temporal bones were collected for analyzing histology of hair cells in the organ of Corti.

Results: Local rhIGF-1 treatment significantly reduced the elevation of ABR thresholds after applying the ototoxic agents. Histological analysis revealed that local rhIGF-1 treatment could promote repair of the hair cells.

Conclusion: These findings demonstrate that local IGF-1 application has potential in repairing outer hair cells damaged by ototoxic agents. On the basis of this study, the clinical use of local IGF-1 application with a gelatin sponge may be useful as a therapeutic option for the treatment of sensorineural hearing loss.

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Introduction

Cochlear hair cells can be damaged by various factors, such as excessive noise, ototoxic agents and aging, all of which trigger sensorineural hearing loss. Hearing aids and cochlear implants, which are currently used to treat hearing loss, show the limitations in the recovery of hearing function and the progression of hearing loss. Regeneration of cochlear hair cells has been proposed as a more rudimentary method for treating hearing loss. Many studies are currently being conducted to examine treatment methods with which appropriate growth factors can be used to regenerate and protect inner hair cells.[1,2]

Insulin-like growth factors (IGFs) play pivotal roles in nervous system development and functional maintenance.[3] IGFs consist of insulin, IGF-1, IGF-2 and IGF-binding protein. ; IGF-1 is expressed during the development of the inner ear and in the cochleovestibular ganglion after birth. In particular, animal experiments have shown that IGF-1 facilitates differentiation of epithelial cells forming the utricle in the inner ear and it strengthens regeneration of vestibular hair cells in the utricle that have been damaged by ototoxic agents.[4] In addition, mutations in the genes encoding these proteins can reportedly lead to sensory hearing loss.[5] Examples include Turner syndrome and Noonan syndrome, in which small stature and hearing loss are associated with low serum concentrations of IGF-1. IGF-1 has been demonstrated to be a neurotrophic factor and its deficiency results in sensorineural hearing loss in humans. Thus, IGF-1 has been used as a therapeutic agent to alleviate neurodegenerative disease.[5]

This study was designed to examine the effect of

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intratympanic IGF-1 application on the regeneration of damaged hair cells and the recovery of hearing function after combined administration of kanamycin and ethacrynic acid in guinea pigs.

Materials and Methods

Experimental animals and deafening procedure

Four male China albino guinea pigs weighing 300-400 g were used. Their auricular reflex and tympanic membranes were normal. All experimental procedures were approved by the university's committee on animal research. Guinea pigs were concomitantly treated with kanamycin (Donga Pharmaceutical Corp., Seoul, Korea) and ethacrynic acid (Sigma Chemical, St. Louis, MO, USA). First, kanamycin (50 ng/mL) was subcutaneously injected into the posterior neck (500 mg/kg). Two hours later, ethacrynic acid (40 mg/kg) dissolved into dimethyl sulfoxide was injected into the tail vein. One week later, hearing loss was defined by observing absence of normal wave III at an amplitude of over 90 dB nHL auditory brain stem response (ABR) test.

Classification of the experimental group and method of intratympanic rhIGF-1 application

In the same animals, the right and left ears were used as the experimental and control groups, respectively. In all four guinea pigs, presence of total deafness was confirmed. The animals were anesthetized before intratympanic rhIGF-1 application. Under a surgical microscope, a tympanotomy was performed at the posteroinferior quadrant of the tympanic membrane. For the right ear, the experimental group, a gelatin sponge (Gelfoam®; Baxter Corp., Deerfield, IL, USA) was immersed in rhIGF-1 (20 μg dissolved in 2 μL physiological saline; R&D Systems, Minneapolis, MN, USA) and was positioned on the round window membrane. Using the same method, in the control group, a gelatin sponge that had been immersed in physiological saline was placed in the left ear.

Auditory brain stem response (ABR) measurements

At weeks 1, 3, and 5 following the administration of rhIGF-1, recordings of ABRs were performed. The Navigator Pro (580 NAVPR2; Biologic System Corp., Mundelein, IL, USA) was used in a soundproof chamber. The animals were anesthetized before testing. An action potential lead was inserted in the parietal region, the reference lead was inserted in the posteroinferior area of both ears, and the ground lead was inserted in the mastoid area of both ears. Thus, the action potential was measured. An insertion-type earphone was placed in the bilateral external ear canal and the electrical impulse was assessed. For auditory stimulation, a click sound of 100 μs was made at a rate of 11.1 times/s, a total of 1,024 times. To measure the hearing threshold, stimulus intensity was lowered by 10 dB from 90 dB nHL in a stepwise manner. Then, the level where the measurable wave III was observed was defined as the hearing threshold.

