3. Adenosine Receptor Agonists

Although information on the cochlea is limited, several mechanisms have been proposed to account for the protective effects of A1 adenosine receptor agonists in nervous tissues. It is thought that deleterious Ca^{2+} influx due to glutamate excitotoxicity is a primary target for neuroprotective adenosine actions in the central nervous system [51-53]. The excitotoxicity of cochlear afferent dendrites induced by kainic acid was attenuated by the application of 2-chloro-N6-cyclopentyladenosine (CCPA), a highly selective adenosine A1 receptor agonist. Ford *et al.* [54] reported the effects of the round window application of R-PIA, a selective adenosine A1 receptor agonist, on cochlear antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase. R-PIA elicited significant increases in the activity of these antioxidant enzymes and significantly reduced the levels of malondialdehyde, a marker of lipid peroxidation, generated in the cochlea. Namely, it is possible that adenosine A1 receptor agonists protect the cochlea against ischemia-reperfusion injury by attenuating glutamate excitotoxicity and through a radical scavenging effect. Indeed, CCPA attenuated cochlear injury induced by transient ischemia [55].

4. Growth Factors

Several authors have shown that growth factors like insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), and fibroblast growth factor may protect hair cells against damage triggered by ototoxic drugs or aging. These growth factors are important for the normal development and survival of cells including hair cells of the organ of Corti [56, 57]. Although the effects of growth factors on cochlear ischemia-reperfusion injury have not been fully elucidated, the protective effects of erythropoietin and IGF-1 have been reported [58, 59].

5. Steroids

Glucocorticoids have been widely used in the treatment of idiopathic sudden sensorineural hearing loss in humans. In a basic animal study, the cochlear function 4 hours after transient ischemia was significantly improved by glucocorticoids, prednisolone and methylprednisolone, at a relatively wide dose range in the case of pre-ischemic administration. On post-ischemic administration, higher doses of glucocorticoids were necessary to ameliorate cochlear ischemia-reperfusion injury [54]. It is considered that glucocorticoids do not promote cochlear blood flow to protect hair cells [60, 61], but directly protect outer hair cells [62].

Dehydroepiandrosterone reportedly exhibits protective effects against cochlear ischemia-reperfusion injury [63]. Interestingly, dehydroepiandrosterone also protects hair cells against acoustic injury [64], suggesting that this steroid protects hair cells and the cochlea against other forms of cochlear injury.

CONCLUSION

An understanding of the mechanisms of cochlear ischemia-reperfusion injury is mandatory for the development of therapeutic interventions directed toward this type of injury. When organs are rendered ischemic, there is a shift from aerobic to anaerobic metabolism, which results in a decrease in cellular ATP. Excitotoxicity induced by glutamate efflux and its failed removal from extracellular spaces during ischemia aggravates the ischemic injury of primary auditory neurons. Outer hair cells are the most vulnerable to cochlear reperfusion injury. Reactive oxygen species including hydroxyl radicals and NO are leading factors in the reperfusion injury of outer hair cells. Once recirculation is achieved after ischemia, efforts are needed to prevent cochlear injury induced by reactive oxygen species and to minimize the loss of cochlear function. Many chemical agents including glutamate receptor antagonists, free radical scavengers and NOS inhibitors, adenosine receptor agonists, and growth factors are under investigation. Further research will clarify the details of the pathogenesis of cochlear ischemia-reperfusion injuries, and efforts should be made to establish strategies to protect the cochlea.

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The influences of sphingolipid metabolites on gentamicin-induced hair cell loss of the rat cochlea

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ABSTRACT

Sphingolipid metabolites inducing ceramide, sphingosine, and sphingosine-1-phosphate (S1P) play important roles in the regulation of cell proliferation, survival, and death. Aminoglycoside antibiotics including gentamicin induce inner ear hair cell loss and sensorineural hearing loss. Apoptotic cell death is considered to play a key role in this injury. The present study was designed to investigate the possible involvement of ceramide and S1P in hair cell death due to gentamicin. In addition, the effects of other metabolites of ceramide, gangliosides GM1 (GM1) and GM3 (GM3), on gentamicin ototoxicity were also investigated. Basal turn organ of Corti explants from p3 to p5 rats were maintained in tissue culture and exposed to 20 or 35 μM gentamicin for 48 h. The effects of ceramide, S1P, GM1, and GM3 on gentamicin-induced hair cell loss were examined. Gentamicin-induced hair cell loss was increased by ceramide but was decreased by S1P. GM1 and GM3 exhibited protective effects against gentamicin-induced hair cell death at the limited concentrations. These results indicate that ceramide enhances gentamicin ototoxicity by promoting apoptotic hair cell death, and that S1P, GM1, and GM3 act as cochlear protectants. In conclusion, sphingolipid metabolites influence the apoptotic reaction of hair cells to gentamicin ototoxicity.

The sphingolipid metabolites ceramide, sphingosine, and sphingosine-1-phosphate (S1P) are known as a new class of lipid second messengers and reportedly play essential roles in the regulation of cell proliferation, survival, and death [15,19]. Ceramide and sphingosine usually inhibit cell proliferation and promote apoptosis, while ceramide-derived S1P acts against apoptosis.

Ceramide has been shown to regulate diverse cellular processes including apoptosis, cell senescence, the cell cycle, and cellular differentiation [12]. The levels of ceramide are increased by stimulating sphingomyelinase which converts sphingomyelin to ceramide. Several signaling molecules, such as tumor necrosis factor, Fas ligand, γ-interferon, interleukin 1, vitamin D, and stressful events including radiation and ischemia, can activate sphingomyelinase and promote ceramide-induced apoptosis. Ceramide activates stress-activated protein kinases (SAPKs) such as jun kinases (JNKs), kinase suppressor of Ras (KSR), and atypical protein kinase C (PKC). Ceramide also activates protein phosphatases such as protein phosphatase 1 (PP1) and protein phosphatase 2A (PPA2) [19]. However, the mechanisms of how ceramide activates protein kinases and phosphatases have never been fully elucidated. Sphingosine is formed only by the deacylation of ceramide [8]. Sphingosine has been reported to be a protein kinase C inhibitor and induce apoptosis [15]. In contrast, another metabolite of ceramide, sphingosine-1-phosphate (S1P), influences opposing pathways of apoptosis and cell survival. For example, S1P stimulates the extracellular signal-regulated kinase (ERK) pathway and counteracts SAPKs [4]. Ceramide, sphingosine, and S1P are interconvertible, and their relative levels are suggested to be important to determine cell fate. It has been shown that sphingosine kinase, the enzyme that phosphorylates sphingosine to form S1P, is a critical regulator of this "sphingolipid rheostat" [15].

Gangliosides are also metabolites of ceramide. Ganglioside GM1 (GM1) has been reported to induce the synthesis of S1P [3]. GM1 has also been considered to exhibit a neurotrophic effect. Namely, GM1 prevents the degeneration of neuronal cells [5], being beneficial in treating stroke [11], spinal cord injuries [7], and Alzheimer's disease [24]. Ganglioside GM3 (GM3) is also a metabolite of ceramide that lies upstream of ganglioside GM1 [20]. There have been many studies on the effects of these sphingolipid metabolites on apoptosis in several cell lines, but their effects on cochlear pathophysiology poorly understood.

Aminoglycoside antibiotics including gentamicin induce sensorineural hearing loss and inner ear hair cell loss. Although apoptotic cell death is considered to play a key role in cochlear injury induced by aminoglycosides, the mechanisms of aminoglycoside-induced hair cell apoptosis have not been fully elucidated. This study was designed to investigate the possible

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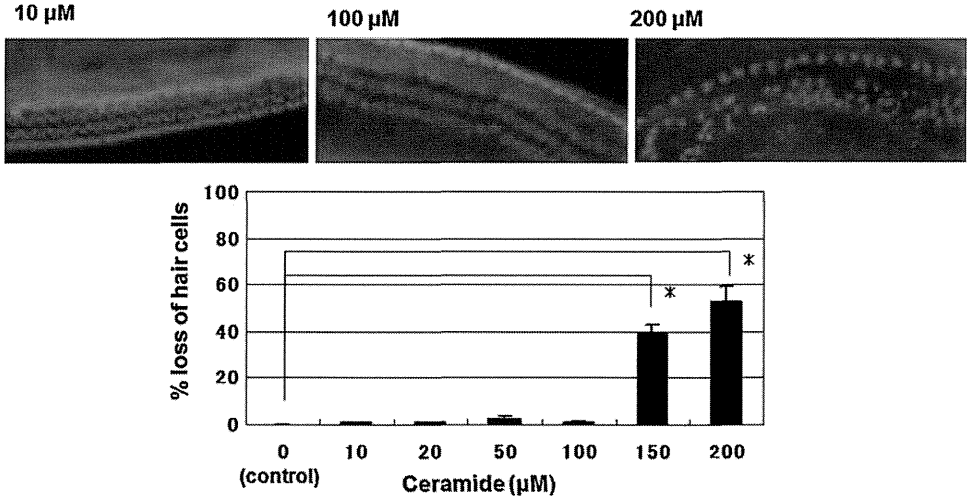

Fig. 1. Effect of ceramide on cochlear hair cells. Representative microphotographs of hair cells cultured with 10, 100, or 200 μM ceramide (without gentamicin) (upper, phalloidin staining). Quantitative analysis of hair cell loss in explants treated with ceramide (without gentamicin) for 48 h (lower). Ceramide itself induced hair cell loss at 150 and 200 μM (*one-way ANOVA and Bonferroni test: $p < 0.05$).

involvement of sphingolipid metabolites in hair cell death due to gentamicin.

