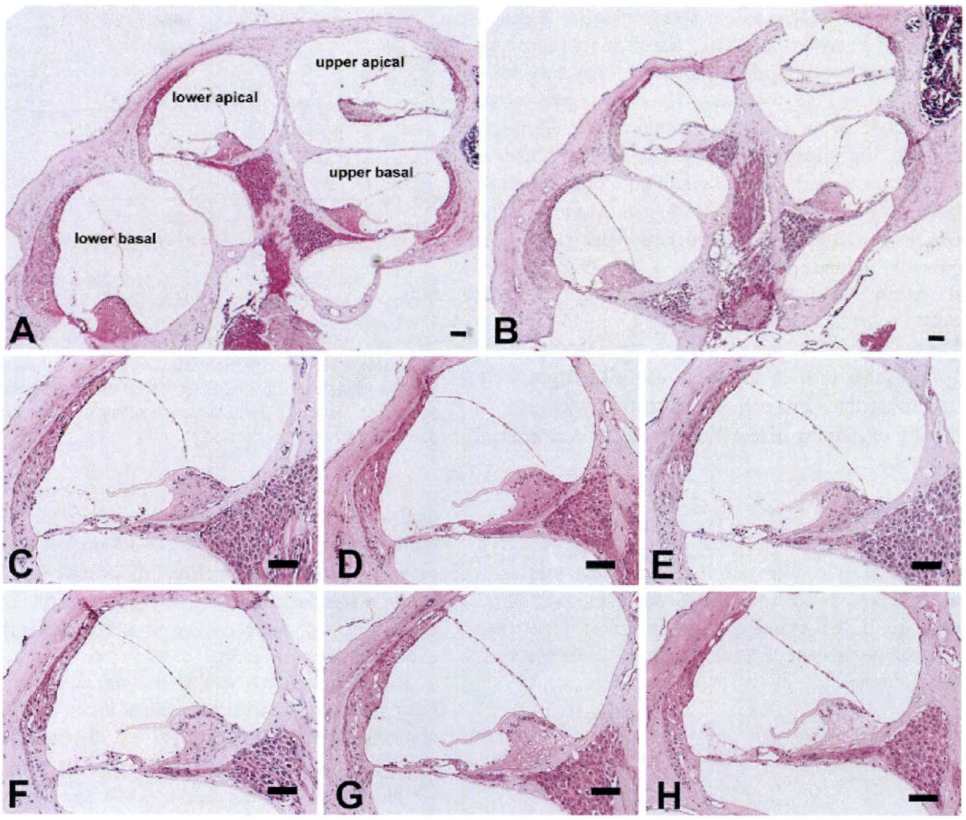

Fig. 2 H&E-staining within the lower apical turn of a mouse cochlea. **a**, **c** Control ear. **b**, **h** 8 weeks after nosie exposure. **d**, **e** Zero and six hours after exposure to 120 dB noise. No significant morphological changes were observed in the spiral limbus. However, 12 h after noise exposure, fibrocytes in the spiral limbus showed signs of degeneration and were noticeably fewer in number (**f**). Twenty-four hours after noise exposure, fibrocytes in the spiral limbus had disappeared completely (**g**). We observed no regeneration of fibrocytes in the spiral limbus 8 weeks after noise exposure (**h**). *Bar*: 25 μm

For SGCs (Fig. 3b) and spiral ligament (Fig. 3c), no significant cell loss was detected even at 8 weeks after noise exposure.

Apoptosis in the spiral limbus after noise exposure

To determine whether the noise-induced disappearance of fibrocytes in the spiral limbus was due to apoptosis, we analyzed cochleas from each group with ssDNA and TUNEL methods. Single-stranded DNA- and TUNEL-labeled cells were only found in the spiral limbus within the apical turns 12 h (Fig. 4c, g, j) and 24 h (Fig. 4d, h, k) after noise exposure. Thereafter, no apoptotic staining was present, even though fibrocyte loss was apparent.

No ssDNA- (data not shown) or TUNEL-labeled cells (Fig. 4) were found in the lateral wall, organ of corti, or spiral ganglion at any time prior to or after noise exposure.

Discussion

The loss of fibrocytes in the spiral limbus has been reported previously with sensory structure damage [1, 11–13]. Ohlemiller et al. noted that the loss of fibrocytes only happened in the apical turn just as our results. But no research paid attention to the time course and mechanism of the cell loss of fibrocytes in the spiral limbus. In our study, we found that the number of fibrocytes in spiral limbus within the apical turns of the cochlea began to decrease 12 h after noise exposure, a time ssDNA and TUNEL labeling was apparent in the fibrocytes of the spiral limbus. This means that the loss of fibrocytes in the spiral limbus within the apical turn was due to apoptosis. Since ssDNA and TUNEL are the common methods for detecting late-stage apoptosis, no TUNEL or ssDNA positive cells were detected until 12 h, initiation and progression of apoptosis

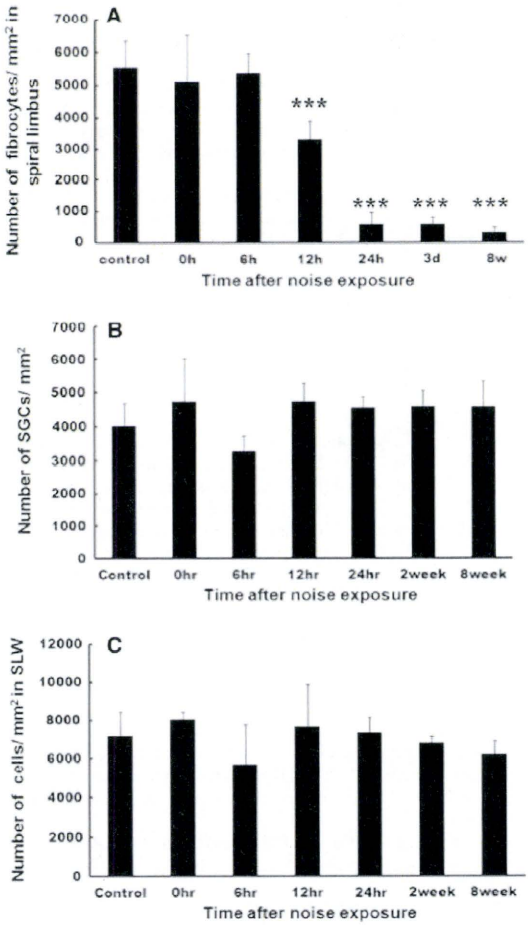

Fig. 3 The numbers of fibrocytes in the spiral limbus (**a**), SGCs (**b**) and cells in spiral ligament (**c**) within the lower apical turns of mouse cochlea. $^{***}P < 0.01$

of the fibrocytes in the spiral limbus may contribute to hearing loss at initial stage after noise exposure.

Noise exposure may lead to insufficiencies of cochlear blood flow [17], leading to ischemia and damage to capillary structure in the cochlea [18]. Ischemic damage leads to increased nitric oxide production, which causes cochlear cell damage [19]. Thus, noise-induced ischemia in cochlea might be associated with apoptosis in spiral limbus fibrocytes.

Ischemic damage, alteration in K^+ concentration, and acute energy failure induced by noise exposure may damage and cause dysfunction of fibrocytes in the spiral limbus. Twenty-four hours after noise exposure, a near complete loss of spiral limbus fibrocytes in the cochlea was detected only in the apical limbus, suggesting that noise-related injury to the limbus begins apically [11]. Interestingly, this may also occur in aging [20, 21], which is commonly associated with hearing loss.

Fibrocytes in the spiral limbus play a role in the inner route of the K^+ recycle in which the K^+ released from inner hair cells flows along the inner sulcus cells, fibrocytes and interdental cells back to the endolymph [6]. Loss of fibrocytes in the limbus induced by noise might disrupt K^+ recycling. Abnormal K concentration in the endolymph and the endocochlear potential (EP) has been reported to influence the activation of the hair cell, and then decrease the hearing level [7]. Minowa et al. [22] found that only the abnormality of the fibrocytes in the cochlea, with the normal appearance of the organ of Corti, the spiral ganglion even at the electron microscopy level, can lead to the sensorineural deafness in the mouse model of DFN3 nonsyndromic deafness. These results support our speculation that the fibrocytes play a critical role in the auditory function.