Preparation of tissue samples for light microscopy

The guinea pigs were anesthetized with 100 mg/kg pentobarbital, given as an intraperitoneal infusion. Then, the heart was exposed and a catheter was inserted into the aorta. Rinsing was done using 0.2 M phosphate buffer solution. Blood was completely removed and 10% formalin solution was infused via the catheter. The temporal bone was fixed in an in vivo setting. The temporal bone of guinea pigs was dissected under a surgical microscope. The cochlea was extracted and fixed in 10% formalin solution at 4°C for 24 h. For 2 weeks, bones were decalcified using a mixture of 25% formic acid and 5% nitric acid. Then, for a further 24 h, the tissue sample was placed in 5% sodium sulfate and then rinsed with 0.2 M phosphate buffer solution. Dehydration was done using graded ethanols, and samples were embedded in paraffin. The paraffin-embedded tissue was sectioned at a thickness of 3 μm and sections were stained with hematoxylin and eosin (H&E) for light microscopy.

Preparation of tissue samples for electron microscopy

Animals were anesthetized with 100 mg/kg pentobarbital, given as an intraperitoneal infusion. The experimental animals were then killed. The temporal bone was harvested and cochlear tissue was extracted under a surgical microscope. The tissue sample was fixed in a mixture of 2.5% glutaraldehyde and 4% paraformaldehyde for 24 h. Rinsing was done using

0.1 M phosphate buffer solution, three times. The anterior bony part of the cochlea, which was fixed, was removed using microsurgical instruments under a microscope. The stria vascularis was dissected, and the organ of Corti was exposed. This was fixed using 2% osmium tetroxide for 2 h. The fixed sample was dehydrated with increasing concentrations of ethanol. After drying at critical points, gold impregnation was done using an ion sputterer. The samples were then examined with a scanning electron microscope (JSM 5410LV; JEOL, Tokyo, Japan).

Results

Auditory brain stem response

After application of rhIGF-1, the ABR was measured at weeks 1, 3, and 5. The ABR threshold showing the normal wave III was decreased maximally down to 50 dB nHL in the experimental ear in which rhIGF-1 was administered (Figure 1). Also, in the experimental ears, decreased hearing thresholds were noted from week 3 on (Figure 2A). The mean threshold measured at week 5 was 60 dB nHL in the experimental ears and 90 dB nHL in the control ears (Figure 2B). This confirmed that the hearing had partially recovered after the application of IGF-1.

Light microscopy findings

The stria vascularis was relatively intact in both the experimental and control groups. In the control group, overall distortion of the organ of Corti was observed.

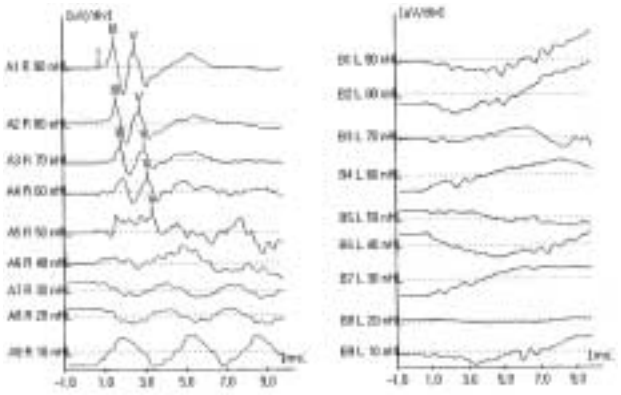

Figure 1. ABR recordings at week 5 in guinea pig treated with intratympanic IGF-1 and saline (A, IGF-1; B, saline). ABR thresholds of the IGF-1- and saline-treated ears were 50 dB nHL and 90 dB nHL, respectively.

Supporting cells, such as Deiter cells, were well arranged, but partial changes and loss in the arrangement of outer hair cells were observed (Figure 3A). In some areas, a loss of inner hair cells was seen, but this was smaller than the loss of outer hair cells. After the application of rhIGF-1, the well organized organ of Corti was observed in the experimental ears. The arrangement of outer hair cells was noted, and the shape of the inner hair cells was well maintained compared to the control group (Figure 3B). Damage to or regeneration of stereocilia on hair cells could not be seen well with H&E staining.

