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ORIGINAL ARTICLE

Prednisolone prevents transient ischemia-induced cochlear damage in gerbils

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Abstract

Conclusions. Prednisolone protects against inner ear damage, even when administered after ischemic injury in Mongolian gerbils. *Objective.* The effect of prednisolone on ischemia-induced cochlear damage was investigated in Mongolian gerbils. *Materials and methods.* The bilateral vertebral arteries were occluded for 15 min to transiently induce cochlear ischemia, followed by an intraperitoneal injection of prednisolone (1 mg/kg) or physiological saline (control). Sequential changes in hearing were evaluated by recording the auditory brainstem response (ABR) before and at 1, 4, and 7 days after treatment. In our histologic analysis, the numbers of dead and intact inner hair cells (IHCs) were counted in specimens stained with rhodamine-phalloidin. *Results.* In control animals, transient ischemia increased the ABR threshold (24.2±8.6 dB) within 7 days of treatment, whereas prednisolone-treated animals exhibited a threshold of 14.2±9.2 dB. Furthermore, the percent IHC loss at the basal turn of the cochlea was 26.5±11.4% in control animals compared with 5.3±3.0% in the prednisolone-treated group.

Keywords: *Glucocorticoid, transient ischemia, inner hair cells, idiopathic sudden sensorineural hearing loss*

Introduction

Idiopathic sudden sensorineural hearing loss (ISSHL) is a medical emergency that requires immediate treatment. At present, however, the etiology of ISSHL remains unclear and hearing impairment is treated empirically. Various hypotheses have been proposed to explain this disease, including vascular disturbance, viral infection, autoimmunity, and perilymphatic fistulas. Because the hearing loss occurs suddenly, we postulated that cochlear ischemia is the most likely cause of ISSHL. Based on this, we developed an animal model for transient cochlear ischemia in Mongolian gerbils, and used it to investigate cochlear pathophysiology and potential therapeutic agents. Our previous studies [1–4] demonstrated that although damage to the stria vascularis is reversible, damage to the organ of Corti and the spiral ganglion is irreversible.

Corticosteroids are the most common clinical treatment for ISSHL. Numerous clinical studies have shown that corticosteroids improve hearing after ISSHL, especially when relatively high doses (>1 mg/kg) are administered. Corticosteroids affect a variety of physiological systems, including the immune response, stress response, inflammation, protein catabolism, carbohydrate metabolism, blood electrolyte levels, and physical behavior. Functionally, they can be classified into glucocorticoids and mineralocorticoids; the former are thought to be the more effective in the treatment of ISSHL. At present, information regarding the effects of glucocorticoids on ischemic cochlear damage is lacking. Thus, we investigated the effects of prednisolone, a synthetic glucocorticoid, on ischemia-induced cochlear damage in gerbils.

Materials and methods

All experiments were conducted in accordance with the Guidelines for Animal Experimentation at the Ehime University School of Medicine. Adult

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male Mongolian gerbils (Meriones unguiculatus) weighing 60–100 g were anesthetized with a mixture of halothane and nitrous oxide. The animals were artificially ventilated using an oral ventilation tube (tidal volume, 1 ml; respiration rate, 70/min). Body temperature was monitored using a PTC-201 thermo-sensor (Unique Medical Corporation, Tokyo, Japan); during surgery, body temperature was maintained at 37–38°C using a heat lamp.

Transient cochlear ischemia was induced using the method described by Hata et al. [5]. Because Mongolian gerbils lack posterior cerebral arteries, the vertebrobasilar arteries supply all blood to the cochleae. These vertebral arteries were bilaterally dissected free of the surrounding connective tissue via a ventral transverse incision in the neck. Silk ligatures (4-0) were looped loosely around each artery and 5g weights were used to tighten these ligatures simultaneously for 15 min, thus inducing ischemia. Then, the sutures were removed to allow reperfusion of the cochleae, which was confirmed by microscopic observation. Following ischemic induction, prednisolone sodium succinate (1 mg/kg dissolved in 1 ml saline; Shionogi & Co. Ltd, Osaka, Japan) was administered intraperitoneally. Control animals were injected with an equal volume of physiological saline.

Hearing was assessed before and at days 1, 4, and 7 after ischemic induction. The auditory brainstem response (ABR) was recorded in a soundproof booth using three stainless steel electrodes inserted subcutaneously in the mastoid (reference), the vertex (indifferent), and the neck (ground). A signal processor (NEC Synax 1200, Tokyo, Japan) was used to measure ABRs in response to an 8 kHz tone burst (rate, 10.5 Hz; plateau, 10 ms; rise/fall time, 0.25 ms; inter-stimulus interval, 50 ms). This frequency was selected based on previous data showing that the high frequency region of the cochlea is more vulnerable to ischemic injury than the middle or low frequency regions [2]. The responses were processed using a 50 to 3 kHz bandpass filter and were averaged 300 times. The minimal stimulus required to evoke the first wave was defined as the ABR threshold.

After assessing ABR at day 7, tissues were collected for histological analysis. Under deep anesthesia, the otic bulla was removed and perfused intrascalarly with 4% paraformaldehyde in phosphate-buffered solution (PBS) at pH 7.4, followed by post-fixation for 2 h in the same fixative at 41°C. The tissue was then immersed in PBS and the organ of Corti was dissected at the basal turn using surface preparation techniques described previously [2]. After serial sectioning, the specimens were stained with rhodamine-phalloidin (Molecular Probes, Eugene, OR, USA) diluted 250 × in PBS containing 0.25% Triton X-100 and 1% bovine serum albumin (BSA) at room temperature for 30 min. Fluorescence was detected using an Olympus BX60 microscope equipped with a green filter (BP 546, FT 580, LP 590 nm) and the images were transferred to a personal computer. The numbers of intact inner hair cells (IHCs) and dead hair cells at the basal turn were counted, and the ratio of dead to whole IHCs (%) was obtained; 150 IHCs at the basal turn were analyzed from each specimen.

The results are expressed as the mean ± standard deviation (SD). A two-tailed Mann–Whitney U test was used to compare ABR results and the ratio of IHC loss between the two groups. A p value of <0.05 was considered statistically significant.

Results

In control animals, the ABR threshold increased immediately after transient ischemia and gradually recovered thereafter, although not to preoperative levels (Figure 1). This initial increase was prevented by post-ischemic administration of prednisolone. On day 7, the average increase in the ABR threshold was 24.2 ± 8.6 dB in the control group, compared with 14.2 ± 9.2 dB in the prednisolone group, indicating that prednisolone treatment may prevent further hearing loss after ischemic injury. However, no statistical difference was observed between the two groups.

Histological specimens showing the basal turn of the cochlea revealed that prednisolone treatment protected against further IHC loss (Figure 2). A surface preparation of the organ of Corti showed that IHCs were sporadically lost in control animals, and

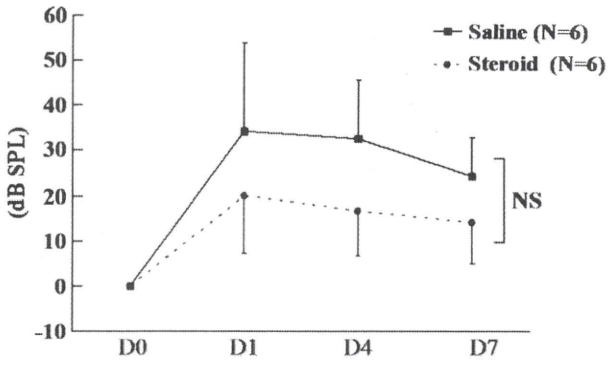

Figure 1. Changes in ABR thresholds following transient cochlear ischemia. Hearing was assessed before (D0) and at days 1 (D1), 4 (D4), and 7 (D7) after ischemic injury. The ABR threshold before ischemia was defined as 0 dB and subsequent changes are shown in dB. The increase in ABR threshold observed on D7 was lower in the prednisolone-treated group than in the control group, although this difference was not statistically significant.