Several previous studies have shown that fibrocytes degenerate and ion transporter expression decreases in the spiral ligament after acoustic trauma [7, 23, 24]. In the present study, no significant changes have been found in

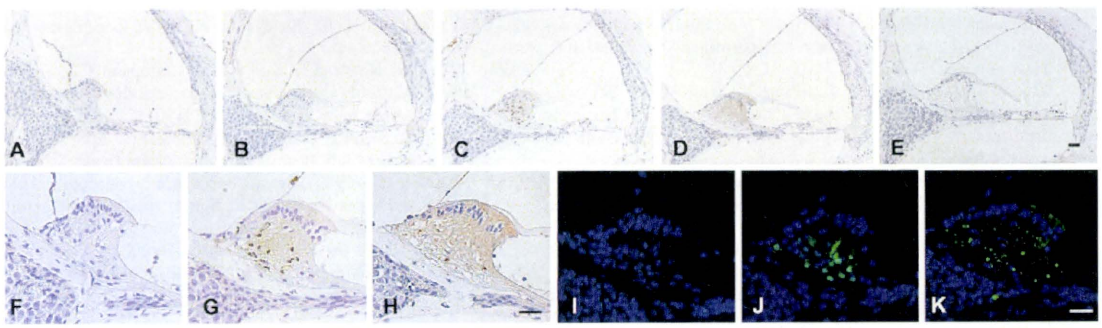

Fig. 4 TUNEL assay (**a–h**) and ssDNA staining (**i–k**) of the lower apical turn of the cochlea after noise exposure. **a**, **f**, **i** Control ear. **b** Six hours. **c**, **g**, **j** Twelve hours. **d**, **h**, **k** Twenty-four hours. **e** Eight weeks. *Bar*: 25 μm

the fibrocytes of spiral ligament. The genetic background of the mice which we used may account for this difference. The cochlear cells and structures in these mice may have different susceptibilities to noise-induced damage. Indeed, different strains of mice show marked variation in noise susceptibility [25, 26]. Ohlemiller [11] showed, for equal acoustic energy exposures, that CBA mice have a reduced EP and show cellular changes in lateral walls, while B6 mice have a normal EP and show little of the pathology seen in CBA mice.

Permanent changes in sensory structures after acoustic injury include hair cell loss, stereocilia damage, and neuronal loss [27], as well as loss of fibrocytes in the limbus and lateral wall, and strial degeneration [11]. In the present study, we did not observe damage in the organ of corti, spiral ganglion, and lateral wall. Several previous studies showed damage to the stereocilia only can lead to the hearing loss, including the PTS and temporary threshold shift (TTS) [27, 28]. Due to the type of histological material used in this study, we could not confirm whether stereocilia damage occurred or not. Although the loss of fibrocytes was only detected in the apical turn, a profound hearing loss was detected at 4, 8 and 16 kHz. Stereocilia damage might attributed to the wide hearing loss in this study.

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Conflict of interest We do not have any financial relationship with the organization that sponsored the research.

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Blockade of interleukin-6 signaling suppressed cochlear inflammatory response and improved hearing impairment in noise-damaged mice cochlea

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ABSTRACT

Hearing impairment can be the cause of serious socio-economic disadvantages. Recent studies have shown inflammatory responses in the inner ear co-occur with various damaging conditions including noise-induced hearing loss. We reported pro-inflammatory cytokine interleukin-6 (IL-6) was induced in the cochlea 6 h after noise exposure, but the pathophysiological implications of this are still obscure. To address this issue, we investigated the effects of IL-6 inhibition using the anti-IL-6 receptor antibody (MR16-1).

Noise-exposed mice were treated with MR16-1 and evaluated. Improved hearing at 4 kHz as measured by auditory brainstem response (ABR) was noted in noise-exposed mice treated with MR16-1. Histological analysis revealed the decrease in spiral ganglion neurons was ameliorated in the MR16-1-treated group, while no significant change was observed in the organ of Corti. Immunohistochemistry for Iba1 and CD45 demonstrated a remarkable reduction of activated cochlear macrophages in spiral ganglions compared to the control group when treated with MR16-1.

Thus, MR16-1 had protective effects both functionally and pathologically for the noise-damaged cochlea primarily due to suppression of neuronal loss and presumably through alleviation of inflammatory responses. Anti-inflammatory cytokine therapy including IL-6 blockade would be a feasible novel therapeutic strategy for acute sensory neural hearing loss.

1. Introduction

Cochlea is the end-organ of the auditory system that encodes sound stimuli to neuronal impulses. It is composed of mechanosensory organs, organ of Corti, primary neurons, spiral ganglion neurons, and surrounding mesenchymal compartments, spiral ligament, limbs and stria vascularis, which coordinately give rise to endocochlear potential that is essential for hair cells in the organ of Corti to generate action potentials in response to sound. When sound vibration reaches the inner ear through material waves of the middle ear ossicular chain, the mechanosensory-gated ion channels at the stereocilial bundles of inner hair cells are stimulated and opened, followed by inward potassium influx that eventually leads to action potential. This is conducted to an impulse of cochlear nerves via synapses between hair cells and axons of spiral ganglion neurons, and finally to central processing in the auditory cortex.

Overexposure to intense sound can cause permanent damage to the auditory system and result in sensorineural hearing loss (Hirose and Liberman, 2003; Saunders et al., 1985; Wang et al., 2002). This damage involves most of the cell types of the cochlea including spiral ligament, hair cells and spiral ganglion. Hair cells die gradually within 2 weeks after the insult (Yamashita et al., 2004), and spiral ganglion neurons become swollen (Parker et al., 2007; Wang et al., 2002). The pathophysiology of noise-induced hearing loss is composed of different types of damage including not only mechanical tissue damage but also secondary damage including excitotoxic-delayed damage (Puel et al., 1998). The molecular mechanisms of noise-induced delayed damage are not fully understood, although the partial contribution of oxidative stresses has been demonstrated (Yamashita et al., 2004).

Previously, the inner ear had been believed to be an immune-privileged organ isolated by the blood–labyrinthine barrier (Juhn and Rybak, 1981), similar to the blood–brain barrier of the central nervous system (CNS) and the blood–eye barrier of the cornea and retina of the eye. Recently, however, the induction of inflammatory responses and up-regulation of pro-inflammatory cytokines in the

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inner ear have been reported in various damaging conditions including noise-over stimulation (Hirose et al., 2005; Keithley et al., 2008; Ladrech et al., 2007; Ma et al., 2000; Satoh et al., 2002; Tornabene et al., 2006). The existence of inflammatory cells at steady state and their increase after insults of the inner ear have been reported by several groups (Hirose et al., 2005; Jokay et al., 2001; Ladrech et al., 2007; Okano et al., 2008; Tan et al., 2008). One explanation of the components for secondary damage would be the local up-regulation of pro-inflammatory cytokines because these molecules are endogenously elevated early stages in the damaged cochlear (within 1 day) and generally induce infiltration of inflammatory cells, of which increases are actually observed in the cochlea up to a maximum level at 3–7 days after the damage. In fact, one of the pro-inflammatory cytokines, tumor necrosis factor α (TNF-α), induces the recruitment of pro-inflammatory cells into the cochlea (Keithley et al., 2008), and the suppression of the infiltration of these cells by inhibiting the TNF-α signal reduces hearing loss in an animal model of immune-mediated labyrinthitis induced by immunization with keyhole limpet hemocyanin (Satoh et al., 2002; Wang et al., 2003).

Another pro-inflammatory cytokine, IL-6, is produced by a variety of cell types during tissue damage, infection and inflammatory diseases (Johnston et al., 2005; Loddick et al., 1998; Yang et al., 2005). Elevated IL-6 levels have been documented in various clinical conditions, indicating that IL-6 is produced in coordination with the disease response (Yoshizaki et al., 1989). Involvement of IL-6 in inner ear damage has also been shown previously. So et al. observed transient up-regulation of IL-6 in cisplatin-treated models, a common damaged cochlea model as well as one with noise-over stimulation (So et al., 2007). We reported that IL-6 is induced in spiral ganglion and lateral wall cells in the early phase of noise-induced cochlea trauma (Fujioka et al., 2006). In the brain and spiral cord, the function of IL-6 is still controversial. In spiral cord injury, the blockade of IL-6 suppressed reactive astrogliosis and ameliorated functional recovery (Okada et al., 2004), whereas, in sharp contrast, it aggravated cerebral damage in brain ischemia (Yamashita et al., 2005). These findings clearly show that the function of IL-6 is largely context-dependent, making it reasonable to address the issue of whether IL-6 acts as an inducer of acute cochlear damage. If it does, inhibition of IL-6 signaling may ameliorate impaired hearing function, which would be of clinical benefit.

IL-6 receptors represent a protein complex consisting of IL-6 receptor subunit (IL-6R) and IL-6 signal transducer (gp130). IL-6R can exist in both soluble and membrane-anchored forms. When ligands bind IL-6R, this complex moves to gp130 anchoring in cell membranes, and therefore the IL-6 signal is transmitted. Recently, a specific humanized neutralizing antibody against a soluble IL-6 receptor (MRA), which efficiently blocks IL-6 signaling, has been clinically used with promising effects in patients with rheumatoid arthritis (RA) (Nishimoto et al., 2004) and inflammatory bowel disease (Ito, 2005).