The basal turn of the organ of Corti was dissected from Sprague–Dawley rats on postnatal days 3 (p3) to 5 (p5) and cultured based on the methods of Van de Water and Ruben [29] and Sobkowicz et al. [22]. All animal procedures were carried out according to guidelines of the Laboratory Animal Research Center at the University of Tsukuba.

Cochlear explants were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 25 mM HEPES, and 30 U/ml penicillin and were cultured in an incubator at 37 °C with 5% CO_2 and 95% humidity. Cochlear cultures were maintained in the above-described medium overnight (8–12 h) and then exposed to a medium containing 20 or 35 μM gentamicin for 48 h to assess the effects of sphingolipid metabolites [25,17].

All sphingolipid metabolites examined in this study were purchased from Sigma (Tokyo, Japan). The tested concentrations of ceramide, S1P, GM1, and GM3 were 10–200, 10–100, 10–1000, and 10–1000 μM, respectively. Ceramide was initially dissolved in ethanol to 15 mM and stored at −20 °C, which was diluted in the culture medium to the final concentration immediately before use. S1P was initially dissolved in methanol to 13 mM and stored at −20 °C. GM1 and GM3 were directly dissolved in the culture medium immediately before use.

After explants were cultured for 48 h in culture media containing 35 μM gentamicin alone or 35 μM gentamicin plus each concentration of sphingolipid metabolites, the explants were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min and then permeabilized with 5% TritonX-100 (Sigma, St. Louis, MO, USA) in PBS with 10% fetal bovine serum (FBS) for 10 min [25,26]. The specimens were stained for phalloidin with a conjugated Alexa Fluro probe (1:100, Molecular Probes Inc., Carlsbad, CA, USA) at room temperature for 1 h. Phalloidin is a specific marker for cellular F-actin and labels the stereociliary arrays and cuticular plates of hair cells [17,25].

To identify apoptosis, the terminal deoxynucleotidyl tranferase-mediated biotinylated UTP nick-end labeling (TUNEL) procedure was applied to cultured explants using the DEAD EndTM Fluorometric TUNEL System (Promega Inc., Madison, WI, USA). At the end of culture, the explants were fixed with 4% paraformaldehyde in PBS for 20 min and then treated with blocking solution containing 5% TritonX-100 in PBS with 10% FBS for 10 min. Using the manufacturer's protocol, after rinsing in PBS and pre-equilibrating, the explants were incubated at 37 °C for 1 h in darkness in the labeling solution which contained terminal deoxynucleotidyl transferase and fluorescein nucleotides. They were then washed again in PBS. The explants were subsequently stained with an Alexa Fluro phalloidin probe to differentiate hair cells from supporting cells.

Hair cells were characterized as missing if no stereocilia and/or no cuticular plate was observed by phalloidin staining. Quantitative results were obtained by evaluating 30 outer hair cells associated with 10 inner hair cells in a given microscopic field [17,25]. The average of three separate counts was used to represent each culture.

All data are expressed as the mean ± S.E.M. Statistical analysis was performed employing unpaired *t*-tests or one-factor ANOVA followed by Bonferroni post hoc tests, as required (StatView 5.0). *p*-Values of less than 0.05 were considered significant. All experiments consisted of $n = 7–12$ explants per experimental group.

In control explants maintained in the initial medium for 48 h without exposure to ceramide, almost all hair cells including one row of inner and three rows of outer hair cells were present. The effects of ceramide and ethanol as a solvent on hair cells were examined and compared with the control. There was no significant hair cell loss when explants were cultured for 48 h in the medium containing 10–100 μM ceramide without gentamicin (Fig. 1). However, 150 and 200 μM ceramide significantly induced hair cell loss (one-factor ANOVA and Bonferroni test: $p < 0.05$ in the 150 and 200 μM subgroups as compared with the control group). Ethanol alone did not induce hair cell loss.

The effect of ceramide on hair cell loss induced by gentamicin of 20 μM (Fig. 2(A)) or 35 μM (Fig. 2(B)) was examined. Ten or 20 μM ceramide did not affect hair cell loss induced by gentamicin. However, hair cell loss significantly increased in the presence of 50 and 100 μM ceramide, although ceramide per se did not have any effect at these concentrations, as shown in Fig. 1.

In TUNEL staining, apoptotic cells were labeled if they had shrunken and fragmented nuclei. Explants exposed to 35 μM gentamicin were labeled by TUNEL staining (Fig. 3). The number of TUNEL-positive cells significantly increased in the specimens cultured with 35 μM gentamicin plus 50 μM ceramide, compared with the gentamicin control group (unpaired *t*-test: $p < 0.05$). Ceramide treatment enhanced the apoptosis of outer hair cells induced by gentamicin.

The effect of S1P on gentamicin-induced hair cell damage was examined. S1P itself did not induce hair cell loss without gentam-

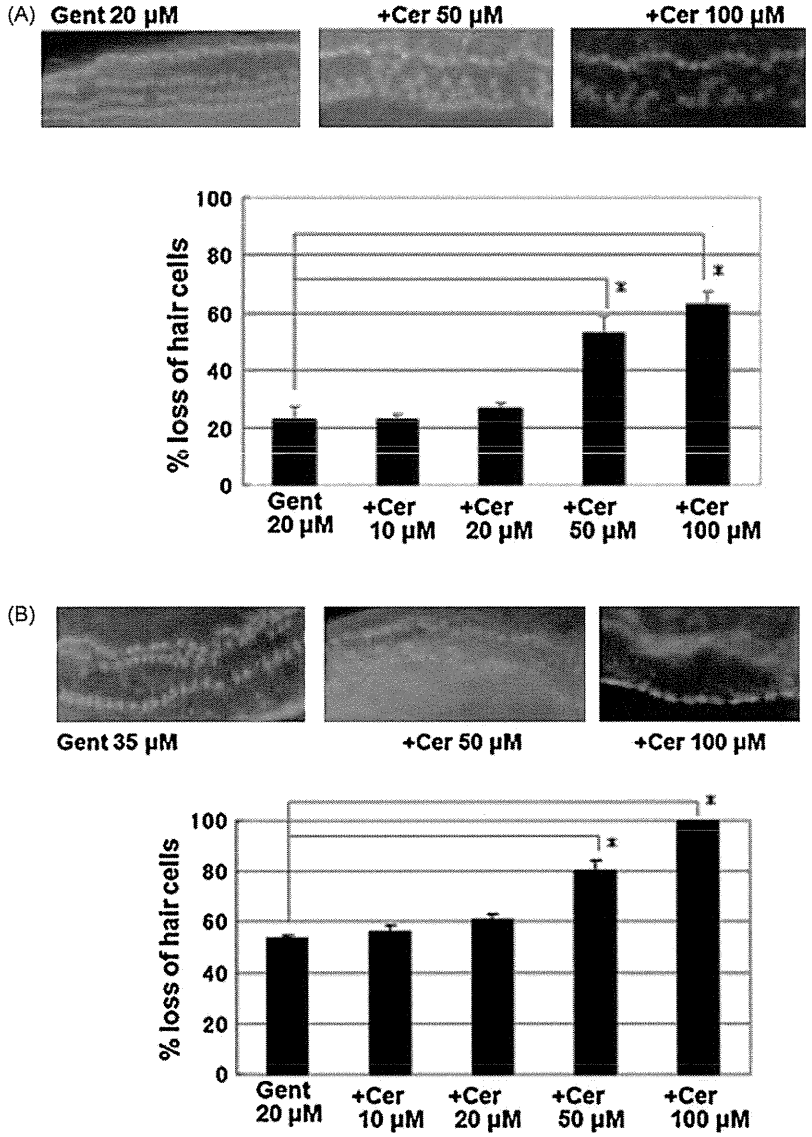

Fig. 2. Effect of ceramide on gentamicin-induced cochlear hair cell loss. (A) Representative photomicrographs of hair cells cultured with 20 μM gentamicin plus 50 or 100 μM ceramide (upper, phalloidin staining). Hair cell loss induced by 20 μM GM significantly increased in the presence of 50 and 100 μM ceramide, although ceramide without gentamicin did not exhibit any effect at these concentrations (lower, one-way ANOVA, Bonferroni test: $^{*}p < 0.05$ at 50 and 100 μM). (B) Representative microphotographs of hair cells cultured with 35 μM gentamicin plus 50 or 100 μM ceramide (upper, phalloidin staining). Hair cell loss induced by 35 μM significantly increased in the presence of 50 and 100 μM ceramide (lower, one-way ANOVA, Bonferroni test: $^{*}p < 0.05$ at 50 and 100 μM). Gent: gentamicin; Cer: ceramide.

icin at a concentration of 10–100 μM (data not shown). Compared to gentamicin alone, treatment with S1P reduced hair cell damage induced by gentamicin at concentrations of 50 and 100 μM of S1P (Fig. 4(A)), and significantly decreased the rate of hair cell loss (two-way ANOVA and Bonferroni test: $p < 0.05$). There were significant differences in the rate of hair cell loss among the 20, 50, and 100 μM subgroups, suggesting the dose dependency of hair cell protection (Bonferroni test: $p < 0.05$).