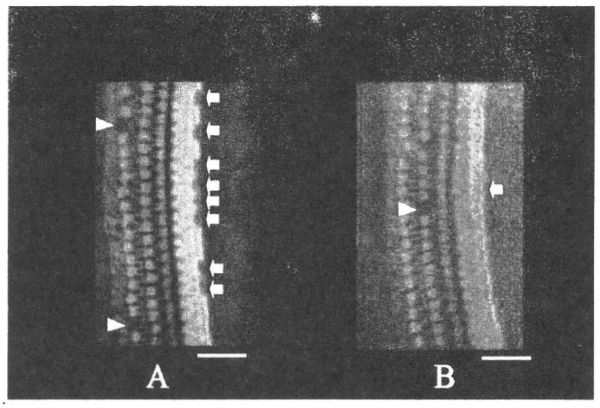

Figure 2. Hair cells at the basal turn stained with rhodamine-phalloidin on day 7 (D7). (A) Control. Stereocilia on inner hair cells (IHCs) disappeared sporadically (arrows), whereas outer hair cells (OHCs) remained almost intact (arrowhead). (B) Prednisolone group. Stereocilia on IHCs and OHCs remained intact (arrow and arrowhead, respectively). Scale bars = 20 μm.

this loss was more severe in the basal turn than in the apical turn. In contrast, IHC loss was reduced by the administration of prednisolone. Throughout the experiment, the involvement of outer hair cells (OHCs) and supporting cells was limited, regardless of whether prednisolone was administered. The percent IHC loss at the basal turn was 26.5 ±11.4% in control animals compared with 5.3 ±3.0% in the prednisolone group, and this difference was statistically significant ($p < 0.001$; Figure 3).

Discussion

Acute interruption of the cochlear blood supply is thought to be one of the major causes of ISSHL. At present, however, the mechanism of ischemic cochlear damage remains unclear, mainly because of difficulties with the available animal models. Chronic animal experiments are often unsuccessful because access to the labyrinthine artery is difficult without disturbing the surrounding neural or vascular structures. During the past 10 years, we have investigated the effects of ischemic cochlear damage in Mongolian gerbils, which are able to tolerate long-term experiments [1–4]. These animals lack the posterior cerebral communicating arteries, leaving only the vertebrobasilar arteries to supply the labyrinthine arteries. As such, occluding the bilateral vertebral arteries at the neck permits transient induction of ischemia. Through intensive studies [1–4], we revealed that IHCs are more vulnerable to ischemic insult than OHCs, and that spiral ganglion neurons undergo apoptosis in two stages, immediately after ischemia and secondarily following the death of hair cells. In contrast, damage to the stria vascularis was reversible. Using this animal model, we can evaluate the effects of candidate drugs for the treatment of ISSHL.

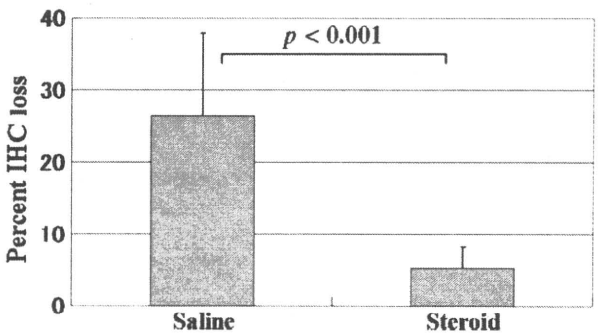

Figure 3. Percent inner hair cell (IHC) loss at the basal turn on day 7 (D7). IHC loss was significantly reduced in the prednisolone-treated group compared with the control ($p < 0.001$).

Here, we showed that the administration of prednisolone reduced transient ischemia-induced cochlear damage. Prednisolone is a synthetic glucocorticoid that is commonly prescribed as a primary treatment for ISSHL, although the mechanism underlying its effect on the inner ear remains uncertain. Tabuchi et al. [6] investigated the effects of corticosteroids such as prednisolone, methylprednisolone, and dehydroepiandrosterone sulfate (DHEAS) on guinea pigs, and revealed that these drugs protected the inner ear against transient ischemia when administered preoperatively. That report also noted that high doses (>10 mg/kg) were less potent than a low dose of 1 mg/kg. Based on these results, we selected a dose of 1 mg/kg prednisolone for the present study.

At present, the effects of prednisolone after ischemic injury remain unclear. In the brain, glucocorticoid receptors have been identified at various sites in the central nervous system (CNS), including the hippocampus, a region thought to be very sensitive to ischemic injury. ffrench-Mullen [7] reported that corticosterone inhibits some types of guinea pig Ca^{2+} channels in hippocampal CA1 neurons in a dose-dependent manner, thus preventing neural damage. Glucocorticoids also suppress the production of free radicals following ischemic insult. In contrast, Xiao et al. [8] showed that glucocorticoids stimulate the MAPK/cJNK pathway in isolated rat hippocampal neurons, thus facilitating apoptosis. These conflicting effects make it difficult to determine the role of glucocorticoids in ischemic injury.

Glucocorticoids are thought to work via two distinct mechanisms: a classic genomic mechanism versus a novel non-genomic mechanism. In the genomic mechanism, glucocorticoids activate the transcription of various genes, thus resulting in

protein synthesis. Because this mechanism requires gene expression, a certain period of time must elapse before an effect can be observed (i.e. from several hours to several days). In the non-genomic mechanism, glucocorticoids act directly on reactive sites, resulting in an observable effect after only seconds or minutes. Because the effects of our post-ischemic prednisolone treatment were observed over several days, it is likely that both genomic and non-genomic mechanisms were active in preventing ischemic damage. It is also possible that prednisolone treatment suppresses the production of free radicals and thus prevents apoptotic cell death in IHCs, minimizing cochlear injury.

Conclusions

The results show that prednisolone protects against cochlear damage due to transient ischemia. Although the exact mechanism of this effect remains unclear, it is likely that both genomic and non-genomic mechanisms are at work and involve the suppression of free radical production and the subsequent inhibition of apoptosis.

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

References

[1] Hakuba N, Gyo K, Yanagihara N, Mitani A, Kataoka K. Efflux of glutamate into the perilymph of the cochlea following transient ischemia in the gerbil. Neurosci Lett 1997;230:69–71.

[2] Koga K, Hakuba N, Watanabe F, Shudou M, Nakagawa T, Gyo K. Transient cochlear ischemia causes delayed cell death in the organ of Corti: an experimental study in gerbils. J Comp Neurol 2003;456:105–11.

[3] Morizane I, Hakuba N, Shimizu Y, Shinomori Y, Fujita K, Yoshida T, et al. Transient cochlear ischemia and its effects on the stria vascularis. Neuroreport 2005;16:799–802.