In the present study, we used rat anti-mouse IL-6 receptor neutralizing antibody (MR16-1) to inhibit IL-6 signals in noise-induced damaged cochlea. MR16-1 binds soluble IL-6 receptor and blocks transmitting signals to gp130 (Okazaki et al., 2002; Tamura et al., 1993).

The aim of the present study is to analyze the functional and pathological effects and the influence on inflammatory cells on inhibiting IL-6 signal in noise-induced damaged cochlea.

2. Materials and methods

2.1. Animals

Four-to-six-week-old male C57BL/6J mice were used ($n = 60$). The animals were purchased from Saitama-Experimental Animal Center and were bred at the Laboratory Animal Center, School of Medicine, Keio University under SPF conditions. All procedures were approved by the ethics committee of Keio University Union on Laboratory Animal Medicine in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health, Bethesda, MD).

2.2. Western blot

For western blotting, animals were killed at pre-noise ($n = 3$), 6 h ($n = 3$) and 24 h ($n = 3$) after noise exposure. Bilateral cochleae were used as a single sample. The whole cochlea was rapidly dissected out, placed in ice-cold lysate buffer (10 mM Tris–HCl, pH 7.6, 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, and protease inhibitor mixture), and then mashed and homogenized. After sonication, the samples were centrifuged at 15000 rpm for 4 min at 4 °C and then stored at −80 °C until electrophoresis. Samples each containing 10 μg protein were subjected to 15% SDS PAGE gel electrophoresis and transferred to membranes. We used rabbit anti-mouse interleukin-6 antibody (Sigma–Aldrich) as a primary antibody and secondary antibodies conjugated with biotin. They were enhanced with ABC Elite complex (Vectastain ABC Elite Kit; Vector Laboratories) and visualized with the ECL Blotting Analysis System (GE Healthcare). β-Actin (Sigma–Aldrich) was used as a primary antibody (standard control).

2.3. Rat anti-mouse IL-6 receptor monoclonal antibody (MR16-1)

The rat anti-mouse IL-6 receptor monoclonal antibody MR16-1 was prepared as described previously (Tamura et al., 1993). MR16-1 was shown to bind to the soluble IL-6 receptor of mice, suppressing IL-6-induced cellular responses in a dose-dependent manner (Okazaki et al., 2002). Other basic characterizations of this antibody have been reported previously (Okazaki et al., 2002; Tamura et al., 1993).

2.4. Administration of MR16-1

Immediately after noise exposure, mice were intraperitoneally injected with a single dose of MR16-1 (100 μg/g body weight; MR16-1 group) or with the same volume and concentration of purified rat IgG (ICN/Cappel; control group).

2.5. Noise exposure and auditory brainstem response (ABR)

In all the experiments, the animals were exposed for 2 h to octave-band noise (OBN) with a peak at 4 kHz, 124 dBSPL. To determine the magnification of the noise-induced hearing loss under the noise conditions in this study, we tested the threshold shift for the control group ($n = 4$) and MR16-1 group ($n = 3$) using the auditory brainstem response (ABR). Noise exposure and the ABR measurements were carried out using methods and equipment reported previously (Fujioka et al., 2006; Kanzaki et al., 2006). ABRs were performed at 3 days before and 3 weeks after noise exposure at 4 and 20 kHz. ABRs were determined on bilateral sides and averaged. The operator measuring the ABRs was unaware of the identities of the animals.

2.6. Preparation of epon-embedded sections

Epon-embedded plastic sections were made to investigate the pathological changes. Animals were killed at pre-noise ($n = 3$) and 3 weeks after noise exposure (MR16-1 group, $n = 6$; control group, $n = 6$). Animals were anesthetized and perfused with 8.6% sucrose in 0.01 mol/L phosphate-buffered (PB), followed by 2% paraformaldehyde (PFA), 2.5% glutaraldehyde and 5% sucrose in 0.1 mol/L PB transcardially, and then the inner ear was dissected from the temporal bone. After overnight fixation following perilymphatic perfusion with 2% PFA and 2.5% glutaraldehyde and 5% sucrose in 0.1 mol/L PB, the inner ears were decalcified in 0.1 mol/L EDTA and 5% sucrose in 0.1 mol/L PB for 7 days. After decalcification, the inner ears were postfixed with 1% OsO_4 and 5% sucrose in 0.1 mol/L PB for 20 min followed by gradual dehydration, and they were then embedded in epon. Epon blocks were sectioned with an ultramicrotome (ULTRACUT type E, Leica) at 1 μm thickness in a roughly horizontal plane parallel to the spiral axis of the modiolar, every five slices were picked up, and then staining with toluidine blue was done. Finally, three semi-thin sections parallel to the mid-modiolar plane (every 5 μm) of each animal were subjected to microscopic evaluation. The number of spiral ganglion neurons and tangent areas of Rosenthal canals were measured to calculate cell densities (number of neurons per area). The average cell density from three sections per one animal was determined and used for the evaluation.

2.7. Immunohistochemistry

Animals were anesthetized and perfused, and inner ears were fixed and decalcified similarly to the epon sections. We used phosphate-buffered saline (PBS) for transcardial perfusion, 4% PFA for fixation, and 1 mol/L EDTA for decalcification. After decalcification, the inner ears were embedded in Tissue-Tek O.C.T. Compound (Sakura Finetechnical) and then frozen. Frozen blocks were sectioned with a cryostat (MICROM HM550, ZEISS) at 8 μm thickness. The mid-modiolar sections were used for immunohistochemistry.

2.7.1. IL-6R and gp130

Animals were killed at pre-noise ($n = 3$). Rabbit anti-IL-6R antibody (Santa Cruz Biotechnology) and rabbit anti-gp130 antibody (Upstate cell signaling solutions)

were used as primary antibodies. 0.1% Triton-X was added to the gp130 solution. Slides were visualized using secondary antibodies conjugated with Alexa 555 [Alexa Fluor® 555 Goat Anti-rabbit IgG (A21429): Invitrogen].

2.7.2. Biotinylated MR16-1

MR16-1 was biotinylated with EZ-Link NHS-Biotin reagents (Thermo Fisher Scientific). Control group animals ($n = 2$) were killed at pre-noise and animals injected with biotinylated MR16-1 ($n = 2$) were killed at 6 h after noise exposure. After quenching with 1.5% hydrogen peroxide, the slides were enhanced using the Elite ABC Kit (Vector Laboratories) and visualized with diaminobenzidine (DAB) solution (Wako Pure Chemical Industries).

2.7.3. Iba1 and CD45 (including count method)

Animals were killed at pre-noise ($n = 3$) and 3, 7, 14 days after noise exposure (MR16-1 group, $n = 3$; control group, $n = 3$). Rat anti-mouse CD45 antibody conjugated with Phycoerythrin (PE) (eBioscience) and rabbit anti-Iba1 antibody (Wako Pure Chemical Industries) were used as primary antibodies. The Iba1-immunostaining was performed using a secondary antibody conjugated with Alexa 488 [Alexa Fluor® 488 Goat Anti-rabbit IgG (A11034): Invitrogen]. We also used a secondary antibody conjugated with Alexa 555 [Alexa Fluor® 555 Goat Anti-rat IgG (A21434): Invitrogen] for CD45-immunostaining to enhance the signal. CD45 is a leukocyte common antigen and Iba1 is specific for microglia/macrophages. Sections were stained every five slices, and three sections were selected randomly from 6 to 8 stained sections. The cell densities of positive cells were calculated similarly to those of epon sections.

2.8. Statistical analysis

Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by the Tukey-test for analysis of spiral ganglion cell density and Iba1 CD45 positive cell density. Mann–Whitney *U*-test was used for analysis of ABR thresholds. $p < 0.05$ was considered statistically significant. All data are presented as mean ± SEM.

3. Results

3.1. Expression of IL-6 and its receptors after noise exposure

In the present study, we first performed Western blot analysis to confirm the induction of IL-6 in the inner ear after noise exposure in the mouse model. IL-6 increased at 6 h and then decreased at 24 h after noise exposure, indicating consistent transient up-regulation in the mouse model (Fig. 1A).

We next examined the expression of IL-6R and gp130 in the inner ear by immunostaining. Both IL-6R- and gp130-immunoreactivities (IRs) were detected in the spiral ganglions and lateral walls (Fig. 1B, D–F, H and I). In the organ of Corti, IL-6R-IR was observed in both hair cells and supporting cells, whereas gp130-IR was detected only in hair cells (Fig. 1C and G). These results confirmed that IL-6R (a target of MR16-1) and gp130 (a signal transducer of IL-6) were expressed in the mouse inner ear.