Scanning electron microscopy findings

In the control ears, partial distortion of the stereocilia

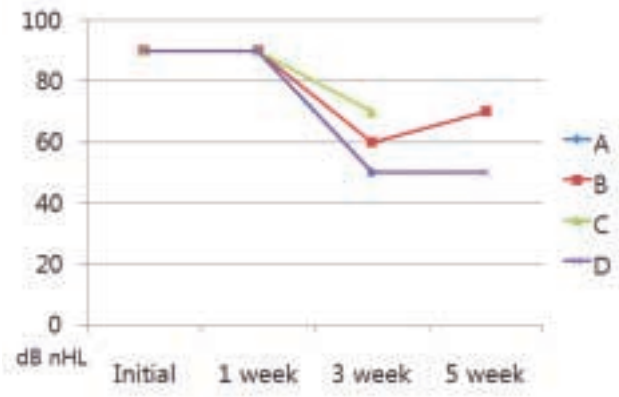

Figure 2A. Individual ABR thresholds at survival time in guinea pigs treated with rhIGF-1 for 1-5 consecutive weeks. Guinea pig D shows the maximal threshold shift (by 40 dB nHL) after 5 weeks.

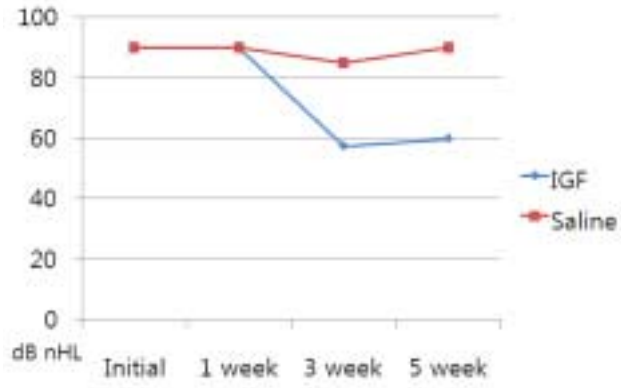

Figure 2B. Mean ABR threshold in guinea pigs treated with rhIGF-1 and saline, respectively. A significant difference in the ABR threshold was detected between the rhIGF-1 and saline groups at 3 weeks after intratympanic application.

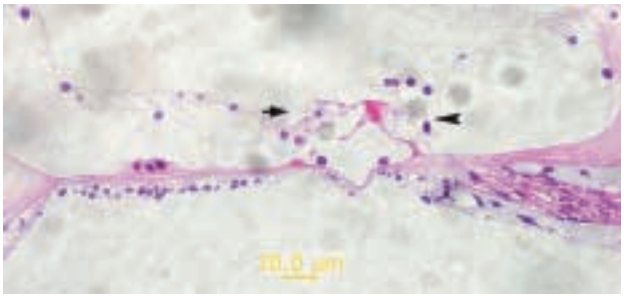

Figure 3A. Light microscopic examination revealed degeneration and loss of cochlear outer hair cells (arrow) and inner hair cells (arrowhead) after 5 weeks in ears receiving intratympanic saline (H&E stain, x400).

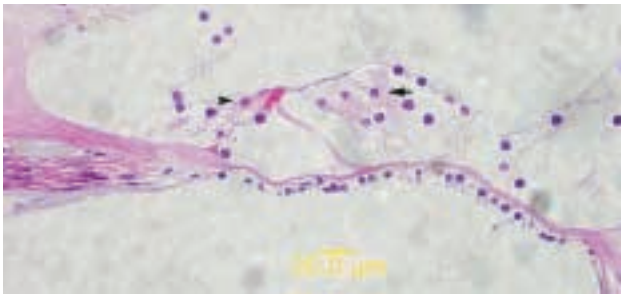

Figure 3B. The cochlear outer hair cells (arrow) and inner hair cells (arrowhead) are relatively repaired in the ear with intratympanic rhIGF-1 application after 5 weeks (H&E stain, x400).

was observed (Figure 4A). As compared with the first row of stereocilia on outer hair cells, on the second and third rows, the deformity and partial loss was more common. Destruction or distortion of stereocilia on the inner hair cells was not observed. In the experimental ear in which rhIGF-1 was applied, damaged outer hair cells had recovered. The arrangement of stereocilia was preserved compared to the control ear. Additionally, self-renewal was apparently achieved and distortion of the outer hair cells was relatively uncommon (Figure 4B). No damage to Deiter cells occurred in either the experimental or control ears.