[4] Fujita K, Hakuba N, Hata R, Morizane I, Yoshida T, Shudou M, et al. Ginsenoside Rb1 protects against damage to the spiral ganglion cells after cochlear ischemia. Neurosci Lett 2007;415:113–7.

[5] Hata R, Matsumoto M, Hatakeyama T, Ohtsuki T, Handa N, Niinobe M. Differential vulnerability in the hindbrain neurons and local cerebral blood flow during bilateral vertebral occlusion in gerbils. Neuroscience 1993;56:423–39.

[6] Tabuchi K, Oikawa K, Uemaetomari I, Tsuji S, Wada T, Hara A. Glucocorticoids and dehydroepiandrosterone sulfate ameliorate ischemia-induced injury of the cochlea. Hear Res 2003;180:51–6.

[7] ffrench-Mullen JMH. Cortisol inhibition of calcium currents in guinea pig hippocampal CA1 neurons via G-protein-coupled activation of protein kinase C. J Neurosci 1995;15: 903–11.

[8] Xiao L, Qi A, Chen Y. Cultured embryonic hippocampal neurons deficient in glucocorticoid (GC) receptor: a novel model for studying nongenomic effects of GC in the neural system. Endocrinology 2005;146:4036–41.

WILEY InterScience DISCOVER SOMETHING GREAT

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Caspase Inhibitor Facilitates Recovery of Hearing by Protecting the Cochlear Lateral Wall from Acute Cochlear Mitochondrial Dysfunction

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We recently showed that acute energy failure in the rat cochlea induced by local administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) causes hearing loss mainly due to degeneration of cochlear lateral-wall fibrocytes. In the present study, we analyzed the effect of the pan-caspase inhibitor z-Val-Ala-Asp(Ome)-fluoromethylketone (Z-VAD-FMK) on 3-NP-induced hearing loss in a model showing temporary threshold shifts at low frequencies and permanent threshold shifts at high frequencies. The model rats received an intraperitoneal injection of either Z-VAD-FMK or vehicle for 3 days starting 1 day prior to 3-NP treatment. One day after the administration of 3-NP, the auditory brain-stem response (ABR) threshold at 20 kHz was elevated to 70 dB in the Z-VAD-FMK group and to 85 dB in controls. The Z-VAD-FMK group completely recovered to the preoperative level within 14 days, whereas in the controls, the ABR threshold remained elevated at 50 dB even 28 days after the administration of 3-NP. Treatment with Z-VAD-FMK also improved recovery of hearing at 8 kHz but did not change recovery at 40 kHz. Histological examination demonstrated that treatment with Z-VAD-FMK inhibited progressive degeneration of the lateral-wall fibrocytes in the cochlear basal turn, as well as apoptosis of these fibrocytes. These results clearly indicate that caspase-dependent apoptosis of fibrocytes in the cochlear lateral wall plays an important role in hearing loss in the present animal model. Moreover, the results of the present study suggest that systemic administration of a caspase inhibitor may be an effective therapy for sensorineural hearing loss caused by acute energy failure such as that observed in cochlear ischemia.

Key words: hearing loss; apoptosis; Z-VAD-FMK; lateral wall of cochlea; caspase

Mitochondria supply cell energy by production of ATP. Mutations in mitochondrial DNA frequently cause both syndromic and nonsyndromic deafness; therefore, the inner ear is considered highly susceptible to deterioration of mitochondrial function (Fischel-Ghodsian et al., 2003; Pickles, 2004; Hsu et al., 2005). This is consistent with the susceptibility of the inner ear to energy failure such as is seen in cochlear ischemia (Seidman et al., 1999). It has been suspected that energy failure, including that caused by cochlear ischemia, may be involved in acute sensorineural hearing loss such as sudden deafness.

To explore the effect of acute mitochondrial dysfunction in the cochlea, we established an animal model of acute cochlear energy failure by administering the mitochondrial toxin 3-nitropropionic acid (3-NP) into the rat round window niche (Hoya et al., 2004). 3-NP is an irreversible inhibitor of succinate dehydrogenase, a complex II enzyme of the mitochondrial electron transport chain (Alston et al., 1977; Coles et al., 1979). Systemic administration of 3-NP produces selective striatal damage (Hamilton and Gould, 1987; Brouillet et al., 1995). In our model, local administration of 500 mM 3-NP into the cochlea led to a permanent auditory brain-stem response (ABR) threshold shift at all frequencies. With the administration of 300 mM 3-NP, we observed a temporary threshold shift at low frequencies, whereas permanent threshold shifts persisted at high frequencies. Histological analysis of cochleas from the permanent

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threshold shift model using 500 mM 3-NP suggested that the degeneration of fibrocytes in the cochlear lateral wall and atrophy of the stria vascularis were the primary causes of permanent hearing loss (Okamoto et al., 2005).

Recently, apoptosis in the cochlear lateral wall was also reported in an endolymphatic hydrop-induced hearing loss model (Labbe et al., 2005) as well as in a presbyacusis model (Alam et al., 2001). Caspase family members function as important signals in the last step of several apoptosis signaling pathways (Vaux and Strasser, 1996; Budihardjo et al., 1999). Caspase-dependent apoptosis in the cochlear cells has been reported in several studies (Devarajan et al., 2002; Van De Water et al., 2004). Moreover, it was recently reported that the cell-permeable pan- caspase inhibitor z-Val-Ala-Asp(Ome)-fluoromethylketone (Z-VAD-FMK) irreversibly binds to the catalytic site of caspase proteases, inhibiting apoptosis (Gregoli and Bondurant, 1999; Schrantz et al., 1999). Z-VAD-FMK also was reported to have a strong antiapoptotic effect in vivo, including providing protection from cell death in murine neurodegenerative disease models (Ona et al., 1999; Li et al., 2000). In an inner ear study, systemic administration of Z-VAD-FMK protected chick vestibular hair cells from aminoglycoside ototoxicity (Matsui et al., 2003). Therefore, we hypothesized that systemic administration of this caspase inhibitor would also protect the cochlear lateral wall. In the present study, we analyzed the effect of Z-VAD-FMK on hearing loss in a model that demonstrates a temporary threshold shift at low frequencies and a permanent shift at high frequencies.

MATERIALS AND METHODS

Animals and Surgery

Experimental procedures reported in this study were approved by the Institutional Animal Care and Use Committee of the National Tokyo Medical Center. We used 6-week-old male Sprague-Dawley rats weighing 160–200 g. Before surgery, the animals were anesthetized with pentobarbital (30–40 mg/kg intraperitoneally), and an incision was made posterior to the left pinna near the external meatus after local administration of lidocaine (1%). The left otic bulla was opened to approach the round window niche. The end of PE 10 tubing (BD-Biosciences, Franklin Lakes, NJ) was drawn to a fine tip in a flame and gently inserted into the round window niche. The pH of a saline solution containing 300 mM 3-NP (Sigma, St. Louis, MO) was adjusted to 7.4 with NaOH. Saline was used as a control. The solution was administered for 2 min at a rate of 1.5 μL/min with a syringe pump. Following treatment, a tiny piece of gelatin was placed on the niche in order to keep the solution inside regardless of head movement after wound closure and awakening from anesthesia. The right cochlea was surgically destroyed to avoid cross-hearing during ABR recording. Starting 1 day before the administration of 3-NP, the animals received a 3-day course of the pan-caspase inhibitor Z-VAD-FMK (1.5 mg/kg a day intraperitoneally; BD-Biosciences, Franklin Lakes, NJ) dissolved in DMSO. Control animals received the same volume of DMSO without Z-VAD-FMK.