The effect of GM1 on gentamicin-induced hair cell damage was examined. GM1 itself did not significantly induce hair cell loss at a concentration of 10–1000 μM (data not shown). GM1 significantly decreased hair cell loss induced by 35 μM gentamicin at concentrations of 100–600 μM. However, the protective effect decreased on an increase in the GM1 concentration, and GM1 significantly increased hair cell loss induced by gentamicin at concentrations of 700 and 1000 μM (Fig. 4(B)).

The effect of GM3 on gentamicin-induced hair cell damage was examined. GM3 itself did not significantly induce hair cell loss at a concentration of 10–1000 μM. GM3 significantly decreased hair cell loss induced by 35 μM gentamicin at concentrations of 100–600 μM. Similarly to GM1 treatment, GM3 significantly increased hair cell loss induced by gentamicin at concentrations of 700 and 1000 μM (Fig. 4(C)).

It has been reported that ceramide causes the death of neuronal cells in the central and peripheral nervous systems. This effect of ceramide to induce cell death was also reported in other cell lines, such as PC12 cells, heart fibrocytes, and tumor cells [2,3,9]. In the present study, it was demonstrated that ceramide also induced the cell death of auditory hair cells.

Ceramide accelerated the cell death of hair cells when administered simultaneously with gentamicin. The results of TUNEL staining strongly suggested that hair cell death was induced via the apoptotic pathway. This apoptosis-inducing effect of ceramide in gentamicin ototoxicity was observed at the low concentrations at which ceramide per se did not induce hair cell death. The precise mechanisms of ceramide-induced apoptosis have not been made clear, but it is currently considered that ceramide induces apopto-

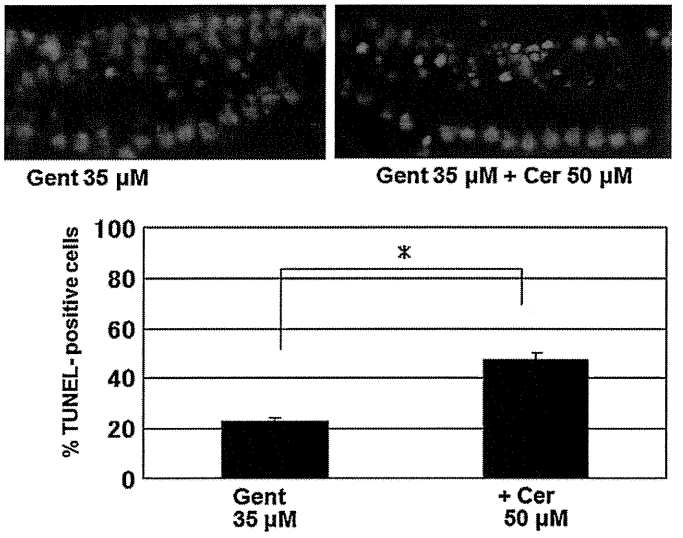

Fig. 3. Effect of ceramide on gentamicin-induced apoptosis. Quantitative analysis employing TUNEL labeling on treatment with gentamicin and ceramide at 50 μM for 48 h. Ceramide significantly increased the TUNEL labeling of outer hair cells (* unpaired *t*-test: $p < 0.05$). Representative photographs of TUNEL labeling (green staining) of the outer hair cells (red phalloidin staining) are shown. Apoptotic cells were noted in outer hair cells treated with 35 μM gentamicin alone. TUNEL-positive hair cells increased on treatment with gentamicin and 50 μM ceramide. Gent: gentamicin; Cer: ceramide.

sis via multiple pathways such as activation of the JNK, PKC, KSR, PP2A, and PP1 pathways [19].

In contrast to the apoptosis-inducing actions of ceramide, S1P protected hair cells against gentamicin. Five subtypes of G protein-coupled S1P receptors have been reported: S1P1 to S1P5. S1P1, S1P2, and S1P3 are reportedly expressed in the cochlea [10]. Recently, three groups independently showed that S1P signaling was essential for the maintenance of the cochlea after birth via the activation of S1P2 [10,13,16]. These findings strongly suggest that the modulation of S1P signaling may affect the survival or degeneration of hair cells associated with exposure to ototoxic drugs. In addition, S1P, generally known to be an anti-apoptotic substance, has been reported to activate ERK and to counteract SAPKs [4]. Recent studies have indicated that the dynamic balance between ceramide and S1P may be important for the determination of cell survival through cell injury [8,15]. Considering the recent studies and present findings, it is considered that the balance of ceramide and S1P may be an important factor to determine the fate of auditory hair cells in terms of gentamicin ototoxicity.

We also examined the effects of GM1 and GM3 on gentamicin ototoxicity. GM1 has been reported to exhibit protective effects through an anti-apoptotic action on not only neuronal cells but also other cell lines [1]. Its protective mechanism is not fully understood, but several reports on central nervous system injury suggest that GM1 is a multifunctional agent. GM1 shows neurotrophic effects, and activates Trk neurotrophin receptors [5]. GM1 induces neurotrophin-3 (NT3) in cultured microglia [18], and also affects sphingolipid metabolism which plays important roles in cell death and survival, as described above. Cavallini et al. [3] demonstrated GM1 promoted the synthesis of S1P through the activation of sphingosine kinase (SK) activity. Furthermore, GM1 exhibited antioxidative activities and reduced oxidative injury [6,21,27], and also regulated the nuclear calcium level [14]. In the present study, we demonstrated that GM1 exhibited protective effects against the gentamicin-induced damage of hair cells at limited concentrations. However, a higher concentration of GM1 adversely increased hair cell loss. This phenomenon was also reported in heart fibroblasts [3]. Although the precise reason why the action of GM1 on the

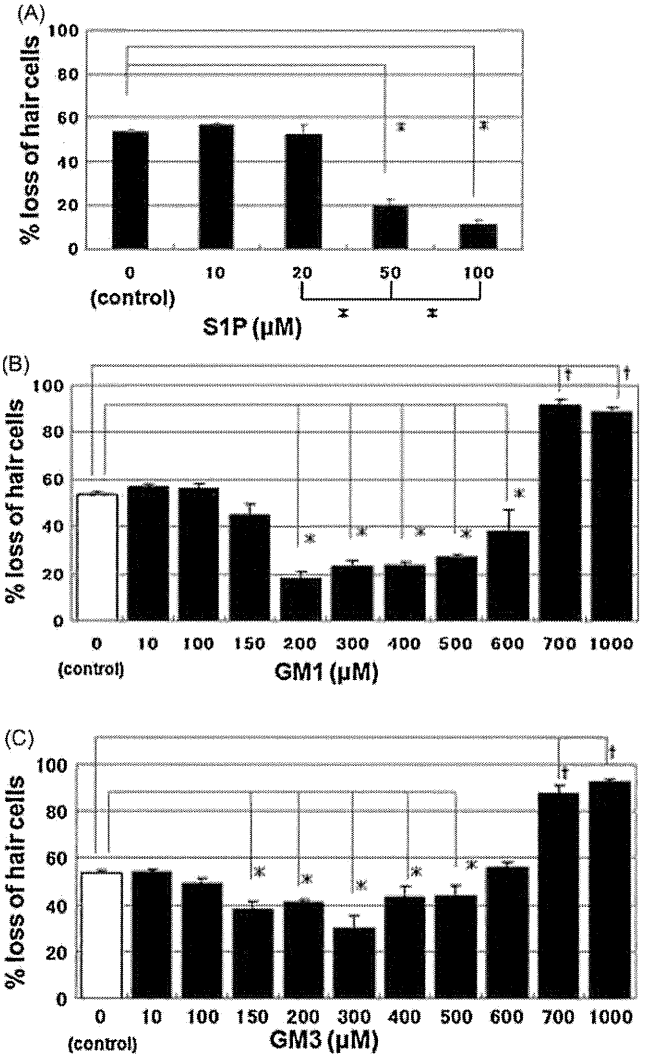

Fig. 4. (A) Effect of S1P on gentamicin-induced cochlear hair cell loss. Hair cell loss significantly decreased in the presence of 50 and 100 μM S1P (Bonferroni test: $^{*}p < 0.05$ at 50 and 100 μM). There were significant differences in the rate of hair cell loss among the 20, 50, and 100 μM subgroups, suggesting the dose-dependency of hair cell protection (Bonferroni test: $p < 0.05$). (B) Effect of GM1 on gentamicin-induced cochlear hair cell loss. Hair cell loss significantly decreased in the presence of 200–600 μM GM1 (Bonferroni test: $^{*}p < 0.05$ at 200 and 600 μM), however, conversely, 700 and 1000 μM GM1 significantly increased gentamicin-induced hair cell loss (Bonferroni test: $^{\dagger}p < 0.05$ at 200 and 600 μM). (C) Effect of GM3 on gentamicin-induced cochlear hair cell loss. Hair cell loss significantly decreased in the presence of 100–600 μM GM3 (Bonferroni test: $^{*}p < 0.05$ at 100 and 600 μM), however, conversely, 700 and 1000 μM GM3 significantly increased gentamicin-induced hair cell loss (Bonferroni test: $^{\dagger}p < 0.05$ at 100 and 600 μM).

cochlea was concentration-dependent is unclear, it was unquestionably derived from the multifunctional nature of GM1. GM3 exhibited a similar protective pattern to GM1, i.e., GM3 protected the cochlea against gentamicin ototoxicity, although it accelerated hair cell loss at high concentrations. It has been reported that mice lacking GM3 synthase show complete hearing loss due to selective degeneration of the organ of Corti [30], suggesting that GM3 may be important for the maintenance of the cochlea. However, it has also been reported that ceramide is produced from GM3 [28], and the overexpression of GM3 results in neuronal cell death in the central nervous system [23]. Further research is necessary to clarify the exact protective and degenerative mechanisms of the gangliosides GM1 and GM3.