3.2. Drug delivery of MR16-1 in the inner ear

To confirm whether injected MR16-1 reaches the inner ear, biotinylated MR16-1 was injected intraperitoneally and its distribution in the inner ear was assessed by immunostaining. Biotin IR was found in spiral ganglions, the organ of Corti and stria vascularis, and lateral walls (Fig. 2A–F), and there was no apparent difference in staining density among the areas in all turns (from apical to basal). These results confirmed the delivery of MR16-1 in the inner ear of noise-exposed mice.

The levels of STAT3 and Erk1, which are down-stream signal cascades of IL-6 receptor, did not change between the control and MR16-1-treated groups, but the levels of the phosphorylated forms of both STAT3 and Erk1 decreased in the sample after administration of MR16-1 (Supplementary Fig. 1).

3.3. Improvement in hearing function

With the noise centered at 4 kHz in a one-octave wide band of which the peak magnitude was 124 dB, we produced a noise-induced hearing loss model in the present experiment. We

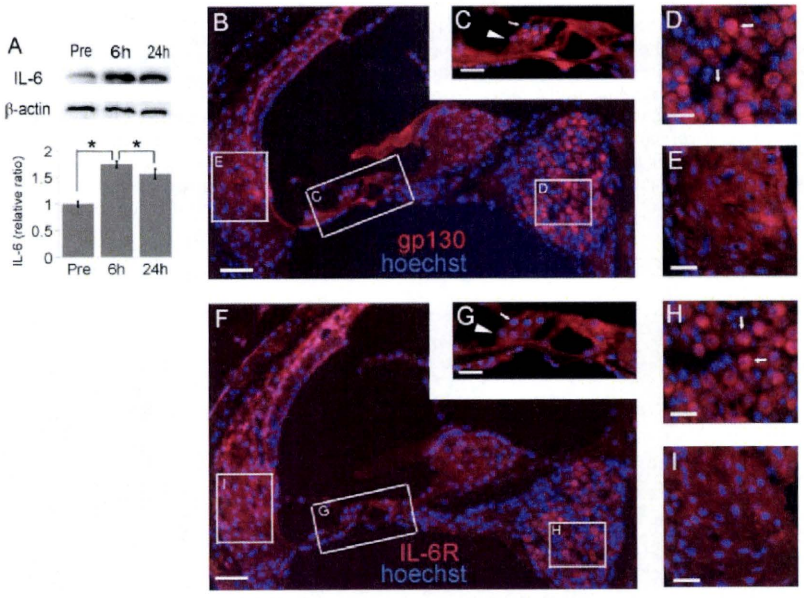

Fig. 1. The expression of interleukin-6, gp130 and interleukin-6R. (A) The results of Western blotting for IL-6 at three different time points. Significant differences in IL-6 expression were observed between pre-noise, 6 and 24 h after noise exposure. The vertical bar represents the relative ratio of IL-6 versus β-actin (internal control). (B–I) The expressions of gp130 (B–E) and IL-6R (F–I). gp130 and IL-6R were expressed in spiral ganglions (E and I), lateral walls (D and H) and the organ of Corti (C and G). Red is IL-6R and blue is hoechst (B–E); red is gp130 and blue is hoechst (F–I). C–E and G–I were high magnifications of boxes in B and F, respectively. Both hair cells (white arrow) and supporting cells (white arrowhead) expressed both gp130 and IL-6R (C and G). Spiral ganglion neurons expressed both gp130 and IL-6R (white arrows) (D and H) (scale bars: B and F, 50 μm; C–E and G–I, 20 μm).

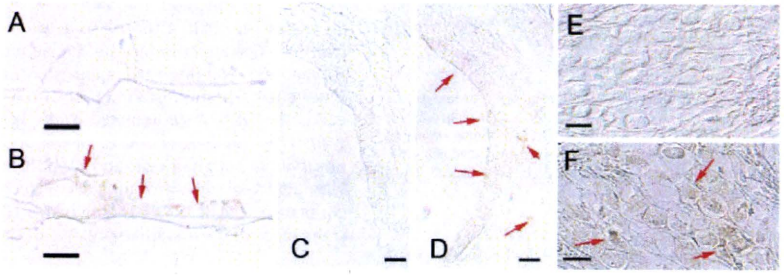

Fig. 2. Drug delivery of MR16-1 in the inner ear: (A, C and E) control group; (B, D and F) biotinylated MR16-1 group. Accumulation in the organ of Corti, stria vascularis and spiral ganglions and spotty staining in the lateral walls were observed (red arrows). Scale bars = A–F, 20 μm.

performed ABR to evaluate hearing function. After noise exposure, the threshold shifts of the IgG-injected control group were 49.4 ± 2.1 dB and 55.6 ± 3.3 dB, and those of the MR16-1 injected group were 33.3 ± 5.8 dB and 50.8 ± 7.1 dB at 4 and 20 kHz, respectively. We observed significant hearing improvement in the MR16-1 group compared with the control group at 4 kHz ($p < 0.05$), but not at 20 kHz, at 3 weeks after noise exposure (Fig. 3). Particularly, hearing function was improved in apical turn by the MR16-1 administration.

3.4. Protection of spiral ganglion treated with MR16-1

The damage caused by noise exposure was seen in many different cell types in the inner ear including spiral ganglion neurons. Previous studies revealed that the extent and intensity of the damage depended on the level, frequency and duration of the exposure. In particular, the severity of vacuoles or swelling observed in the spiral ganglion neurons varied depending on the strength of the noise (Parker et al., 2007; Wang et al., 2002). In the present study, we found decreased cell density in the spiral ganglion due to the disappearance of ganglion neurons in the noise-exposed animals. In contrast, the cell density of spiral ganglion neurons in the MR16-1-treated group was well preserved. Although a significant protective effect was observed in the apical turn of spiral ganglion neurons by MR16-1 administration, the spiral ganglion neurons in the middle or basal turn were damaged in a similar way to the non-treatment control group (Fig. 4).

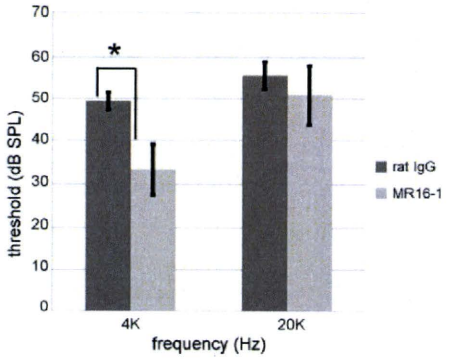

Fig. 3. Hearing function (auditory brainstem response). Functional improvement with MR16-1 measured by auditory brainstem response (ABR). We gave a tone burst sound stimulus, lowered the volume, recorded ABR waveforms, and then determined the threshold. Significant hearing improvement was observed at 4 kHz with MR16-1 treatment 3 weeks after noise exposure ($p < 0.05$).

To evaluate the damages in the hair cells after noise exposure, we performed surface preparation of the inner ear. Both inner and outer hair cells were decreased by noise exposure, but no significant difference was observed between MR16-1 treatment group and the control group in all turns (Supplementary Fig. 2).

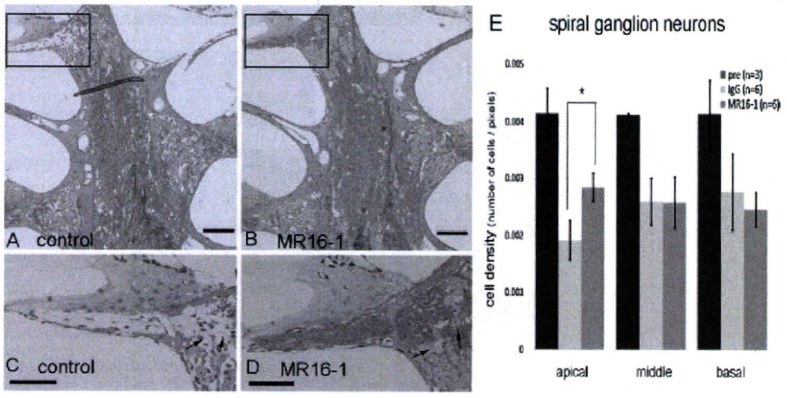

Fig. 4. Pathological changes in spiral ganglions. Spiral ganglion neurons in the apical turn were decreased in the control group after noise exposure (C: black arrow), not in MR16-1 treatment group (D: black arrows): A and C, control group; B and D, MR16-1 treatment group; C and D are high magnifications of the boxes in A and B. Scale bars = A and B, 100 μm; C and D, 50 μm. The decrease in spiral ganglion neuron densities was attenuated significantly in the MR16-1 treatment group ($p < 0.01$) (E).