ABR Recording

Before surgery and 2 hr and 1, 3, 7, 14, 21, and 28 days after surgery (n = 5 rats/treatment group at each time), ABRs were recorded using waveform storing and stimulus control with Scope software on a PowerLab system (PowerLab2/20, AD Instruments, Castle Hill, Australia). We performed electroencephalograph (EEG) recordings with the extracellular amplifier Digital Bioamp system (BAL-1, Tucker-Davis Technologies, Alachua, FL). Sound stimuli were produced by a coupler-type speaker (ES1spc, Bio Research Center, Nagoya, Japan) inserted into the left ear canal of the rats. Tone bursts of 8, 20, and 40 kHz (0.2 msec rise/fall time and 1 msec flat segment) were generated, and the amplitude was specified by a real-time processor and programmable attenuator (RP2.1 and PA5, Tucker-Davis Technologies, Alachua, FL). Sound level calibration was performed using a sound-level meter (NL32, RION, Tokyo, Japan). Maximum output at each frequency of 8, 20, and 40 kHz was 87, 86, and 96 dB, respectively. For recording, stainless-steel needle electrodes were placed ventrolaterally to each ear. Waveforms of 512 stimuli at a frequency of 9 Hz were averaged, and the visual detection threshold was determined with increases or decreases in the sound pressure level in 5-dB increments.

Statistical Analysis

Statistical analyses of the ABR threshold were performed using one-way analysis of variance, followed by a multiple-comparison procedure, the Turkey-Kramer test, using STAT-VIEW J 4.5 (Abacus Concepts, Inc.). Significance for all statistical procedures was set at $P < 0.05$.

Histological Analysis

Histological analysis was performed 2, 7, 14, 21, and 28 days after surgery (n = 2 rats/treatment group at each time). The rats treated with 3-NP and control rats were deeply anesthetized with pentobarbital and transcardially perfused with 0.01M phosphate buffer (pH 7.4) containing 8.6% sucrose, followed by fixative consisting of freshly depolymerized 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) containing 5% sucrose. After decapitation, left temporal bones were removed and immediately placed in the same fixative. Small openings were made at the round window, oval window, and apex of the cochlea. After immersion in the fixative overnight, the temporal bones were decalcified by immersion for 6 days at 4°C in 0.1M ethylenediamine tetraacetic acid (EDTA; pH 7.4) containing 5% sucrose with stirring. The bones were then rinsed overnight in 0.1M phosphate buffer containing 5% sucrose, postfixed in 1% osmium tetroxide in the same buffer for 150 min, dehydrated in a graded ethanol series, and embedded in Epon 812 resin. For light microscopy, semithin sections were cut in the horizontal plane parallel to the modiolus and stained with toluidine blue.

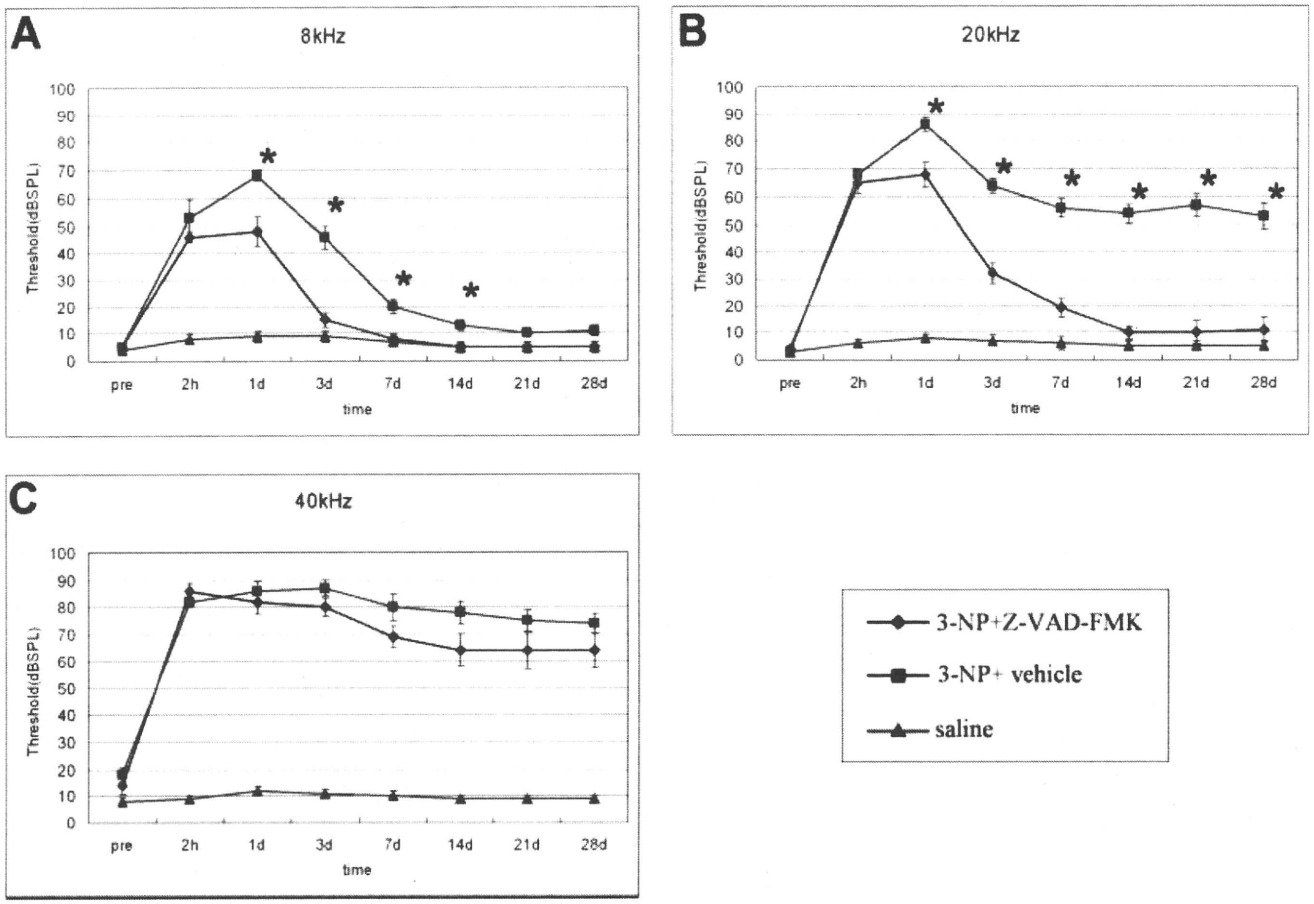

Fig. 1. Time course of ABR threshold shift. ABR threshold changes at 8 kHz (**A**), 20 kHz (**B**), and 40 kHz (**C**) for rats treated with saline (saline), with 3-NP and DMSO (3-NP+ vehicle), and with 3-NP and Z-VAD-FMK (3-NP+Z-VAD-FMK) (*$P < 0.05$ significant difference between rats treated with 3-NP and DMSO and rats treated with 3-NP and Z-VAD-FMK; error bars indicate standard error of the mean).