In conclusion, the effects of sphingolipid metabolites on gentamicin ototoxicity were examined in the present study. Ceramide

enhances the apoptotic death of outer hair cells, although S1P protected hair cells in the presence of gentamicin ototoxicity. GM1 and GM3 exhibited protective effects against gentamicin-induced hair cell death at the limited concentrations. Although the function of sphingolipid metabolites is still obscure in the cochlea, regulation of their metabolism has the possibility to suppress gentamicin ototoxicity.

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Daily Short-Term Intratympanic Dexamethasone Treatment Alone as an Initial or Salvage Treatment for Idiopathic Sudden Sensorineural Hearing Loss

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Key Words

Idiopathic sudden sensorineural hearing loss · Intratympanic dexamethasone treatment · Initial treatment · Salvage treatment · Laser-assisted myringotomy

Abstract

Objective: Intratympanic (IT) steroid therapy has been proposed as an alternative treatment option for patients with idiopathic sudden sensorineural hearing loss (ISSNHL). However, the number and frequency of IT treatments and drug delivery methods remain to be determined. The purpose of this study was to evaluate the efficacy of daily short-term IT dexamethasone (DEX) treatment alone in ISSNHL patients using laser-assisted myringotomy (LAM) for the drug delivery route as an initial and/or salvage treatment. ***Study Design:*** Retrospective study. ***Setting:*** University hospital. ***Patients:*** Seventy-six ISSNHL patients receiving IT DEX. Patients with low-tone hearing loss, unilateral or bilateral fluctuating hearing loss or contralateral hearing loss were excluded. ***Intervention:*** DEX (4 mg/ml) was injected through a perforation made by LAM. IT DEX administration was performed on 8 sequential days. ***Main Outcome Measures:*** Pre- and postprocedure hearing levels. The average hearing level was determined by 5 frequencies (250, 500, 1000, 2000 and 4000 Hz). ***Results:*** Nineteen out of 76 patients fit the criteria for initial treatment in the study (group I), while 24 patients, who had failed systemic therapy, received salvage treatment (group S). The mean age of the patients in groups I and S was 56.2 years with a range from 31 to 73 years of age and 46.0 years with a range from 11 to 76 years of age, respectively. The mean number of days from onset of symptoms to IT therapy in groups I and S was 4.8 days with a range of 1–23 days and 15.3 days with a range of 6–28 days, respectively. In group I, 18 of the 19 patients (95%) showed improvement of more than 10 dB in the pure-tone audiogram, with a mean improvement of 40 dB. Twelve patients (63%) recovered completely and 16 patients (84%) demonstrated successful results with an improvement of more than 30 dB. In group S, 14 of the 24 patients (58%) showed improvement of more than 10 dB with a mean improvement of 16 dB. Two (8%) of the 7 patients (29%) with successful results recovered completely. ***Conclusions:*** Daily short-term IT DEX administration using LAM for ISSNHL patients without concurrent therapy showed a high response rate and high cure rate and proved to be an alternative therapeutic option to high-dose systemic steroids as a first- and/or second-line treatment.

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Introduction

Idiopathic sudden sensorineural hearing loss (ISSNHL) is the most frequent acute sensorineural hearing loss and is one of the few types of sensorineural hearing loss which can be cured. One of the main problems in the treatment of ISSNHL is that the therapeutic options are limited [Conlin and Parnes, 2007]. Systemic glucocorticoids have been the mainstay for treatment of ISSNHL [Wilson et al., 1980], but as the etiology of ISSNHL is thought to be multifactorial and the mechanism of action of glucocorticoids in the inner ear remains unclarified, the optimal dose is currently unknown. Up to now, higher concentrations of glucocorticoids in the inner ear have been considered desirable because they were thought to exert a stronger effect on hearing recovery. However, systemic application of glucocorticoids, oral or intravenous (IV), had limitations in providing higher concentrations in the perilymph [Parnes et al., 1999; Chandrasekhar et al., 2000; Niedermeyer et al., 2003] because of the blood-labyrinthine barrier. In addition, high-dose systemic administration of glucocorticoids causes higher occurrences of undesirable side effects, in particular, the potential risk of avascular necrosis of the femoral head, and should be avoided in patients with diabetes mellitus, hypertension, gastric ulcer, tuberculosis and so on. Given this background, intratympanic (IT) injection of glucocorticoids for ISSNHL has attracted attention. It could induce higher concentrations of the agent in the target organ and produce less side effects in other parts of the body. Several lines of evidence in animal models and human studies revealed that IT administration results in significantly higher perilymph concentrations of steroids than IV or oral administration [Parnes et al., 1999; Chandrasekhar et al., 2000; Chandrasekhar, 2001; Niedermeyer et al., 2003].

Since Parnes et al. [1999] reported efficacy of IT steroid (ITS) in animal models and a human study, clinical reports of ITS therapy for ISSNHL have been increasing (see review by Hu and Parnes [2009]). Most of the studies have focused on the ITS therapy as a salvage treatment option for patients with ISSNHL [Parnes et al., 1999; Chandrasekhar et al., 2000; Gianoli and Li, 2001; Guan-Min et al., 2004; Dallan et al., 2006; Haynes et al., 2007; Kilic et al., 2007; Plaza and Herraiz, 2007; Van Wijck et al., 2007; Plontke et al., 2009; Dallan et al., 2010]. On the other hand, there are several studies which investigated the efficacy of the ITS treatment alone as an initial treatment [Kakehata et al., 2006; Battaglia et al., 2008; Han et al., 2009; Hong et al., 2009; Kara et al., 2010], although some reported efficacy of the concurrent use of ITS with high-dose steroids as an initial treatment [Battista, 2005; Lautermann et al., 2005; Ahn et al., 2008; Battaglia et al., 2008].

An ideal treatment of ISSNHL should have a high cure rate as well as a high response rate. A high cure rate is important because patients are not satisfied with results, even an improvement of 30 dB, if their hearing ability does not return to its previous level.

We previously reported efficacy of IT dexamethasone (DEX) treatment on 8 sequential days in ISSNHL patients with diabetes mellitus using laser-assisted myringotomy (LAM) for the drug delivery route as an initial treatment [Kakehata et al., 2006]. The administration of DEX through the small perforation made by LAM [Kakehata et al., 2004] is an easy, secure and confirmable delivery with minimal or no pain. Although a blinded, controlled and randomized study is preferred, as the number and frequency of IT treatments and drug delivery methods remain to be determined, we undertook a retrospective study to ascertain the effective protocol for the treatment of ISSNHL.

The purpose of this study was to evaluate the efficacy of daily short-term IT DEX treatment using LAM without concurrent therapy in ISSNHL patients as an initial treatment as well as a salvage treatment and whether it could be a therapeutic option to high-dose systemic steroids.

Materials and Methods

Seventy-six ISSNHL patients were treated with IT DEX between April 2002 and December 2009. Patients were included in this study if they had a sensorineural hearing loss of 30 dB or more with over 3 contiguous audiometric frequencies that occurred in fewer than 3 days. Patients with low-tone hearing loss, unilateral or bilateral fluctuating hearing loss or contralateral hearing loss and Ménière's disease were excluded. Patients with no identifiable cause of sudden hearing loss were considered to have ISSNHL. Additionally, patients with inadequate follow-up after the treatment (less than 4 weeks) or late IT DEX therapy (beginning more than 4 weeks after onset) were excluded. In the initial treatment group (group I), patients who had received any preceding therapy were excluded. On the other hand, patients who had received systemic steroids for more than 5 subsequent days before the IT DEX therapy were included in the salvage group (group S).

Criteria for Outcome

Pure-tone audiograms were obtained before, during and after the procedure and at periodical checkups. Average hearing was determined by 5 frequencies (250, 500, 1000, 2000 and 4000 Hz). A final audiogram was obtained at least 4 weeks after the final injection. Criteria for audiologic improvement were based on the classification prepared by the Acute Severe Hearing Loss Study

Table 1. Patient profiles

	Group I	Group S
Patients	19	24
Sex (male/female)	12/7	10/14
Age, years		
Mean ± SD	56.2 ± 9.7	46.0 ± 18.7
Range	31 – 73	11 – 76
Vertigo	4 (21%)	12 (50%)
Diabetes mellitus	14 (74%)	3 (13%)
Initial hearing level, dB		
Mean ± SD	77.7 ± 18.2	74.6 ± 15.3
Range	49 – 102	42 – 102
Duration between onset and IT DEX, days		
Mean ± SEM	4.8 ± 5.0	15.3 ± 6.4
Range	1 – 23	6 – 28

Group [Kanzaki et al., 2003] of Japan, which classifies the outcomes as complete recovery, marked recovery, slight recovery, or nonrecovery. Complete recovery is defined as recovery of hearing to within 10 dB of the contralateral pure-tone average, marked recovery as an improvement of the average hearing of 30 dB or more, and slight recovery as an improvement of the average hearing of between 10 and 30 dB. Complete recovery or marked recovery is considered successful treatment.