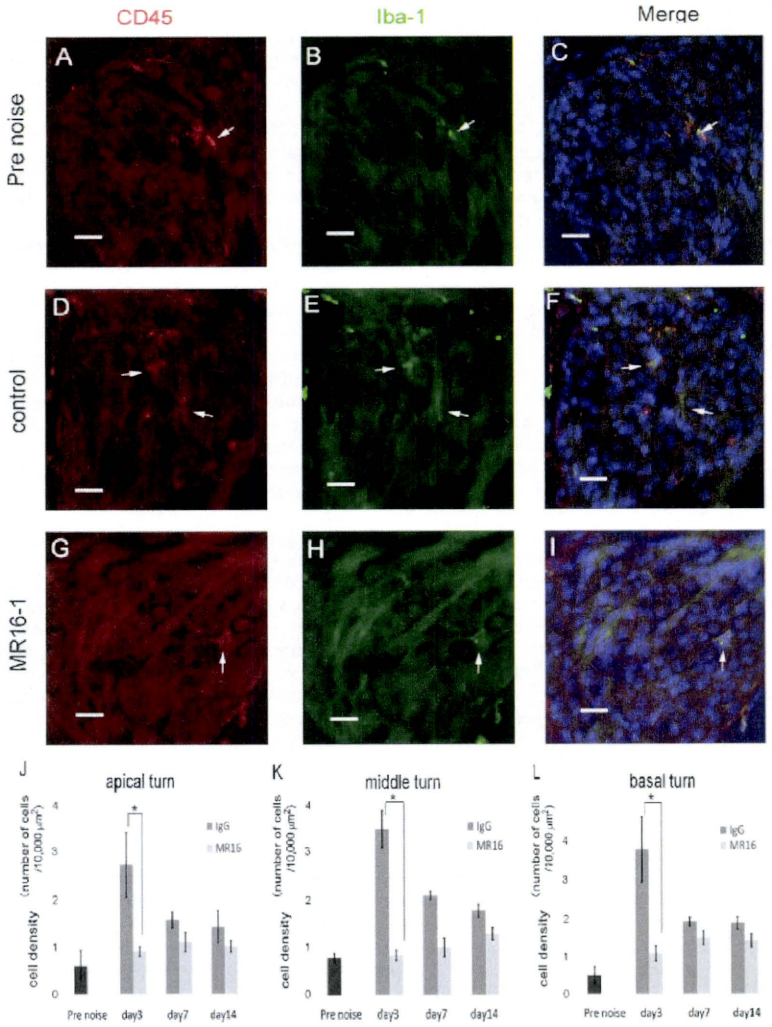

Fig. 5. Inflammatory cells in the spiral ganglions. A few double positive cells were observed at pre-noise (A–C). Three day after noise exposure, double positive cells were increased in the control group (D–F), but not so much increased in the MR16-1 treatment group (G–I). Double positive cells are indicated by white arrows. Blue is hoechst, and red is CD45 (A, D and G), Iba-1 (B, E and H); C, F and I are merged images. Scale bars = 20 μm. Significant differences were observed between the control group and MR16-1 treatment group in all turns on day 3, and in middle turn on day 7 (J–L). Cell densities of the MR16-1 treatment group were lowered at all time points and turns compared with the control group in apical (J), middle (K), and basal turn (L), respectively.

3.5. MR16-1 suppressed inflammation in inner ear

We assessed the number of Iba1/CD45- double positive cells by immunostaining to examine whether MR16-1 affected inflammatory cells in the inner ear. Following noise exposure, Iba1/CD45-double positive cells in the spiral ganglion increased to a peak at day 3, then decreasing at days 7 and 14 after noise exposure in apical, middle and basal turns. Treatment with MR16-1 significantly lowered the number of Iba1/CD45-double positive cells at day 3 after noise exposure in all turns (Fig. 5). In the spiral ganglions of all groups, Iba1 was detected in 99.1 ± 0.7% of CD45 positive cells, and CD45 was positive in 99.1 ± 0.8% of Iba1-positive cells. Almost all positive cells expressed both CD45 and Iba1, with very few cells expressing only either CD45 or Iba1. These results indicated that MR16-1 effectively suppressed the infiltration of cochlea macrophages in the spiral ganglion after noise exposure.

Similarly, the lateral walls also exhibited the infiltration of cochlea macrophages after noise exposure. Iba1/CD45-double immunostaining was detected mainly in the inferior region and adjacent to the junction of the ligament with the bony cochlear capsule among type III fibrocytes. Following noise exposure, cochlea macrophages were found throughout the spiral ligament. On the other hand, MR16-1 treatment did not significantly reduce the number of Iba1/CD45-double positive cells in the lateral walls compared with the control group (Supplementary Fig. 3).

4. Discussion

4.1. Protective effect of MR16-1 in NIHL

Our present data clearly showed that the blockade of IL-6 attenuated inflammatory reactions within the cochlea. In general, the pathophysiology of noise-induced hearing loss is diverse, including not only primary physical tissue damage, but also secondary damage including excitotoxic-delayed damage in hair cells, spiral ganglion cells and lateral wall (Hirose and Liberman, 2003; Wang et al., 2002). Infiltration of inflammatory cells, especially macrophages, may also be another pathophysiological source of secondary damage. We have shown herein that MR16-1 exerts a protective effect both functionally and pathologically in the area of most intense sound exposure (4 kHz). However, we did not observe any change in the number of hair cells (Supplementary Fig. 2), suggesting that MR16-1 could not prevent delayed hair cell loss; this assumption is also supported by the in vitro result that recombinant IL-6 protein did not affect cell viability onto an organ of Corti-derived cell-line, HEI-OCI, which has been used for the screening of ototoxity for many years (data not shown). Over the past few years, Liberman and colleagues have revealed that the damage of cochlea is, at least partially, primarily by the loss of spiral ganglion cells independently from hair cell damage (Kujawa and Liberman, 2006). In the present study, the only area where significant histopathological changes were observed was in the spiral ganglion, which was accompanied by significant decreases of Iba1/CD45-double positive cells specifically in the Rosenthal canal where the preserved neurons were located. Subtle changes of lateral wall fibrocytes in the type II area were also observed, but the difference was not significant. As a consequence, we concluded that the effect of MR16-1 against noise-over exposure would presumably be due to the attenuation of primarily neuronal loss in the cochlea through strong suppression of immune cell recruitment, which is distinctively seen in the Rosenthal canal.

4.2. Involvement of cochlear macrophages in MR16-1 treatment

Several groups have reported the infiltration of inflammatory cells into the inner ear at peak periods of 3–7 days after several types of damage including noise exposure, drugs, and surgical stress (Fujioka et al., 2006; Hirose et al., 2005; Ma et al., 2000; Okano et al., 2008; Sato et al., 2008; Satoh et al., 2002; Tan et al., 2008; Tornabene et al., 2006). Our results were compatible with those reports, although none of them reported the strong suppression of macrophage infiltration. Hirose et al. reported that the increase of inflammatory cells with CD45 or Iba1 in the inner ear after noise exposure arises from migration from the circulation (Hirose et al., 2005), and they proposed that these cells were "cochlear macrophages". Lang et al. first reported that those cells were recruited from bone marrow by prospective labeling experiments (Lang et al., 2006), and Tan et al. observed that those cells expressed CD45 (Tan et al., 2008). Therefore, we considered that CD45 and Iba1 were the best markers for cochlear macrophages and assessed the number of Iba1- and CD45-double positive cells. In the animal model we used in this study, their densities were increased four to five times compared to pre-noise, and were decreased by more than 90% by MR16-1 treatment. In spinal cord injury, some studies have reported that hematogenous macrophages were cytotoxic, and that their excessive infiltrations into the lesion were rather detrimental (Gris et al., 2004; Popovich et al., 1999; Saville et al., 2004). Most recent findings showed that the excessive infiltration of hematogenous macrophages in the cochlea resulted in severer damage in the cochlea after ototoxic insult, revealed by bone marrow transplantation from CX3CR1 knock-out mice (Sato et al., 2009). Taking our successful attenuation of hearing loss into account, we assume that reduced cochlear macrophages were possibly cytotoxic for spiral ganglion neurons and that the blockade of IL-6 signal by MR16-1 reduced deteriorative infiltrating hematogenous macrophages, resulting in the improvement of noise-induced hearing loss both functionally and pathologically.

The underlying mechanisms of how MR16-1 reduced the number of macrophages remains to be elucidated. IL-6 is widely recognized as a multifunctional pro-inflammatory cytokine that has a variety of roles in immune responses and inflammations, such as antibody production through B-cell activation, T-cell differentiation and macrophage infiltration to local damaged areas (Kishimoto, 1989; Muraguchi et al., 1988). Several reports concerning CNS showed a strong impact of IL-6 on inflammatory responses, leading to translational application in a clinical setting: in spinal cord injury, blockage of IL-6 using MR16-1, reduced inflammatory cells and reactive astrogliosis, resulting in functional recovery (Okada et al., 2004). IL-6-null mice are less vulnerable to brain trauma by cryo-ablation (Morganti-Kossmann et al., 2002) and are resistant to experimental autoimmune encephalomyelitis due to a significant decrease in inflammatory reactions (Mendel et al., 1998). The underlying mechanisms for many of those phenomena are not yet fully understood, although increasing knowledge about this classical cytokine is gradually leading to a new conceptualization: the pivotal pro-inflammatory cytokine IL-6 brings the immune system from "steady state" to "inflammatory phase", and CD4-positive T-cells from "arresting" state to "armed" inflammatory T-cells, so-called Th17 (Chen and O'Shea, 2008), that enhance multiple inflammatory reactions including chemokine secretion. B-cells are also affected, so antibody production will be increased. Our results clearly demonstrated that blockage of IL-6 signaling dramatically suppressed inflammatory reaction in the damaged cochlea. Although we lack the evidence, a similar phenomenon seemed to have occurred in our model, suggesting that MR16-1 works as an "anti-inflammatory" agent in regard to noise damage. Further investigations are warranted to address this issue.