Terminal Deoxynucleotidyl Transferase–Mediated dUTP Nick-End Labeling Assay and Immunohistochemistry

A terminal deoxynucleotidyl transferase (TdT)–mediated dUTP nick-end labeling (TUNEL) assay and immunohistochemistry were used for active caspase-3 analysis 2 days after administration of 3-NP ($n = 2$ per group). The rats treated with 3-NP and the rats treated with Z-VAD-FMK or DMSO were deeply anesthetized with pentobarbital and transcardially perfused with 0.01M phosphate buffer (pH 7.4) containing 8.6% sucrose, followed by immersion in fixative consisting of freshly depolymerized 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4). After the rats were decapitated, their left temporal bones were removed and immediately placed in the same fixative. Small openings were made at the round window, oval window, and apex of the cochlea. After immersion in the fixative overnight, the temporal bones were decalcified by immersion for 14 days at 4°C in 0.1M EDTA (pH 7.4) containing 5% sucrose with stirring. The specimens were dehydrated in a graded ethanol series, embedded in a paraffin block, and sectioned into 5-μm sections. The sections were analyzed by TUNEL assay and immunohistochemistry for active caspase-3. The TUNEL assay was performed using an ApopTag Peroxidase In Situ Apoptosis Detection Kit (Chemicon International Inc., Temecula, CA). The specimens were digested with 20 μg/mL proteinase K in PBS for 3 min, followed by incubation in PBS with 3% H_2O_2 for 5 min and then with TdT at 37°C for 1 hr. The sections were incubated with anti–digoxigenin conjugate at room temperature for 30 min and then an avidin-biotin-horseradish peroxidase complex (Vector Laboratories, Burlingame, CA) for 30 min and then were stained with DAB (Vector Laboratories, Burlingame, CA) for 5 min and hematoxylin for 1 min. The specimens were viewed with a light microscope (Eclipse E600; Nikon, Tokyo, Japan) equipped with a digital camera (COOLPIX 990; Nikon, Tokyo, Japan). The negative control was stained without active TdT but with proteinase K digestion to control for nonspecific incorporation of nucleotides or nonspecific binding of enzyme-conjugates and with distilled water substituted for TdT enzyme reagent at the same volume. For immunohistochemistry for active caspase-3, the deparaffinized specimens were heated and boiled for 5 min in

0.01M citrate buffer (pH 6.0) in a microwave and were incubated with blocking solution (1.5% normal goat serum in PBS) for 30 min at room temperature before incubation with anti–active caspase-3 (1:40 in PBS, rabbit anti–active caspase-3 polyclonal antibody; Chemicon International Inc., Temecula, CA) for 1 hr. Alexa 488–conjugated antirabbit IgG (1:400; Molecular Probes, Eugene, OR) was used as a secondary antibody. For nuclear staining, 4′,6-diamidino-2-phenylindole (DAPI) (Dojindo Laboratories, Kumamoto, Japan) was used. Negative control was stained without primary antibody for nonspecific binding of secondary antibody. The specimens were viewed with a confocal laser microscope (Radiance 2100, Bio-Rad, Richmond, CA,).

RESULTS

Threshold Shift by 3-NP and the Effect of Z-VAD-FMK

We first examined the ABR threshold at 8, 20, and 40 kHz of rats treated with 3-NP in our hearing loss model. Whereas the rats treated with saline did not show significant changes in the ABR threshold at any frequency or time, rats treated with 3-NP and DMSO (vehicle group) exhibited elevated ABR thresholds at all frequencies beginning 2 hr after administration of 3-NP and reaching a peak threshold 1 day after 3-NP administration (Fig. 1A–C). At 8 kHz, the ABR threshold recovered to the preoperative level by 14 days after administration of 3-NP. At 20 kHz, the threshold recovered gradually, reaching approximately 55 dB by 14 days after 3-NP administration and remaining at that level thereafter. At 40 kHz, the threshold recovered to 75 dB by 28 days after 3-NP administration. We noted a significant difference between the threshold 1 and 28 days after 3-NP administration for both 20 and 40 kHz ($P < 0.001$ and $P = 0.04$, respectively).

In rats treated with 3-NP and Z-VAD-FMK (Z-VAD-FMK group), the ABR threshold also rapidly elevated beginning 2 hr after administration of 3-NP (Fig. 1A–C). However, the peak threshold values 1 day after 3-NP administration at 8 and 20 kHz were significantly lower than those of the vehicle group (Fig. 1A, B). Moreover, improved recovery of the ABR threshold was observed at 8 and 20 kHz. At 8 kHz, the ABR threshold recovered to the preoperative threshold 3 days after administration of 3-NP. At 20 kHz, the ABR threshold recovered to the preoperative threshold by 14 days after 3-NP administration. However, at 40 kHz, no significant differences between the Z-VAD-FMK and vehicle groups were observed at any time (Fig. 1C).

Histological Changes in Cochlear Lateral Wall

In the rats exposed only to DMSO (vehicle), loss and degeneration of fibrocytes in the lateral wall associated with enlarged extracellular spaces were observed 2 days after administration of 3-NP in the basal turn (Fig. 2A). These changes were most prominent in the area of type 2 fibrocytes. Moreover, we observed nuclear condensation suggestive of apoptosis in the fibrocytes surrounding the acellular area. Seven days after 3-NP administration, we noted degeneration of all types of lateral-wall fibrocytes (Fig. 2B). Fourteen days after 3-NP administration, the loss of all types of fibrocytes became more prominent (Fig. 2C). Moreover, the stria vascularis, which remained structurally normal up to 7 days after 3-NP administration, also demonstrated atrophic changes at this point. There were similar histological findings 28 days after 3-NP administration (Fig. 2D). In the middle turn and apex of the cochlea, the fibrocytes and stria vascularis revealed no morphological changes. In the Z-VAD-FMK group, on the other hand, the fibrocytes and stria vascularis revealed no morphological changes in any turn of cochlea at any time (Fig. 2E–H).

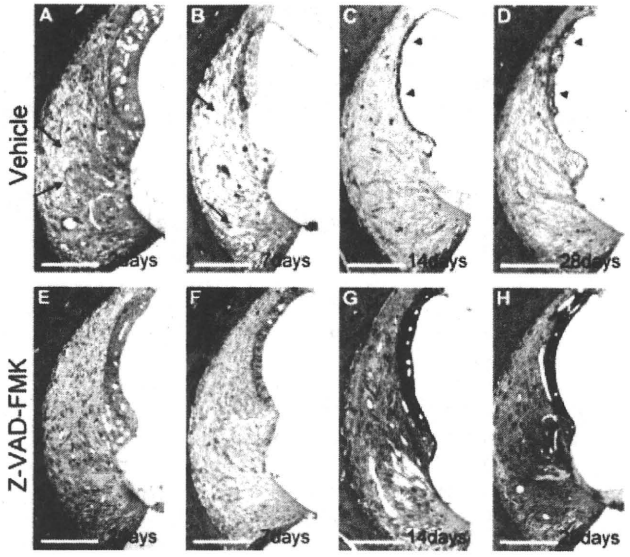

Fig. 2. Changes in the lateral wall in of the basal turn. Histological evaluation of the cochleas of rats in the vehicle group (**A–D**) and in the Z-VAD-FMK group (**E–H**) 2, 7, 14, and 28 days after administration of 3-NP. **A:** Two days after administration of 3-NP, mild degeneration, nuclear condensation, and loss of fibrocytes in the area of type 2 fibrocytes (arrows) 2 days after administration of 3-NP in rats treated with vehicle only. **B:** Loss of fibrocytes evident in all areas of the spiral ligament (arrows) 7 days after administration of 3-NP in rats treated with vehicle only. **C, D:** Severer degeneration of fibrocytes and also atrophy of the stria vascularis (arrowheads) apparent 14 and 28 days, respectively, after administration of 3-NP in rats treated with vehicle only. **E–H:** Neither degeneration of fibrocytes nor atrophy of the stria vascularis evident in rats treated with Z-VAD-FMK 2, 7, 14, and 28 days, respectively, after administration of 3-NP. Scale bar = 50 μm.