Treatment Procedure

Patients only received IT DEX treatment and did not undergo additional treatment. Delivery methods were described elsewhere [Kakehata et al., 2006]. Briefly, DEX was injected through a perforation made by LAM, or through a tympanostomy tube (Paparella type II) for the first 6 cases. In the outpatient clinic, a perforation with a diameter of 1.4–2 mm was made in the tympanic membrane with a CO_2 laser unit (OtoLAM; LUMENIS, Yokneam, Israel) using a single pulse of 10–13 W after tympanic membrane anesthesia with iontophoresis [Kakehata et al., 2004]. The location of the perforation was between the oval window and the round window (RW).

The patient lies flat with the affected ear upward and the head is tilted 45° away with the chin upward so that the RW membrane is bathed. 0.5 ml of DEX (4 mg/ml) is injected through the perforation made by LAM using a 1-ml tuberculin syringe with a 26-gauge needle. DEX 4 mg/ml was used because it is the only DEX available in our country. Under direct visualization with an otomicroscope or a magnifying otoscope, one can confirm that the tip of the needle is inserted through the perforation and the mesotympanum is filled with DEX, replacing the air. 0.5 ml is usually more than enough to fill the mesotympanum and a small amount of DEX spills to the outside of the tympanic membrane as a reservoir. The patient is instructed to remain in this position for 30 min without swallowing. DEX administration is performed on 8 sequential days because our previous study showed that daily IT DEX treatment for 8 sequential days was at least as effective as IV DEX treatment (tapering over 8 days) for ISSNHL patients with diabetes mellitus [Kakehata et al., 2006].

Results

Profile of Patients

Table 1 summarizes the profiles of the patients in groups I and S. Nineteen out of 76 patients fit the criteria outlined in Materials and Methods for group I, while 24 patients, who had failed systemic therapy, received the IT DEX treatment as a salvage treatment (group S). Twelve men and 7 women were included in group I and 10 men and 14 women in group S. The mean age of groups I and S was 56.2 years with a range from 31 to 73 and 46.0 years with a range from 11 to 76, respectively. The mean number of days from onset of symptoms to IT DEX treatment in group I was 4.8 days with a range of 1–23 days and in group S it was 15.3 days with a range of 6–28 days.

The average hearing level before the IT DEX treatment in group I was 77.7 ± 18.2 dB (mean ± standard deviation) with a range from 49 to 102 dB. There were 4 (21%) patients who had vertigo and 14 (74%) patients with diabetes mellitus. On the other hand, in group S, the average hearing level before the IT DEX treatment was 74.6 ± 15.3 dB with a range from 42 to 102 dB. There were 12 (50%) patients with vertigo and 3 (13%) patients with diabetes mellitus. There was no significant difference in the hearing level before the IT DEX treatment between the 2 groups (Welch's t test). The difference in duration between onset and the IT DEX treatment was significant ($p < 0.001$).

In group S, the mean duration between onset of the symptoms and the initial systemic steroids treatment was 1.9 days with a range from 0 to 20 days. All patients but 1 started the initial treatment within 6 days. Twelve (50%) patients received the treatment on the day of onset. The patients in group S received various kinds of systemic steroids at least 5 consecutive days before being referred to our hospital as well as other therapies such as agents that decrease blood viscosity (dextran), prostaglandin E_1 (PGE_1), ATP and vitamin B_{12}. In 18 out of 24 patients, DEX was used intravenously or orally, usually starting from an amount of 8 mg/day followed by tapered doses for 8 days, with a total amount of 40 mg. Prednisolone or betamethasone was also used in other patients. Six patients received PGE_1 therapy after the failure of the initial steroid therapy. One patient who failed the initial systemic IV DEX therapy starting from an amount of 32 mg received PGE_1 therapy and oxygen at hyperbaric pressure as a salvage treatment.

Clinical Outcomes

Figure 1a depicts the overall results in group I. Eighteen of 19 patients (95%) showed improvement in the

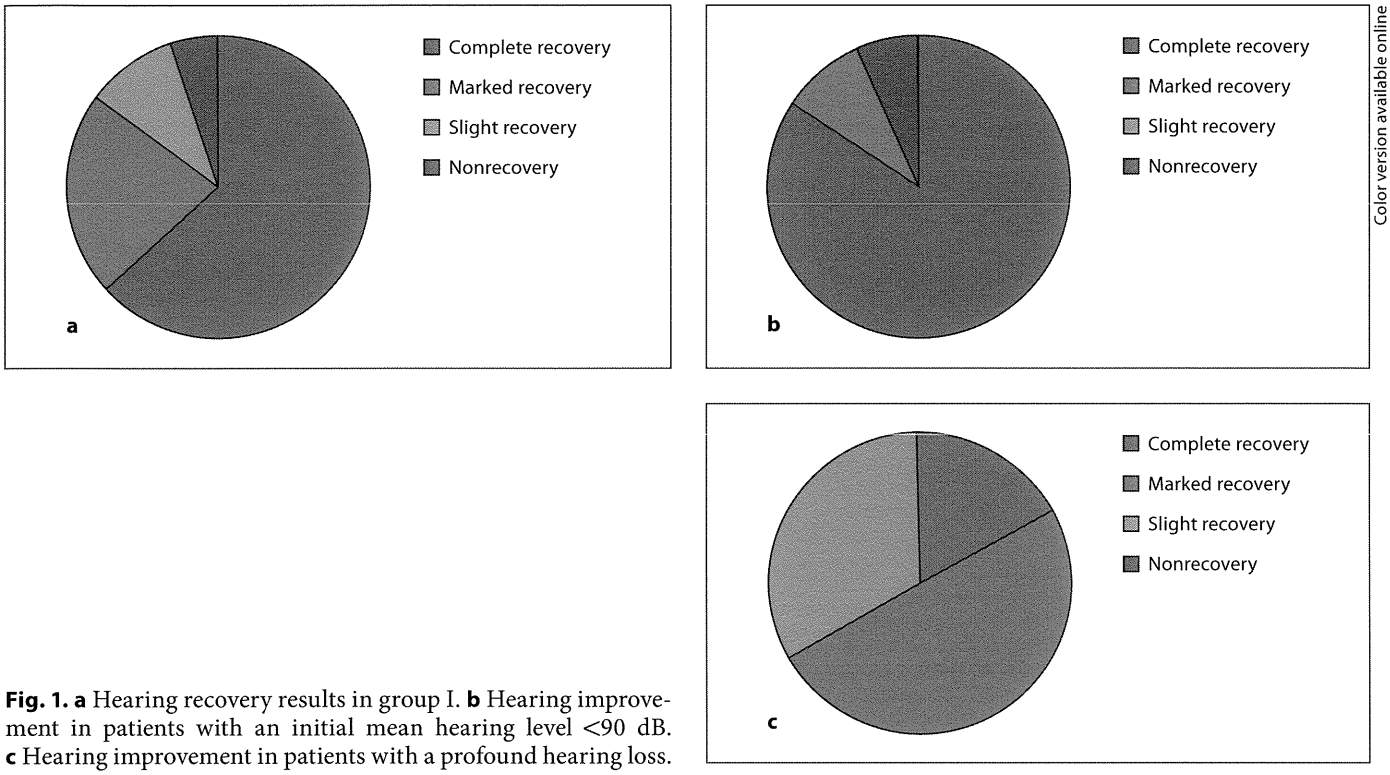

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Fig. 1. a Hearing recovery results in group I. **b** Hearing improvement in patients with an initial mean hearing level <90 dB. **c** Hearing improvement in patients with a profound hearing loss.

pure-tone audiogram of more than 10 dB. After the treatment, the mean pure-tone average reached 38.8 dB from 77.7 dB with a mean improvement of 39.7 ± 18.4 dB. Sixteen (84%) patients demonstrated successful treatment and 12 (63%) patients recovered completely, reaching a hearing level identical to that of the unaffected ear. However, a 62-year-old man, who underwent treatment 23 days after the onset with an initial hearing level of 61 dB without vertigo, showed no improvement. As it is known that patients with a profound hearing loss (initial pure-tone average worse than 90 dB) have a poor prognosis, those patients were evaluated separately. There were 13 patients who did not have a profound hearing loss. This group had 11 (85%) with complete recovery, 1 (8%) with marked recovery and 1 (8%) with no hearing recovery (fig. 1b). The mean hearing improvement was 40.5 ± 19.2 dB. In the profound hearing loss group, all patients responded to the treatment with a mean hearing improvement of 37.8 ± 18.2 dB; 1 (17%) with complete recovery, 3 (50%) with marked and 2 (33%) with slight recovery (fig. 1c). Hearing improvement between the 2 groups was not statistically different.

The results for all patients in group S are presented in figure 2a. Fourteen of 24 (58%) patients showed improvement. The mean pure-tone average reached 57.8 from 74.6 dB with a mean improvement of 16.8 ± 21.6 dB. Seven (29%) patients demonstrated successful treatment, including 2 (8%) with complete recovery. Ten patients did not show improvement. There were 20 patients who did not have a profound hearing loss, whose mean hearing improvement was 16.9 ± 23.6 dB. This group had 11 (55%) with improvement and 7 (35%) with successful treatment and 2 (10%) with complete recovery (fig. 2b). In the profound hearing loss group, all patients responded to the treatment with a mean improvement of 16.5 ± 7.2 dB.