In our model, functional and pathological improvement was observed only in the apical turn. Generally, when a band noise is chosen for damaging cochleae, cellular loss in spiral ganglion neurons is observed in the part corresponding to the frequency (Wang et al., 2002). In this study, we used 4 kHz octave-band noise, of which the expected damage was possibly at a maximum level in the apical turn. Severer damage in the apical turn may change the cytokine expression and result in harmfulness of hematogenous macrophages being stronger than in another turn. We propose that MR16-1 inhibited this stronger harmfulness and improved hearing function and pathological changes.

Iba1 and CD45 positive cells shown in lateral walls were derived from mainly the inferior region and adjacent to the junction of the ligament with the bony cochlear capsule among the type III fibrocytes at pre-noise. After noise exposure, double positive cells were found throughout the spiral ligament (Supplementary Fig. 3). These findings were compatible with those of previous reports (Hirose et al., 2005; Tan et al., 2008). In lateral walls, significant suppression of infiltrating cells was not found after treatment with MR16-1. This suggests that MR16-1 treatment was not effective in inhibiting recruitment of macrophages but that there might at least be some influence at the cytokine level.

4.3. Drug delivery

Our result shows that the systemic application successfully delivered MR16-1 into the cell membrane throughout the cochlea and blocked the induction of IL-6 signaling in the damaged cochlea, even though endogenous IgG level in the labyrinth is at a very low

concentration. Previous reports showed that vascular contusion or disruption frequently occurred after intense noise-induced damage, and thus we assume that this drug entry is mediated by the disruption of the blood–labyrinth barrier. Currently, more than 20 monoclonal antibodies are being used in clinics or are undergoing clinical trials. Our findings suggested that monoclonal antibody treatment will be a feasible option for acute inner ear disorder including noise-induced hearing loss.

4.4. Clinical relevance—translational study

One of the advantages of anti-IL-6 receptor antibody treatment is that the humanized anti-IL-6R antibody (MRA; Atlizumab) has already been used in clinics and showed high efficacy in Castleman's disease and rheumatoid arthritis (Nishimoto et al., 2004). The results of our study might be of help in the development of anti-IL-6 receptor antibody treatment for the clinical setting, presenting the possibility of minimizing the risk for sensorineural hearing loss.

5. Conclusion

In conclusion, we have successfully demonstrated the significance of anti-IL-6 treatment as a novel strategy for acute sensorineural hearing loss. This treatment would be accompanied by a decrease in infiltrating macrophages through non-steroidogenic anti-inflammatory response. Different from the variety of adverse effects of corticosteroids, IL-6 antibody treatment agents have less side effects, thus potentially offering a clinically preferable option.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neures.2009.12.008.

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原　著

前庭・蝸牛症状を呈したアブミ骨ならびに代用アブミ骨前庭陥入例の画像所見と治療経験

福留　真二[1]・東野　哲也[2]

Imaging findings and treatment of invagination of the stapes and stapes prosthesis into the vestibule

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We experienced cases of invagination into the vestibule of a Teflon piston following stapes surgery as well as of the stapes itself caused by an earpick-related injury. In all three cases, the pathology was accurately determined by diagnostic imaging centered around high-resolution CT. All patients had severe invagination, and stapedectomy was considered necessary for improvement of their symptoms. Although the vestibular symptoms improved postoperatively, one patient developed hearing loss and relapse of nystagmus two months later. Our examination of the problems involved in the treatment of invagination of the stapes or stapes prosthesis into the vestibule for the acute, subacute, and chronic phases showed that different problems existed during each phase.

Key words: stapes invagination, teflon piston, high resolution CT

はじめに

近年側頭骨画像診断の向上は著しく，高分解能CTならびに多断面再構成画像（MPR）の活用によりアブミ骨病変の詳細な描出が可能となった[1]。今回我々はアブミ骨手術後にテフロンピストンが前庭腔に陥入した症例および耳掻きによる外傷によりアブミ骨が前庭腔に陥入した2症例を経験し，いずれの症例も側頭骨CTで明瞭な病態診断が可能であった。これらの症例は発症直後から7年に及び，症例毎に異なった治療上の問題点を有していたので報告する。

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[2]宮崎大学附属病院耳鼻咽喉・頭頸部外科

症　例

症例1：52歳，女性。

主訴：めまい，左難聴。

家族歴：特記事項なし。

既往歴：特記事項なし。

現病歴：平成18年11月，耳掻き中にねこが飛びかかってきて左耳を突き，直後より回転性めまいと嘔気，左難聴を自覚。近医にて3日間入院加療

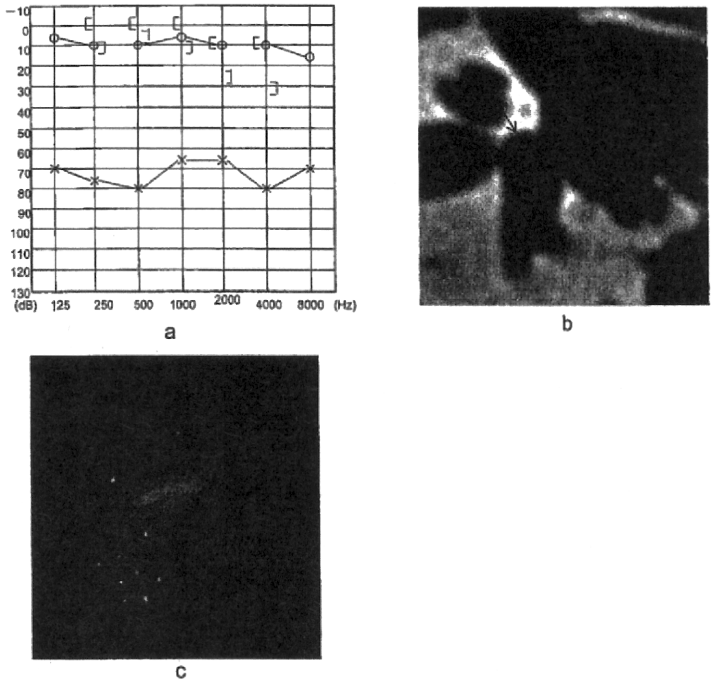


図1　症例1

a：術前の聴力像にて，左に気骨導差と高音域での軽度骨導閾値上昇を認める
b：術前CT画像にてアブミ骨が前庭内に陥入している所見（矢印）が認められる
c：手術所見にてキヌタ骨長脚の骨折およびアブミ骨の前庭内陥入（矢印）が認められた

を受けた。その後もふらつきが持続したため，3ヶ月後に公立病院耳鼻咽喉科で左鼓室試験開放術，鼓膜閉鎖術を受けた。その際アブミ骨脱臼所見があったが，術者がアブミ骨手術の経験がないため，当院を紹介受診した。

初診時所見：注視眼振は認めなかったが，頭位・頭位変換眼振検査では，全頭位で，左向きの水平性定方向性眼振を認めた。左形成鼓膜は良好であったが，オージオグラムでは40〜70 dBの気骨導差を認め，高音域での骨導域値の軽度上昇を伴っていた（図1a）。CT所見でアブミ骨陥入像（図1b）を確認後，アブミ骨手術による聴力悪化の可能性について同意を得た上で手術を施行した。

手術所見：平成19年4月23日左アブミ骨手術を施行した。耳後切開より鼓室に入ると，キヌタ骨長脚は骨折し，豆状突起の付着したアブミ骨は前庭腔内に陥入しており（図1c），ツチ骨とキヌタ骨も離断していた。慎重にアブミ骨を摘出し筋膜片で前庭窓を閉鎖，キヌタ骨も摘出し，シュクネヒトのマレウスアタッチメントを用いてStapedectomy-Mの形とした。

術後経過：術後めまい症状は改善し，1週間目に行った検査では，術前にみられた左向き水平性眼振は消失していた。術後の純音聴力検査において気骨導差は改善したが，高音域の骨導閾値上昇がみられた。