Histological Analysis of Organ of Corti and Spiral Ganglion

In the vehicle group, atrophy of outer hair cells and Deiters cells was observed in the organ of Corti 2 days after administration of 3-NP (Fig. 3A). The pillar cells, Corti tunnel, and inner hair cells showed no morphological changes. Twenty-eight days after 3-NP administration, no remarkable changes were observed

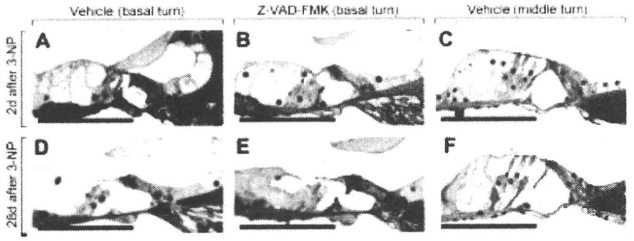

Fig. 3. Morphological changes in the organ of Corti. Histological evaluation of the organ of Corti in the vehicle group (**A, C, D, F**) and the Z-VAD-FMK group (**B, E**) 2 and 28 days after administration of 3-NP. Two and 28 days after administration of 3-NP, atrophy of the outer hair cells and Deiters cells without other obvious changes in the organ of Corti in the cochlear basal turn was detected in rats treated with 3-NP and vehicle (**A, D**) and in rats treated with 3-NP and Z-VAD-FMK (**B, E**). In the vehicle group, no structural changes were detected in the organ of Corti in the cochlear middle turn 2 and 28 days after administration of 3-NP (**C, F**). Scale bar = 50 μm.

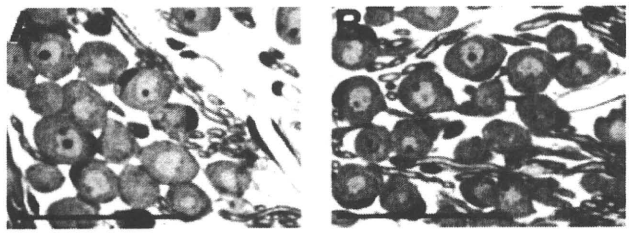

Fig. 4. Structural changes in the spiral ganglion. Histological evaluation of the spiral ganglion cells in the cochlear basal turn of rats treated with 3-NP and vehicle revealed no structural changes 2 days (**A**) and 28 days (**B**) after administration of 3-NP. Scale bar = 50 μm.

(Fig. 3D). We found the same morphological findings in the Z-VAD-FMK group, that is, atrophy of the outer hair cells and the Deiters cells, 2 and 28 days after administration of 3-NP (Fig. 3B, E). In the middle and apical turns of the cochlea, the organ of Corti revealed no morphological changes at any time (Fig. 3C, F). The spiral ganglion cells exhibited no morphological changes in any turn of cochlea at any time (Fig. 4A, B).

Detection of Apoptosis in Cochleas of Vehicle and Z-VAD-FMK Groups

A TUNEL assay was used to compare the extent of apoptosis 2 days after administration of 3-NP between vehicle- and Z-VAD-FM-treated rats. In the vehicle group, TUNEL-positive cells were observed 2 days after 3-NP administration in the area of type 2 fibrocytes in the lateral wall of the basal turn (Fig. 5A), but not in the organ of Corti or the spiral ganglion in the cochlear basal turn (Fig. 5C, D). In the Z-VAD-FMK group, no TUNEL-positive cells were observed after 3-NP administration (Fig. 5B), and no TUNEL-positive cells were observed in the middle or apical turns of the cochlea in

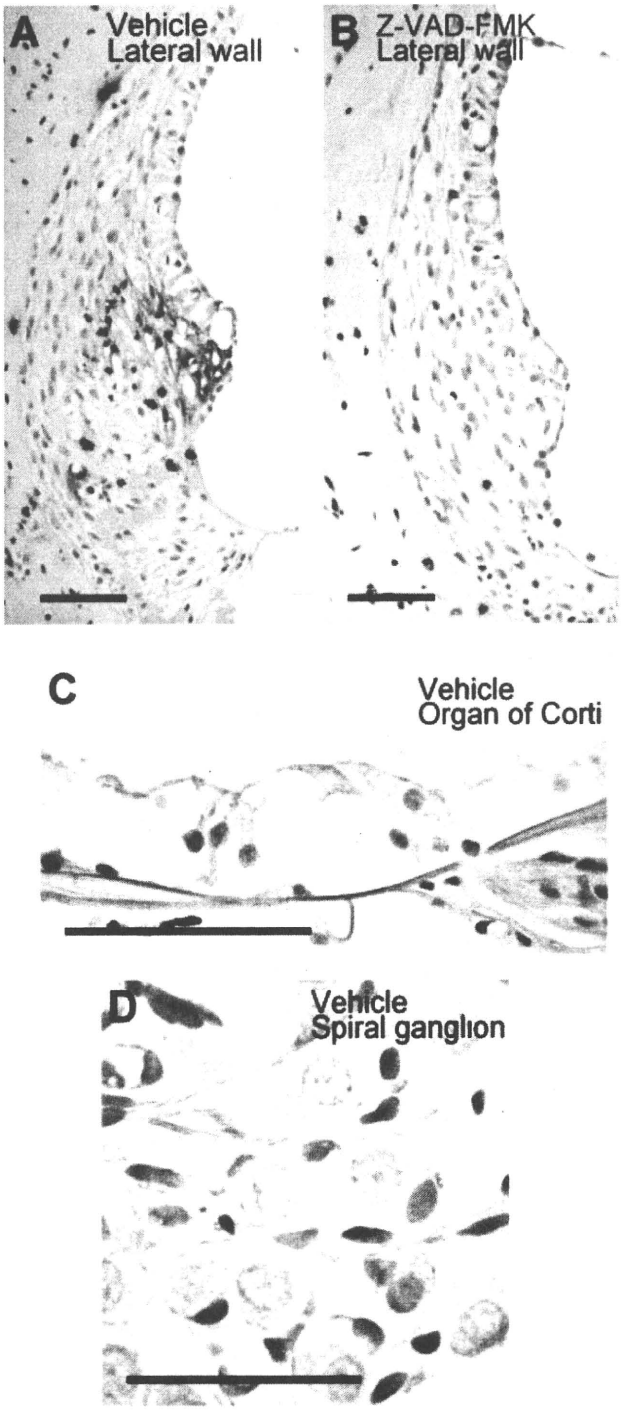

Fig. 5. Detection of apoptosis by TUNEL assay in fixed cochlear sections. TUNEL-positive cells in the area of type 2 fibrocytes in the lateral wall were evident 2 days after administration of 3-NP in rats treated with vehicle (**A**) but not in the organ of Corti (**C**) or the spiral ganglion (**D**). No TUNEL-positive cells were detected in the cochlear lateral wall of rats treated with 3-NP and Z-VAD-FMK (**B**). Scale bar = 50 μm.