Discussion

The purpose of this study was to examine potential benefits of local rhIGF-1 application in a gelatin sponge for the treatment of hearing loss caused by ototoxic agents. Drug delivery methods for the inner ear can primarily be divided into systemic and local infusion. In systemic drug application, the blood-inner ear barrier inhibits the transport of drugs from serum to the inner ear. Thus, achieving an appropriate concentration of drugs is difficult. For local drug application, an implantable mini-pump is often used in animal experiments. However, a complex sequence of surgical treatments is necessary and this technique cannot be used in the clinical setting. One method for the local application of drugs, intratympanic application, is a novel treatment modality with which a drug can be effectively delivered into the site in patients with inner ear diseases. It has been used to

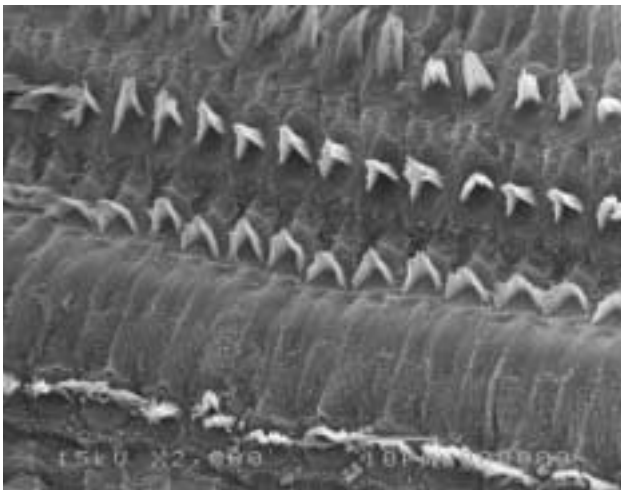

Figure 4A. Scanning electron microscopic findings in a control ear: fusion and distortion of stereocilia of outer hair cells (arrows) was seen after 3 weeks (x2000).

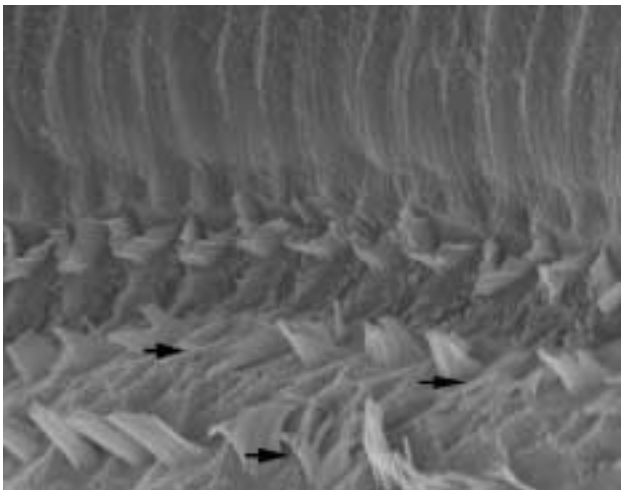

Figure 4B. Scanning electron microscopic findings in an IGF-1-treated ear: the morphology of the sterocilia of hair cells was repaired after 3 weeks (x2000).

treat various inner ear conditions, such as sudden sensorineural hearing loss, autoimmune inner ear disease and intractable tinnitus. If the drugs are to be supplied to the middle ear cavity, they can be absorbed through the round window membrane. They pass through a blood-labyrinth barrier; drugs can be delivered at high concentrations to the endolymph and perilymph. Recent studies have reported the use of a biodegradable hydrogel for the sustained delivery of drugs.[6] In the current study, gelatin sponge (Gelfoam®) insert was used to continuously administer the growth factor into the inner ear, through the round window membrane. Gelfoam® is a polymer gelatin sponge manufactured using collagen. It is a water-insoluble polymer and is then typically absorbed within 4-6 weeks.[7] The use of an implantable mini-pump is advantageous in making changes in the concentration of drugs and administering drugs both objectively and accurately. However, we used a gelatin sponge because it can also be used in the clinical setting. The concentration of drugs can be an important factor in treatment, and studies have reported that the hearing threshold significantly decreased when rhIGF-1 was administered at a dose of 10 μg/μL in the middle ear cavity of guinea pigs.[6] In the current study, we did not examine this issue. Instead, we administered the drugs at the same dose and examined whether this was associated with recovery from hearing loss.