In the responders of group S, the mean hearing improvement was 30.1 ± 18.3 dB. Several factors which are known to affect the prognosis of patients were compared between the responders and nonresponders. The mean duration between onset of symptoms and IT DEX treatment was 14.5 ± 5.9 days and 16.5 ± 7.1 days in the responders and nonresponders, respectively, which is not statistically different. On the other hand, the average hearing level before the IT DEX treatment was 82.0 ± 12.4 dB and 64.2 ± 13.0 dB in the responders and nonresponders, respectively, which is statistically different ($p < 0.01$).

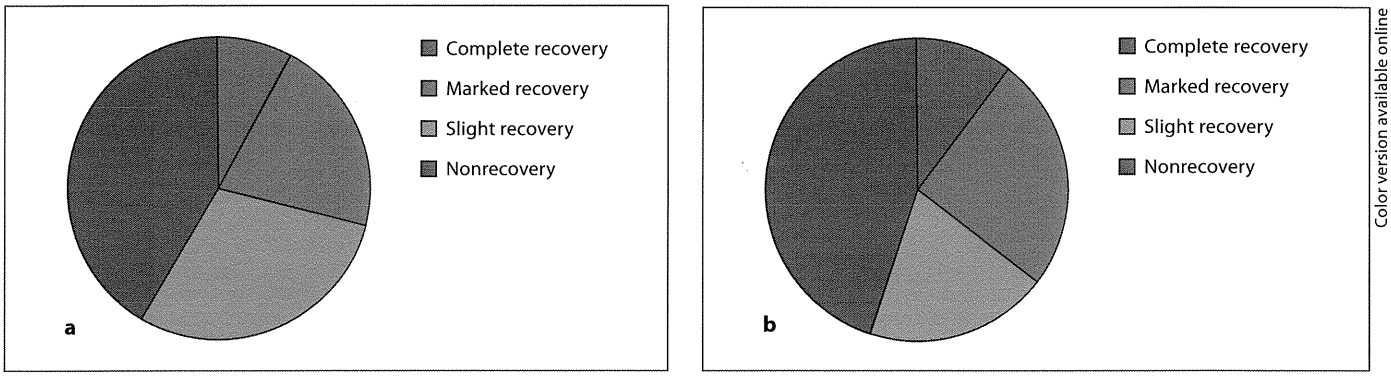

Fig. 2. a Hearing recovery results in group S. **b** Hearing improvement in patients with an initial mean hearing level <90 dB.

Side Effects

There was transient vertigo of about a minute associated with the injections. Some patients had tolerable pain after the injection for a minute. No patients had otitis media. The perforations were closed spontaneously or with a chitin sheet patch in most patients. For 12 patients, autologous serum ear drops and/or basic fibroblast growth factor with a chitin sheet patch therapy in the outpatient clinic [Kakehata et al., 2008] were performed and the perforations were closed successfully except in 1 patient. Small perforations remained in 2 patients. One patient underwent myringoplasty.

Discussion

Recently, there has been increased interest in IT administration of steroids for the treatment of ISSNHL, whether as an initial or salvage treatment [Parnes et al., 1999; Gianoli and Li, 2001; Guan-Min et al., 2004; Battista, 2005; Lautermann et al., 2005; Dallan et al., 2006; Kakehata et al., 2006; Haynes et al., 2007; Kilic et al., 2007; Plaza and Herraiz, 2007; Van Wijck et al., 2007; Ahn et al., 2008; Battaglia et al., 2008; Han et al., 2009; Hong et al., 2009; Plontke et al., 2009; Dallan et al., 2010; Kara et al., 2010], since ITS seems to be a potent alternative treatment option to systemic steroid therapy. As a first-line treatment, several clinical studies reported efficacy of combination therapies of ITS with systemic steroid therapy [Battista, 2005; Lautermann et al., 2005; Ahn et al., 2008; Battaglia et al., 2008]. However, there has been no ITS treatment protocol that seems to be superior [Hu and Parnes, 2009]. Thus, before discussing the efficacy of combination therapy, it seems necessary to elucidate a preferable ITS treatment protocol without concurrent or previous treatments. Here, we performed daily short-term IT DEX treatment on fresh ISSNHL patients without concurrent treatments in order to investigate the efficacy of ITS alone.

An ideal treatment of ISSNHL should have a high cure rate as well as a high rate of response. To improve the cure rate is especially important because patients are not satisfied, even if their hearing level is improved by 30 dB, if it does not improve to near the hearing level of the unaffected ear. In this study, the rate of response and the cure rate of daily short-term IT DEX administration alone as an initial treatment reached 95 and 63%, respectively. This cure rate is higher than that of IT DEX treatment without concurrent systemic steroids in the recently published studies. Battaglia et al. [2008] reported a cure rate of 29% (5/17) by IT DEX therapy of 3 weekly injections. In their study, a high-dose oral prednisone treatment (tapering from 60 mg for 14 days; total 660 mg) and a combination therapy (IT DEX plus high-dose oral steroids) were also attempted. The cure rate of the oral steroid therapy and the combination therapy was 17% (3/18) and 63% (10/16), respectively. Battaglia et al. [2008] advocated the efficacy of the combination therapy over the systemic high-dose steroid therapy. Our cure rate was higher than that of the IT DEX therapy of 3 weekly injections and comparable to that of the combination therapy. This suggests that daily administration through LAM for 8 days is more effective than 3 weekly injections. In addition, in patients who did not have a profound hearing loss, our cure rate of 85% is higher than that of the combination therapy (63%). In the profound hearing loss group, the mean hearing improvement reached 37.8 dB, which was not statistically significantly different compared to the 40.5 dB in the other group. However, the cure rate was

low (17%) compared to that of 66% (deduced from figure 3 in Battaglia et al. [2008]) in the combination therapy, which might suggest a limitation of the IT DEX therapy in this form for patients with a profound hearing loss. This may also suggest that there are other pathological conditions in the case of profound hearing loss which cannot be reached by ITS treatment.

On the other hand, Ahn et al. [2008] reported an additional effect of IT DEX (3 injections on days 1, 3 and 5) through a 25-gauge spinal needle on oral methylprednisolone therapy (tapering from 48 mg for 14 days) in a study with a larger number of subjects. The cure rate by the combination therapy of IT DEX plus oral methylprednisolone was 25% (15/60), which did not show significant improvement compared to the oral methylprednisolone alone (27%, 16/60).

Battaglia et al. [2008] and Ahn et al. [2008] used a treatment protocol of 3 transtympanic injections of DEX on infrequent days. In most studies, steroids were administered by transtympanic injection through a fine needle under local anesthesia at 1–5 injections over 1–3 weeks [Hu and Parnes, 2009]. Recently, Kara et al. [2010] reported a high cure rate of 48% (14/29) by 5 transtympanic injections of IT DEX on 5 consecutive days as an initial treatment. Taking into account our high cure rate by 8 injections on 8 consecutive days, daily application of ITS may be more effective than infrequent application.

Recent studies on cochlear pharmacokinetics with local ear drug delivery revealed that after the end of the 30-min application, the concentration in the base of the scala tympani rapidly declines due to clearance from the cochlea and diffusion into other compartments and apical regions [Plontke et al., 2007]. To conquer this drawback, several delivery methods have been devised for the sustained application of the drug to the RW membrane, such as the Silverstein MicroWick [Silverstein et al., 1997] or the MicroCatheter [Kopke et al., 2001; Plontke et al., 2009]. However, the latter is no longer available because the FDA removed it from the market. With the Silverstein MicroWick, the patient can apply the steroid several times a day. In our previous study, we speculated that our high response rate and the degree of improvement might be due mainly to the delivery methods used and/or the frequency of the applications. The delivery method with a wide opening to the mesotympanum assures the certain filling of the mesotympanum with the treatment agent and allows the air to escape from the mesotympanum, permitting the treatment agent to contact the RW membrane. The high cure rate as an initial treatment in this study might support this speculation.

As a salvage treatment, improvement of more than 10 dB was achieved in 58% and the cure rate was 8% with successful treatment in 29%. The mean improvement was 16.8 dB. In the responder group, the mean improvement reached 30.1 dB. There are a number of studies of ITS as a salvage treatment. Reported response rates were between 27.5 and 73.6% [Parnes et al., 1999; Chandrasekhar et al., 2000; Gianoli and Li, 2001; Guan-Min et al., 2004; Dallan et al., 2006; Haynes et al., 2007; Kilic et al., 2007; Plaza and Herraiz, 2007; Van Wijck et al., 2007; Plontke et al., 2009; Dallan et al., 2010].

Initial severity of hearing loss is one of the known prognostic factors. Although there were no significant differences between the initial and salvage groups regarding the hearing level before the IT DEX treatment (77.7 ± 18.2 vs. 74.6 ± 15.3 dB) ($p < 0.01$), the hearing level after the treatment (38.8 ± 22.3 vs. 57.8 ± 21.8 dB) and gain of improvement (39.7 ± 18.4 vs. 16.8 ± 21.6 dB) were significantly higher in group I ($p < 0.01$). One of the reasons for these better results is the shorter duration between the onset and IT DEX treatment (4.8 ± 5.0 vs. 15.3 ± 6.4 days; $p < 0.01$). However, between the patients with successful treatment and those with no response in group S, the difference in the mean duration between onset of symptoms and IT DEX treatment (12.3 ± 3.5 vs. 16.5 ± 7.1 days) was not statistically significant, although the patients starting IT DEX treatment later than 19 days after onset did not have successful outcomes, suggesting the therapeutic window of this treatment. It is also likely that group S may include those with poor response to steroid therapy, which was not overcome by the high dose of steroid induced by the ITS therapy.