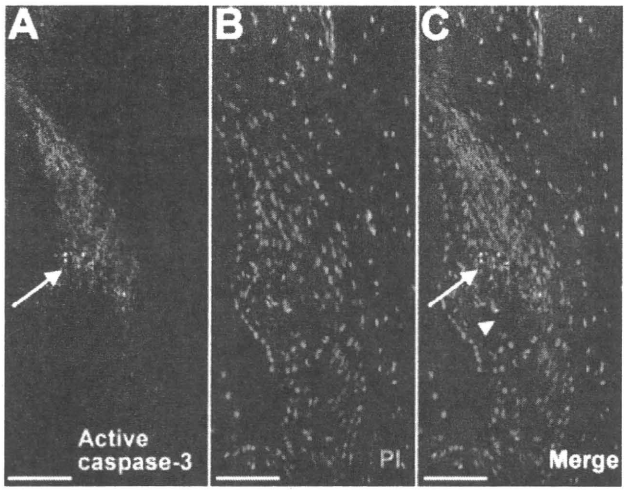

Fig. 6. Expression of active caspase-3. **A:** Immunofluorescence microscopy image of fixed cochlear sections using anti–active caspase-3 antibody. **B:** Propidium iodide (PI) nuclear staining. **C:** Merge of **A** and **B**. Expression of active caspase-3 (arrow) detected in the area of type 2 fibrocytes around the acellular space (arrowhead) in the spiral ligament of the cochlear basal turn 2 days after administration of 3-NP. Scale bar = 50 μm.

either the vehicle or the Z-VAD-FMK group (data not shown).

Expression of Active Caspase-3 in the Cochlea after 3-NP Administration

To confirm the TUNEL assay result, we performed additional immunohistochemical analysis of the apoptosis marker active caspase-3. In the vehicle group, immunoreactivity to active caspase-3 was detected in the area of type 2 fibrocytes in the basal turn 2 days after administration of 3-NP (Fig. 6A–C), but not in the organ of Corti, the spiral ganglion, or the middle or apical turns of the cochlea.

DISCUSSION

Apoptosis in Acute Cochlear Energy Failure and Its Inhibition by a Pan-Caspase Inhibitor

In a rat model of acute cochlear mitochondrial dysfunction, active caspase-3-positive fibrocytes were observed in the spiral ligament 2 days after administration of 3-NP. Caspase-3 is a downstream effector caspase that is activated by upstream initiators, including caspase-8 and caspase-9. Activated caspase-3 cleaves a number of vital proteins, including Bcl-2 and inhibitors of deoxyribonucleases and cytoskeletal proteins, and carries out the program of apoptosis (Cheng et al., 1997; Kothakota et al., 1997; Thornberry and Lazebnik, 1998). Activated caspase-3-positive cells were mostly detected in the area of type 2 fibrocytes, which also demonstrated apoptosis by the TUNEL assay 2 days after 3-NP administration. Histological examination also showed degeneration of type 2 fibrocytes 2 days after administration of 3-NP. Taken together, these results suggest that degeneration of lateral-wall fibrocytes was mediated by caspase-dependent apoptosis. The almost complete inhibition of degeneration and apoptosis of lateral-wall fibrocytes by systemic administration of a pan-caspase inhibitor supports the idea that degeneration of lateral-wall fibrocytes was caused by apoptosis. Furthermore, these results indicate that Z-VAD-FMK which was administered systemically, was efficiently delivered into the lateral wall of the cochlea. Moreover, in addition to fibrocytes, Z-VAD-FMK protected the stria vascularis.

Active and passive potassium ion transport through these lateral-wall fibrocytes is essential for maintaining a high potassium concentration and endocochlear potential in the endolymph (Wangemann, 1995; Wangemann and Schacht, 1996). To sustain this important role, the lateral-wall fibrocytes have various combinations of ion pumps, ion channels, ion transporters, and gap junctions. Type 2 fibrocytes express Na,K-ATPase and have numerous mitochondria (Nakazawa et al., 1995; Spicer and Schulte, 1996). Furthermore, impairment of potassium ion recycling, for example in the mouse model of DFN3 nonsyndromic deafness, results in degeneration of cochlear fibrocytes and severe hearing loss (Minowa et al., 1999). In this model, type 2 fibrocytes demonstrate positive immunoreactivity to active caspase-3 and also demonstrate apoptosis by the TUNEL assay. These features of type 2 fibrocytes support that this cell type is highly vulnerable to mitochondrial toxins.

The present study has demonstrated delayed atrophy of the stria vascularis 2 weeks after administration of 3-NP. In the mouse model of DFN3 nonsyndromic deafness, atrophy of the stria vascularis was also observed, followed by degeneration of lateral-wall fibrocytes (Xia et al., 2002). Considering the time course of stria vascularis atrophy and fibrocyte degeneration in the model in the present study, atrophy of the stria vascularis may occur secondarily to degeneration of lateral-wall fibrocytes rather than from 3-NP directly damaging the stria vascularis.

Atrophy of Sensory and Nonsensory Cells in Organ of Corti

In the present animal model, atrophy of outer hair cells and Deiters cells was detected in the basal cochlear turn 2 days after administration of 3-NP and remained for at least 28 days. Neither cells immunoreactive to active caspase-3 nor TUNEL-positive cells were detected in the organ of Corti. Furthermore, this atrophy could not be inhibited by administration of Z-VAD-FMK. These results indicate that atrophy of cells in the organ of Corti was caused by a mechanism different from caspase-dependent apoptosis. This atrophy is most likely caused by a direct effect of 3-NP on the organ of Corti. It is known that the concentrations of drugs such as gentamicin and ionic markers administered through the round window membrane are higher in the basal turn than in the middle and apical turns (Salt and Ma, 2001; Plontke et al., 2002). Because 3-NP was administered through the round window membrane, the con-

centration of 3-NP in the basal turn was probably higher than that in the middle and apical turns, which may explain that atrophy occurred only in the basal turn.

Mechanism of Hearing Loss Caused by Acute Energy Failure

The elevation of the ABR threshold at 8 and 20 kHz induced by the administration of 3-NP was suppressed by the administration of Z-VAD-FMK. At 20 kHz, the peak threshold 1 day after 3-NP administration was suppressed by about 20% with Z-VAD-FMK. Furthermore, the ABR threshold had recovered to its preoperative level by 14 days after 3-NP administration in the Z-VAD-FMK group compared to a ABR threshold of about 55 dB even 28 days after 3-NP administration in the vehicle group. In rats, 20-kHz sound was received on the apical side of the basal turn, where both the organ of Corti and spiral ganglion cells were structurally normal in our study. Thus, the threshold shift at 20 kHz was likely caused by degeneration of the lateral wall of the basal turn. Ion transport in the cochlea is most active in the basal turn (Salt, 2001). When the lateral wall of the cochlear basal turn is damaged, endocochlear potential decreases in the middle and basal turns (Wu and Hoshino, 1999). By the same mechanism, the low endocochlear potential in the cochlear middle turn may be caused by 3-NP-induced lateral-wall damage in the basal turn, resulting in elevation of the ABR threshold at 20 kHz in the vehicle group. In agreement with this hypothesis, administration of Z-VAD-FMK preserved the lateral wall of the basal turn and accelerated recovery of the ABR threshold at 20 kHz. In this case, temporary hearing loss may be explained by ATP shortage causing temporary impairment of potassium transport, followed by recovery to normal endocochlear potassium concentrations. Previously, the furosemide-induced sensorineural hearing loss model also showed temporary impairment of ion transport of the cochlear lateral wall, which was associated with elevation of the threshold for the compound action potential of the cochlea (Lang et al., 2003).