症例2：47歳，男性。

主訴：めまい，右難聴。

家族歴：特記事項なし。

既往歴：特記事項なし。

現病歴：平成19年5月26日，耳掻き中に誤って右耳をついた。その直後よりめまいと右難聴が出現し，翌日近医耳鼻咽喉科を受診し，当院紹介受診となった。

初診時所見：右鼓膜は下方に不整形の穿孔あり

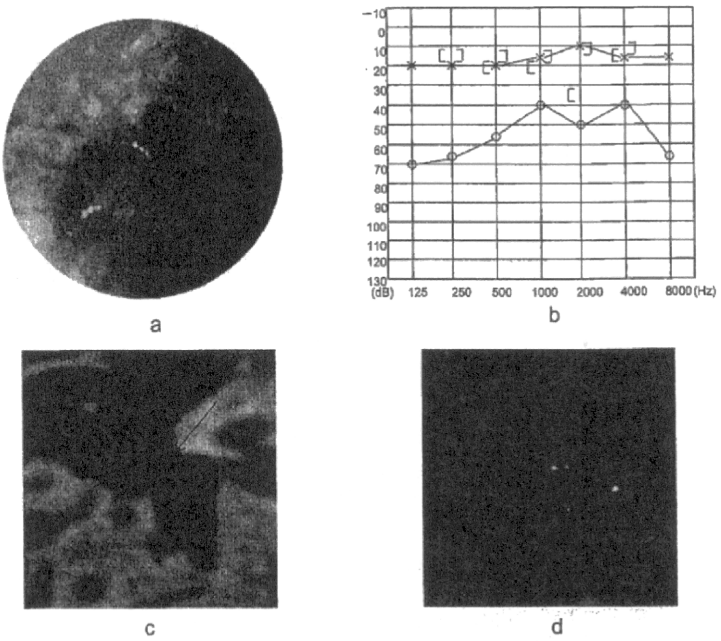


図2 症例2
a：右鼓膜には下方に不整形の穿孔（矢印）が認められた
b：術前の聴力像で，右は低音域での気骨導差と2000 Hzでの骨導閾値の軽度上昇を認めた
c：術前CT画像にてアブミ骨が前庭内に陥入している所見（矢印）が認められた
d：手術所見にてアブミ骨が前庭内に陥入している所見（矢印）が認められた

（図2 a），鼓室粘膜の発赤と水様性貯留液を少量認めた。注視眼振検査において左向きのI°の水平性定方向性眼振を認め，頭位・頭位変換眼振検査でも，左向きの水平性定方向性眼振を認め，眼振は右下頭位で増強した。聴力検査では低音域で30～50 dB，中音域から高音域にかけて15～20 dBの気骨導差を認め，2000 Hzの骨導閾値上昇を伴っていた（図2 b）。側頭骨CTを撮影したところ，図2 cのようにアブミ骨の前庭内陥入が認められたため，アブミ骨脱臼を伴う外傷性鼓膜穿孔と診断し，鼓室粘膜の炎症を伴っていたので抗生剤投与と安静を促した。めまい症状が強く，手術的治療を強く希望したため，難聴悪化の可能性を充分に理解頂いた上でアブミ骨摘出術を施行した。

手術所見：平成19年6月4日右鼓膜形成術，右アブミ骨手術を施行した。鼓膜穿孔部は炎症性肉芽と皮膚の鼓室面へのめくれ込みを伴って肥厚。鼓室内にも粘膜肥厚と少量の黄色調貯留液を認めた。アブミ骨は前庭内に陥入しており，脱臼部からは外リンパの漏出を認めた。ツチ骨とキヌタ骨が離断していたため，キヌタと連結したアブミ骨を慎重に持ち上げ，前庭窓部を筋膜片で閉鎖した。シュクネヒトのマレウスアタッチメントをツチ骨柄近位端に連結してアブミ骨手術を行った。

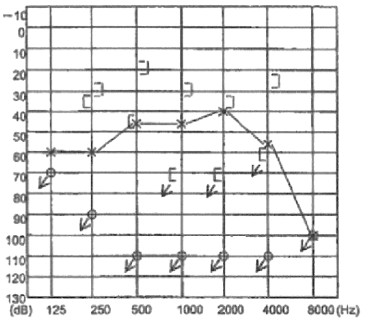
術後経過：術後早期においてめまい症状消失し，術前にみられた左向き水平性眼振は消失，聴力も保存された。しかし，術後2ヶ月目に再診した際，約20 dBの骨導閾値の上昇を認め，Frenzel眼鏡での頭位・頭位変換眼振検査において，左向き水平性眼振の再出現が確認された。日常生活に問題となる前庭症状はなくなったが，最終受診時（術後5ヶ月目）にも眼振は認められている。

症例3：43歳，女性。

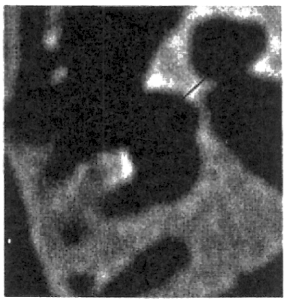
主訴：めまい，右難聴。

家族歴：娘に伝音難聴，耳瘻孔と頸瘻あり（当科でBranchio-Oto-Renal（BOR）症候群と診断）

現病歴：幼少時より両難聴を指摘されていた。



a



b

c

図3 症例3
a：術前の聴力像で，右は高度混合性難聴を呈している
b：術前CT画像にてテフロンピストンの前庭内陥入（矢印）が認められた
c：手術所見にてテフロンピストンの陥入（矢印）が認められた

左伝音難聴に対して26歳時に某公立病院耳鼻科にて伝音再建手術が施行され，左聴力改善が得られた。この時にアブミ骨の奇形も指摘されたが，アブミ骨は保存された。36歳時に右伝音難聴に対して某病院にて右アブミ骨手術を受けたが，その直後から右聾となった。当時の術者からの説明では外リンパガッシャーのため，聴力を保存することが不可能とのことであった。術後から鼻をかんだり，急激に頭を動かしたりすると，くらっとするめまいを感じるようになり，走るとそれにあわせてウォンウォンと頭の中で鳴るような感じがあった。

初診時所見：両耳後部切開痕，両耳前部に瘻孔摘出後瘢痕あり。両鼓膜は正常で，注視眼振および頭位・頭位変換眼振検査において眼振は認められなかった。純音聴力検査では左43。8dB，右聾であった（図3a）。CT検査で右側キヌタ骨豆状突起～アブミ骨が認められず，右前庭腔内にテフロンピストン様の形状を呈する高吸収域を認めた（図3b）。T2強調MRIでは同部位は線状の低信号として描出された。左側はキヌタ骨長脚下端とアブミ骨頭との間を繋ぐ大きな骨構造が認められており，また両側とも蝸牛の軽度低形成も認められた。右前庭腔内に陥入したテフロンピストンが前庭症状の原因になっている可能性を説明し，手術の同意が得られた。

手術所見：平成19年8月22日手術を施行した。右外耳道，鼓膜を剥離すると，鼓室内にテフロンピストンのフック部を認めたため（図3c），慎重に抜去すると，膜状に閉鎖した前庭窓のテフロンピストン抜去部位より外リンパの漏出を認め，この部を筋膜片で閉鎖した。

術後経過：術直後より前庭症状は消失し，術後2年以上経過した現在も再発はない。

表1，2に3症例のまとめを記す。

表1　アブミ骨・代用アブミ骨前庭陥入3症例の病歴のまとめ

症例	年齢	性別	受傷原因	症状	初診までの経緯	初診時所見
1	52	F	耳掻き	左難聴回転性めまい	近医で鼓膜閉鎖術時にアブミ骨陥入認めた	左向き眼振 左混合性難聴
2	47	M	耳掻き	右難聴回転性めまい	近医耳鼻科より紹介	右鼓膜穿孔，左向き眼振 右混合性難聴
3	43	F	アブミ骨手術	右難聴くらっというめまい	手術を施行した病院より紹介	眼振なし右聾

考　察

1）アブミ骨脱臼診断に対する高分解能CT，多断面再構築画像（MPR）の有効性

近年側頭骨画像診断の向上は著しく，高速らせんCTにより短時間で空間分解能の高い描出が可能となり，耳小骨病変，軽度の奇形や耳硬化症などの診断能が向上してきた[1)~3)]。これまで鼓室試験開放術以外に確かめようのなかった耳小骨病変の診断も可能になってきている。また多断面再構築画像（MPR）によりテフロンピストンやアブミ骨に沿った任意のCT断面を作成することができ，術前病態把握だけでなく，病態説明にも役立っている。今回提示した症例もMPRを作成することによって，卵円窓内に深く陥入したテフロンピストンやアブミ骨を描出し，正確な病態把握に役立った。術中所見も側頭骨CT所見と一致しており，画像診断が正確に行われたことが確認できた。病変抽出のためのスライス面の作製には，従来はCT室内のワークステーションで放射線科医と連携して行わなければならなかったが，コーンビームCT等の改良により，耳鼻咽喉科医単独でも微細な画像処理が可能となりつつあり，今後の普及が望まれる[1)2)]。