There are at least 3 requirements for successful IT treatment. Firstly, a secure and confirmable delivery method is necessary. For the agent to perfuse via the RW membrane, it is important to replace the air around the RW membrane [Nomura, 1984; Silverstein et al., 1997] with the solution containing the agent. Secondly, sequential or continuous administration of the drug might be desirable because it is expected to maintain the concentration of the drug in the target cells at a high level [Plontke et al., 2007]. Finally, it should be an easy and painless delivery method. The daily short-term IT DEX treatment using LAM seems to meet these 3 requirements.

In conclusion, daily short-term IT DEX treatment using LAM for ISSNHL patients without concurrent therapy is effective as an initial treatment as well as a salvage one and proved to be an alternative therapeutic option to high-dose systemic steroids. However, a prospective study is necessary to validate the conclusion.

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原 著

TRT後のMCL・UCL検査の変化

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Tinnitus Retraining Therapy（TRT）は、耳鳴を消失させるのではなく、順応により気にならなくさせることが目的の治療である。TRTにより、耳鳴以外の外界からの音に対しても馴化を起こし、most comfortable loudness level（MCL）やuncomfortable loudness level（UCL）が変化するか否かを検討した。9名（男性4名、女性5名）にTRTを4－22カ月（平均12カ月）施行した。Tinnitus Handicap Inventoryスコアは、59.7±21.8から20.2±10.1へと改善したが、MCL・UCL検査は両者とも明らかな変化を認めなかった。治療前のUCLが特に低値を示した症例においても、TRTによるMCL・UCLの変化を認めなかった。

Key words：耳鳴、TRT、MCL・UCL検査、順応

はじめに

TRT（Tinnitus Retraining Therapy）は、神経生理学的モデルに基づいた耳鳴の順応療法で、TCI（Tinnitus Control Instrument）による音響療法とカウンセリングから成り立っている[1]。TCIによる音響治療は、雑音を与えることにより耳鳴と周辺雑音とのコントラストを減弱させ、大脳辺縁系での耳鳴に対する過敏性を減少させ、耳鳴に順応させる（habituation）治療である。これは耳鳴を消失させるのではなく、気にならなくさせることが目的である[2]。一方、most comfortable loudness level（MCL）検査・uncomfortable loudness level（UCL）検査は、通常外界からの純音に対して音が快適に聞こえる大きさ（MCLレベル）と、これ以上聞いてはいられない不快な大きさ（UCLレベル）を測定する検査で、補聴器のフィッティングの際に使われることが多い。また、内耳機能検査の一つとして、リクルートメント現象の有無の判定に使われることもある[3]。

TRTにより耳鳴に順応し気にならなくなった場合、耳鳴以外の外界からの音に対しても馴化され、MCL・UCLレベルが変化する可能性がある。今回われわれは、耳鳴に対してTRTを行った前後で、MCL・UCL検査に変化が起こるか否か検討した。

対象と方法

対象は九州大学病院耳鼻咽喉科外来を耳鳴で受診し、TRTを施行され治療前と治療後にMCL・UCL検査を行った男性4名、女性5名の計9名である。年齢は34－71歳（平均60.0歳）、耳鳴耳の聴力レベルは8.3－66.7 dBで、平均29.7 dBであった。治療期間は4－22カ月（平均12カ月）であった。TRTの判定は、自覚症状については耳鳴の大きさと耳鳴の気になり方を標準耳鳴

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検査法1993（耳鳴研究会作成）の5段階評価に従って行った[4]。また、耳鳴の心理的苦痛度・生活障害度はNewmanのTinnitus Handicap Inventory（THI）scoreの日本語訳にて評価した[5]。MCL・UCL検査は250 Hz、1,000 Hz、4,000 Hzの連続音を用い、上昇法で測定した。測定順序は、左右のMCL検査を行った後に左右のUCL検査を行った。TRTのサウンドジェネレータは、シーメンスヒヤリングインストルメンツ社のTCIを使用した。統計処理はOrigin 6.1 Jにて解析し、t検定にて行った。

結　果

5段階評価では、耳鳴の大きさに関しては治療前の3.8 ± 0.8から治療後の2.7 ± 1.0へ、耳鳴の気になり方に関しては治療前の3.3 ± 1.0から治療後の2.1 ± 1.3へと変化した（図1）。両者とも統計学的に差は認めないものの（大きさ：$p = 0.06$、気になり方：$p = 0.09$）、減少傾向を認めた。THIスコアは、対象のすべての症例でスコアの減少を認め（図2A）、平均で59.7 ± 21.8から20.2 ± 10.1へと有意差をもって減少した（図2B）。障害度分類では、severe handicap（58 − 100）からmild handicap（18 − 36）まで改善したことになる。

MCL・UCL検査は、平均で右耳はMCLが48.3 dBから48.3 dB（250 Hz）、45.0 dBから46.6 dB（1,000 Hz）、47.2 dBから45.0 dB（4,000 Hz）、UCLが77.7 dBから75.0 dB（250 Hz）、78.3 dBから78.8 dB（1,000 Hz）、77.7 dBから75.5 dB（4,000 Hz）へと、MCL、UCLの両者とも変化を認めなかった（図3A）。左耳もMCLが49.4 dBから48.8 dB（250 Hz）、45.5 dBから43.8 dB（1,000 Hz）、51.6 dBから50.0 dB（4,000 Hz）、UCLが77.7 dBから75.0 dB（250 Hz）、77.2 dBから77.2 dB（1,000 Hz）、81.6 dBから76.2 dB（4,000 Hz）へと、MCL、UCLの両者とも変化を認めなかった（図3B）。

特に治療前のUCLが低値を示した症例について、TRTにてMCL・UCL値が変化するか否かをみた（図4）。症例は34歳、女性、聴力は左右とも正常、TRT前のUCLは45 − 65 dBと低値であった。6カ月間TRTを施行し、THIは38点から26点へとmoderate handicapからmild handicapまで改善した。しかし、MCL・UCL値は右250 Hzと右1,000 HzのMCLが上昇したものの、UCLは40 − 70 dBと明らかな変化を認めなかった。

考　察

TRTによりTHIスコアは平均で59.7から20.2へと減少した（図2B）。20点以上の改善で臨床的に意義があるとされており[6]、耳鳴に対してTRTの有用性が確認された。しかし、MCL・UCL値についてはTRTによる変化を認めなかった（図3）。内因性の耳鳴と外界からの音に対する中枢の感受性には相違があることが考えられる。ラウドネス・バランス検査で、耳鳴のラウドネスは6 dB以内が70%、9 dB以内が84%とされており[7]、耳鳴の音量は外界音に換算すると非常に小さい。そのために、耳鳴に対してはTRTによる順応が可能であるが、MCL・UCL検査で与える大きな音に対しては、大脳辺縁系での過敏性を変化させることができず、MCL・UCL値が不変であった可能性が考えられる。また、耳鳴のマスキング検査（遮蔽検査）において、いかなるマスキング音でも耳鳴の完全なマスキングができない症例が存在するといわれている[8]。このことは耳鳴と外界からの音を感受する中枢の部位が異なることを意味し、そのために耳鳴において有効であったTRTがMCL・UCL検査に対しては影響を与えなかった可能性が考えられる。

耳鳴患者の平均UCLは、TRT前で77.2 dB − 81.6 dB、TRT後で75.0 dB − 78.8 dBであった。通常UCLは90 dB前後とされるが[9]、今回の症例では低値となっている。耳鳴患者の20 − 45 %程度は聴覚過敏が合併するといわれており[10]、UCLの低下は聴覚過敏の側面を反映しているの