To induce prompt loss of cochlear hair cells in an experimental animal model of deafness, the coadministration of aminoglycoside antibiotics and loop diuretics is often used.[8] In the current study, to make an animal model of deafness, we used kanamycin (an aminoglycoside antibiotic) and ethacrynic acid (a loop diuretic). When ethacrynic acid and kanamycin are coadministered, the former increases the blood flow of the inner ear. Thus, the former prolongs the time during which the latter act on the hair cells. The effects of aminoglycoside antibiotics occur primarily in outer hair cells. According to histopathological studies, the most susceptible region is known to be the outer hair cells, located at the base of the cochlea. Once progressed, the destruction of spiral ganglion cells subsequently occurs.[9]

In this study, the findings confirmed that the threshold as measured using the ABR decreased significantly from week 3 on after applying rhIGF-1. The threshold decreased down to a maximum value of 50 dB nHL. At week 5, however, the threshold was not lower with regard to that at week 3. Indeed, the threshold was actually elevated. Whether a sufficient dose of the drug reached the inner ear could not be confirmed. Further studies are warranted to measure the period during which a sufficient concentration of drugs is present in the perilymph and the time at which the maximal drug concentration is reached using the drug delivery system described here.

No significant decrease in the threshold was observed in the control group. These findings indicate that the injection of ototoxic agents caused irreversible hearing loss. In animal experiments, the local administration of rhIGF-1 has been reported to be effective in protecting the outer hair cells in noise-induced hearing loss.[6] In this study, however, IGF-1 was effective in inducing the repair of damaged hair cells, rather than protecting the hair cells from damage by ototoxic agents.[10] These findings are consistent with previous studies stating that no protective effect on inner hair cells was observed by the administration of growth factors in an experimental animal model of the damage of vestibular hair cells by gentamicin.[4]

A histopathological analysis showed some distortion and partial loss of the hair cells in the control ear. In an experimental ear, the rearrangement of hair cells, which had been deformed, was noted at week 5 after administration of rhIGF-1. These findings indicate that IGF-1 acts on the organ of Corti and thereby has an effect on repairing the deformed hair cells. According to the scanning electron microscopy results, distortion of stereocilia was primarily observed in rows 2 and 3 of the outer hair cells. Distortion of stereocilia of the inner ear hair cells was not seen. Scanning electron microscopy showed that the deformed stereocilia of

the outer hair cells had a normal arrangement 3 weeks after supplying rhIGF-1. Hearing recovery, as confirmed by the ABR, was mainly achieved from week 3 on. These findings suggest that the rearrangement of hair cells following the delivery of rhIGF-1 is possible prior to week 3. In this study, however, whether the lost hair cells could be regenerated was not confirmed. The three mechanisms by which damaged hair cells are replaced are regeneration, transdifferentiation, and repair.[11] Lost hair cells in experiments with birds are replaced by the regenerative proliferation of supporting cells and the transdifferentiation of mature supporting cells into hair cells. In mammals, the process for the regeneration and repair of hair cells has been reported to occur as a result of the transdifferentiation of supporting cells into hair cells and the self-repair of hair cells that received sublethal damage.[12] In this study, scanning electron microscopy showed almost no loss of hair cells. This suggests that the hair cells that received a sublethal insult were repaired following delivery of rhIGF-1.

Finally, we acknowledge the limitations of this study, including the small number of subjects and our not demonstrating a relationship between the concentration of rhIGF-1 in the inner ear and hearing recovery.

Conclusions

The results confirmed that a partial recovery in hearing was achieved after intratympanic application of a gelatin sponge immersed in rhIGF-1 in an experimental animal model of hearing loss due to ototoxic agents. The drug delivery method described here showed no particular adverse effects in the animal experiment. Also, this non-invasive method could readily be repeated in the clinical setting. Based on the results of the current study, we suggest that the intratympanic application of rhIGF-1 may be useful for patients with sensorineural hearing loss. However, the exact mechanisms by which IGF-1 acts in hair cells are unclear and require further research. Further studies are also warranted to examine whether hearing recovery can be achieved following the delivery of IGF-1 even in cases of chronic damage of hair cells and auditory neurons. In addition, studies to identify other neurotransmitters involved in the effective recovery of hearing loss and to achieve complete hearing recovery are needed.

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