2）アブミ骨陥入の急性期～亜急性期に対する治療の問題点

耳掻きなど外傷により耳小骨を損傷する症例はまれであり，とりわけアブミ骨を損傷する例はさらに少ない。しかし，その治療方針に関しては以前より議論のあるところであるが，我々は受傷後1ヶ月以内の急性期，1ヶ月～半年の亜急性期，それ以上時間の経過した慢性期に病期を分け，それぞれの時期における治療と問題点について検討してみた。症例1は受傷後3ヶ月目という亜急性期，症例2は受傷9日目という急性期に手術を施行した。ともにアブミ骨摘出し，ツチ・キヌタ関節離断を伴っていたためシュクネヒトのマレウスアタッチメントを用いてstapedectomy-Mを施行した。症例1はオージオグラム上は術前の骨導閾値の上昇が高音域に限られていたが，3ヶ月間前庭症状，耳鳴症状が改善しないため，保存的には治療困難と考え，また前庭窓の完全閉鎖のためにはアブミ骨摘出以外に確実な方法はないと考えた。手術によって前庭症状は消失し気骨導差は改善したが，軽度の骨導閾値上昇はみられた。一方症例2は鼓室粘膜の炎症を伴っていたが，めまい症状強かったために本人は手術的治療を強く希望した。術中も鼓室粘膜の炎症所見がみられ，術直後はめまい症状消失したが，術後聴力は軽度低下している。また術後2カ月目に再診した際，右高度難聴の状態となっており，麻痺性眼振も再出現した。外リンパ瘻の再発や内耳炎を起こしたことが考えられる。

これまでの報告では，急性期の外傷性外リンパ瘻は一般的には約2週間の安静，ビタミン剤や細胞代謝賦活剤，ステロイドの投与など保存的治療を行い，難聴，めまいが改善しない場合には外科的治療に踏み切るとする報告が多い[4)5)]。これは内耳窓の修復力の高さによるためである。しかし，アブミ骨が前庭内に陥入した場合は，その陥入の程度，アブミ骨の状態によって治療方針が様々であり，相対する意見がある。Vanderstockら[6)]は，聾で前庭症状のあるものはただちに手術すべきだが，聴力が残存し前庭症状の軽いものはベッド上安静にすべきであると述べているが，早期に手術を施行すべきだとの意見も少なくない[7)8)]。外傷後の中耳の急性炎症が十分に消退していない状況で

表2　アブミ骨・代用アブミ骨前庭陥入3症例の治療経過のまとめ

症例	発症から手術までの期間	手術所見	術式	術後前庭症状	術後眼振	術後聴力
1	5ヶ月目	アブミ骨陥入	左アブミ骨手術	めまい改善	眼振消失	骨導閾値軽度上昇 気骨導差改善
2	9日目	右鼓膜穿孔 アブミ骨陥入	右アブミ骨手術	めまいは一旦改善するも再燃	眼振は一旦消失するも再燃	術後2ヶ月目に骨導閾値軽度上昇
3	7年目	テフロンピストン陥入	右アブミ骨手術	くらっというめまい消失	眼振なし	右聾のまま

の内耳窓の処理は危険性が高いが，一方で鼓膜穿孔による感染の助長や高度のめまい症状等を考慮すると，早期の手術もやむを得ない場合もあろう。また，時間を置くと膜迷路とアブミ骨が癒着するために，後日，アブミ骨手術が必要となった場合には，かえって内耳障害を起こす危険性が高くなる可能性もある[4]。手術手技に関しても，アブミ骨の除去または整復を行うべきであるとの報告もあるが，陥入が軽度であればアブミ骨を保存する再建方法が好ましいという意見もあり，症例に応じた対応が要求される[4)7)]。本症例1，2のようにアブミ骨の前庭内陥入が高度で単純な整復が困難なときには，アブミ骨を摘出して前庭窓を閉鎖する手技が外リンパ瘻防止策として信頼性が高いと考えられる。急性期に手術を施行したアブミ骨陥入例の過去の報告を調べてみると，11例の報告のうち，アブミ骨を摘出しなかった例が6例あり[4)7)]，stapedectomyを行った例が5例あった[4)9)]。アブミ骨を摘出しなかった6例はいずれも陥入が軽度ということもあるが，6例中5例に聴力改善を認めている。残りの1例は術後に聴力改善を認めたが，その後再び骨導の低下が起こってしまった症例で，術中も外リンパ液の漏出を止めるのが困難であったと記されている[7]。一方stapedectomyを施行した5例中聴力の改善がみられたのは2例のみであり[4)9)]，残りの3例は最終的には骨導聴力は低下している[4)7)]。これらの症例はアブミ骨の陥入が高度である例が多く，本症例も含めてアブミ骨の陥入が高度でstapedectomyを要するような症例の急性期手術の聴力予後は不良と考えられ，アブミ骨を摘出せずに瘻孔の閉鎖が可能な症例は，できるだけ保存的に処理すべきと考えられた。

これに対して亜急性期のアブミ骨陥入に対する手術は4例あり，伝音難聴の残存が1例[10]，前庭症状・伝音難聴の持続が2例[5)7)]，受傷後2ヶ月して生じた高度難聴とめまいが1例であった[2]。伝音難聴のみが残存していた1例は手術にてアブミ骨はそのまま保存し，術後聴力改善が得られたが，それでも2年後に骨導低下がみられている。前庭症状・伝音難聴が持続した症例はいずれも手術においてアブミ骨はそのまま保存され，前庭症状・聴力の改善が得られている。受傷後2ヶ月して高度難聴・めまいを認めた症例は，アブミ骨が前庭内に深く陥入しており，アブミ骨を摘出して再建を行い，眼振・めまい感は消失したが，聴力改善は得られなかった。亜急性期の手術は比較的予後良好といえるが，受傷後2ヶ月経過して内耳症状が悪化する例もあることから，急性期の手術はその予防的な意義も念頭に置く必要がある。

3）アブミ骨陥入の慢性期に対する治療法の問題点

慢性期に手術を行った症例は2例あり，いずれも残存した伝音難聴に対する手術が施行されている[7)11)]。1例はアブミ骨の陥入は軽度で，卵円窓を筋膜でカバーした上で連鎖再建が行われ[7]，もう1例はアブミ骨陥入が高度であったために，手術による危険性を十分に説明した上でstapedectomyが施行されている[11]。術後経過は良好で，いずれも聴力改善が得られている。本症例3においては，アブミ骨の前庭陥入ではなく，アブミ骨手術の際のテフロンピストンの陥入という点で異なるが，右聾になったのは比較的鋭利なテフロンピストンが陥入して膜迷路損傷を生じた可能性がある。術後7年経過して，既に術直後の前庭症状の大部分は消失していたが，頭の動きや圧変化で

の前庭症状だけが残っていた。テフロンピストンが平衡斑を直接刺激するものか，またはMRI上では前庭腔内は内耳液の存在が示唆されることから，外リンパ液の流動を介した機序も想定される。テフロンピストンが折れて前庭内に落ち込んだままの状態でも，外リンパ液の漏出を防げば，めまい症状も起こらず，聴力が保たれた例も報告されているが[12]，陥入の方向や深さ等，前庭腔内でのピストンの微妙な位置関係に依存するものと思われる。本症例は聴力が聾で，前庭症状は持続しており，術中所見からもテフロンピストンの摘出は症状改善に不可欠であったと思われる。慢性期の治療としては，骨導聴力が残存しており，気骨導差を改善するための手術においては，可能な限りアブミ骨を保存して再建するのが望ましいと考えられるが，深く落ち込んでいる場合は摘出もやむを得ない場合もある。その場合は慎重な手術操作と聴力低下の可能性を術前に十分に患者に説明する必要がある。

また本症例3においてはBOR症候群との診断を得ているが，左側は16年前にアブミ骨を温存して鼓室形成術が施行されている。詳細は不明で聴力の改善を得ているが，残存している気骨導差の原因として，アブミ骨固着や内耳低形成の存在も考えられる。

ま と め

耳掻きによる外傷によりアブミ骨が前庭内に陥入した2症例と，アブミ骨術後にテフロンピストンが前庭内に陥入した症例を経験した。いずれの症例も高分解能CTを中心とした画像診断により，病態を正確に把握しえた。3症例とも陥入が高度であり，症状改善のためにアブミ骨及びテフロンピストンの摘出が必要であった。術後より前庭症状の改善を認めたが，1例は2ヶ月後に聴力低下し，眼振も再出現した。

アブミ骨，代用アブミ骨の前庭内陥入に対する治療の問題点について，急性期・亜急性期・慢性期に分けて考察した。

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