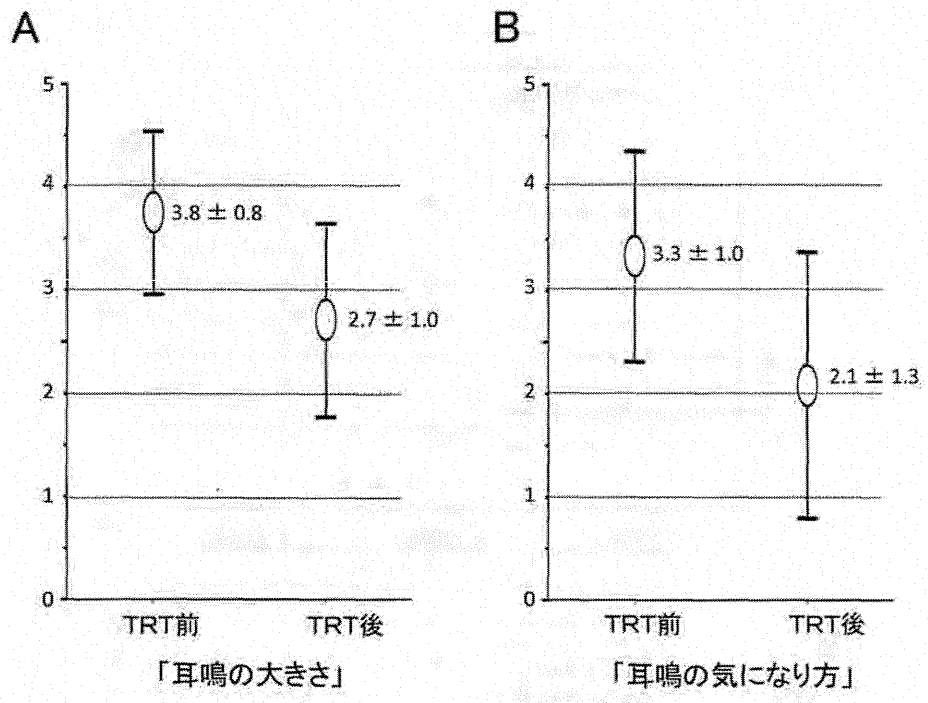

図1　TRTによる5段階評価の変化

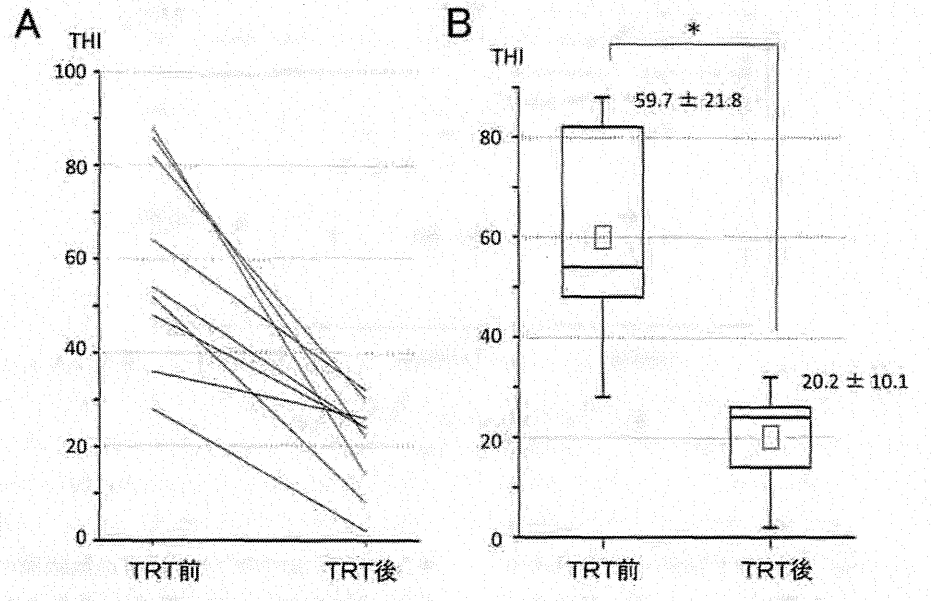

図2　TRTによるTHIスコアの変化
A：全症例のTHIの変化（n = 9）　B：Aのボックスチャート。最大値：75パーセンタイル、中央値：25パーセンタイル、最小値を示す。□は平均値、数字は平均±標準偏差を示す。

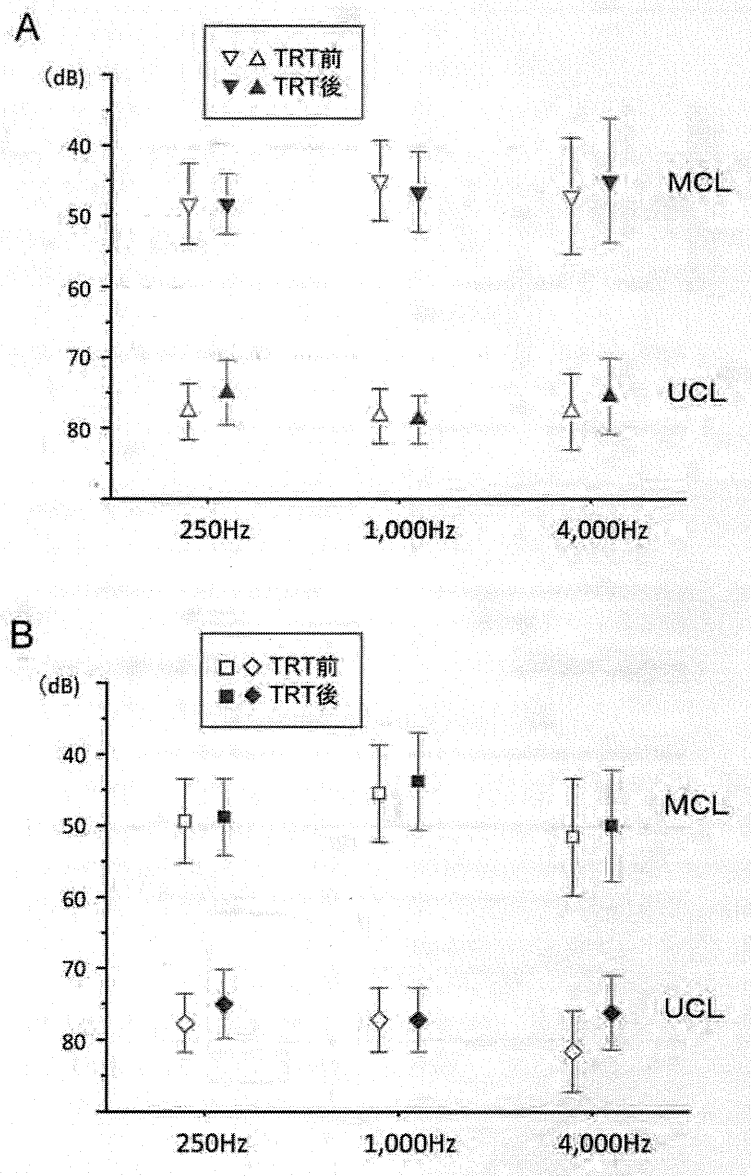

図3　TRT前後のMCL・UCL平均値
A：右耳　B：左耳

かもしれない。UCL値の低下が著しい症例について、TRTにより外界からの音に対しても順応しUCL値が上昇することも考えられたが、実際は変化しなかった（図4）。UCL低値は、内耳障害におけるリクルートメント現象を反映している[3]。リクルートメント現象の発生源についてはいまだ定説はないが、Tonndorf[11]は、有毛細胞の感覚毛と蓋膜の関係を唱えている。すなわち、感覚毛と蓋膜が離れていると低音圧刺激では刺激が伝わらず反応閾値が上昇するが、強音圧刺激に

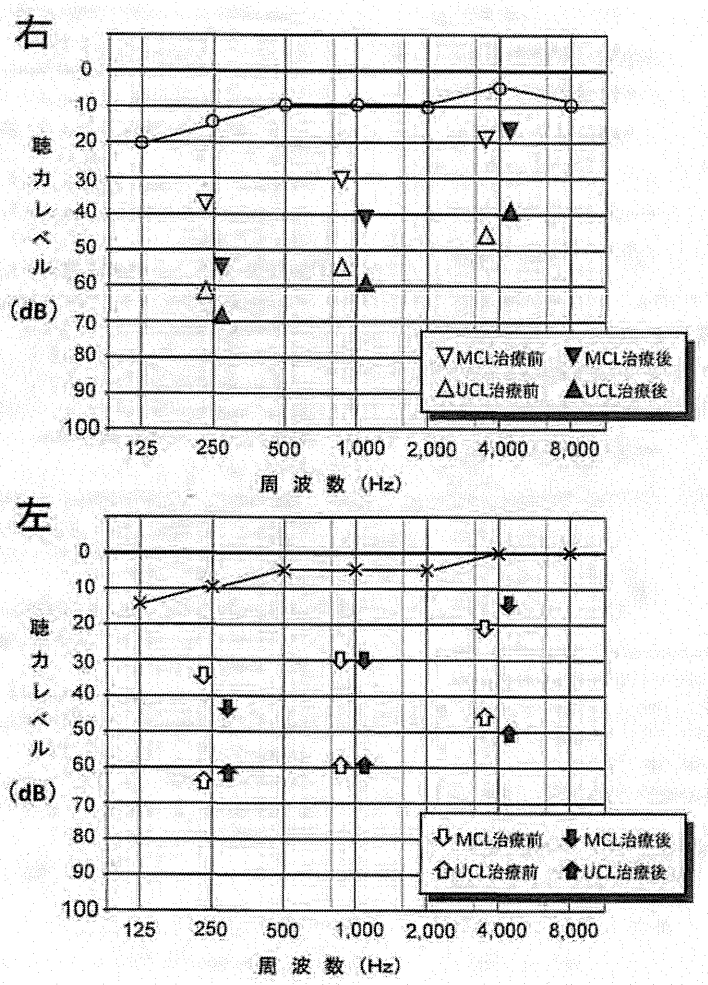

図4　症例のMCL・UCL変化

おいては感覚毛が蓋膜に接し、出力曲線の急峻なのびを示し、リクルートメント現象として観察されるという説である。またCenter-clippingという内有毛細胞に特異な現象についても言及している。いずれにしても、低値を示すUCLは内耳に起因しているため、TRTで大脳辺縁系での過敏性を変化させても、UCL自体には影響を与えなかったと考えられる。

耳鳴については不明の点が多く、原因についても諸説があるが、近年、内有毛細胞から神経伝達物質を直接受けるラセン神経節細胞の受容体の変化が、耳鳴の原因の一つであると報告された[12)-14)]。内有毛細胞からラセン神経節細胞への神経伝達物質はグルタミン酸であり、ラセン神経節細胞には、グルタミン酸レセプターのサブタイプであるAMPA（α-アミノ-3-ヒドロキシ-5-メソオキサゾール-4-プロピオン酸）レセプターが存在する。しかし、サリチル酸による耳鳴を発症させた場合、動物モデルでも[12)]、in vitro研究でも[13)] AMPAレセプターのほかにNMDA（N-メ