At 8 kHz, the threshold recovered to the preoperative level by 14 days after 3-NP administration in the vehicle group. Because of the relatively long distance between the apical turn and the oval window, the effect of lateral-wall damage in the basal turn on the homeostasis of endolymph in the apical turn is weaker than on that in the lower region. Thus, complete recovery without Z-VAD-FMK protection may be explained by compensation of a relatively mild decrease of endocochlear potential and potassium ion concentration in the apical turn by local potassium recycling. In the Z-VAD-FMK group, the peak threshold 1 day after 3-NP administration was reduced about 30% compared to that in the vehicle group and by 3 days after the administration of 3-NP had recovered to the preoperative level. This accelerated recovery of the ABR threshold at 8 kHz is likely a result of the same mechanism described for the ABR threshold at 20 kHz.

At 40 kHz, we found little improvement in the ABR threshold in either the vehicle or the Z-VAD-FMK group, although apoptosis and degeneration of lateral-wall fibrocytes and atrophy of the stria vascularis were almost completely inhibited in the Z-VAD-FMK group. Thus, poor recovery of ABR threshold at 40 kHz is probably a result of the atrophy of cells in the organ of Corti in the basal turn.

We did not detect any protective effects of Z-VAD-FMK on the 3-NP-induced ABR threshold shift 2 hr after 3-NP administration. At this early time, elevation of the ABR threshold can be considered unrelated to apoptosis of the lateral wall. It is reasonable to assume that the observed threshold shift was related to impairment of active cochlear cell function because of the lack of ATP.

Cochlear energy failure as the result of cochlear ischemia is a possible mechanism of acute sensorineural hearing loss. The present study has demonstrated a protective effect of a pan-caspase inhibitor on elevation of the ABR threshold, although it is currently not known whether administration of Z-VAD-FMK after hearing loss exhibits the same effect. The present results suggest that inhibition of caspase activity might be a useful strategy for improving hearing after hearing loss caused by acute cochlear energy failure.

CONCLUSIONS

A rat model of cochlear energy failure induced by the local administration of the mitochondrial toxin 3-NP exhibited a temporary ABR threshold shift at 8 kHz and permanent ABR threshold shifts at 20 and 40 kHz. In this model, we observed caspase-3-dependent apoptosis in lateral-wall fibrocytes of the basal cochlear turn and atrophy of sensory and nonsensory cells in the basal turn of the organ of Corti. Furthermore, systemic administration of a caspase inhibitor for 3 days starting 1 day before the administration of 3-NP almost completely prevented apoptosis of fibrocytes in the cochlear lateral wall and partially inhibited the ABR threshold shift. The present results suggest that inhibition of apoptosis by a caspase inhibitor may be a useful strategy for improving hearing after hearing loss caused by acute cochlear energy failure such as that observed in cochlear ischemia.

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REFERENCES

Alam SA, Oshima T, Suzuki M, Kawase T, Takasaka T, Ikeda K. 2001. The expression of apoptosis-related proteins in the aged cochlea of Mongolian gerbils. Laryngoscope 111:528–534.

Alston TA, Mela L, Bright HJ. 1977. 3-Nitropropionate, the toxic substance of Indigofera, is a suicide inactivator of succinate dehydrogenase. Proc Natl Acad Sci U S A 74:3767–3771.

Brouillet E, Hantraye P, Ferrante RJ, Dolan R, Leroy-Willig A, Kowall NW, Beal MF. 1995. Chronic mitochondrial energy impairment produces selective striatal degeneration and abnormal choreiform movements in primates. Proc Natl Acad Sci U S A 92:7105–7109.

Budihardjo I, Oliver H, Lutter M, Luo X, Wang X. 1999. Biochemical pathways of caspase activation during apoptosis. Annu Rev Cell Dev Biol 15:269–290.

Cheng EH, Kirsch DG, Clem RJ, Ravi R, Kastan MB, Bedi A, Ueno K, Hardwick JM. 1997. Conversion of Bcl-2 to a Bax-like death effector by caspases. Science 278:1966–1968.

Coles CJ, Edmondson DE, Singer TP. 1979. Inactivation of succinate dehydrogenase by 3-nitropropionate. J Biol Chem 254:5161–5167.

Devarajan P, Savoca M, Castaneda MP, Park MS, Esteban-Cruciani N, Kalinec G, Kalinec F. 2002. Cisplatin-induced apoptosis in auditory cells: role of death receptor and mitochondrial pathways. Hear Res 174:45–54.

Fischel-Ghodsian N, Kopke RD, Ge X. 2004. Mitochondrial dysfunction in hearing loss. Mitochondrion 4:675–694.

Gregoli PA, Bondurant MC. 1999. Function of caspases in regulating apoptosis caused by erythropoietin deprivation in erythroid progenitors. J Cell Physiol 178:133–143.

Hamilton BF, Gould DH. 1987. Nature and distribution of brain lesions in rats intoxicated with 3-nitropropionic acid: a type of hypoxic (energy deficient) brain damage. Acta Neuropathol (Berl) 72:286–297.

Hoya N, Okamoto Y, Kamiya K, Fujii M, Matsunaga T. 2004. A novel animal model of acute cochlear mitochondrial dysfunction. Neuroreport 15:1597–1600.

Hsu CH, Kwon H, Perng CL, Bai RK, Dai P, Wong LJ. 2005. Hearing Loss in Mitochondrial Disorders. Ann N Y Acad Sci 1042:36–47.

Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, McGarry TJ, Kirschner MW, Koths K, Kwiatkowski DJ, Williams LT. 1997. Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. Science 278:294–298.

Labbe D, Teranishi MA, Hess A, Bloch W, Michel O. 2005. Activation of caspase-3 is associated with oxidative stress in the hydropic guinea pig cochlea. Hear Res 202:21–27.

Lang H, Schulte BA, Schmiedt RA. 2003. Effects of chronic furosemide treatment and age on cell division in the adult gerbil inner ear. J Assoc Res Otolaryngol 4:164–175.

Li M, Ona VO, Guegan C, Chen M, Jackson-Lewis V, Andrews LJ, Olszewski AJ, Stieg PE, Lee JP, Przedborski S, Friedlander RM. 2000. Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. Science 288:335–339.

Matsui JI, Haque A, Huss D, Messana EP, Alosi JA, Roberson DW, Cotanche DA, Dickman JD, Warchol ME. 2003. Caspase inhibitors promote vestibular hair cell survival and function after aminoglycoside treatment in vivo. J Neurosci 23:6111–6122.

Minowa O, Ikeda K, Sugitani Y, Oshima T, Nakai S, Katori Y, Suzuki M, Furukawa M, Kawase T, Zheng Y, Ogura M, Asada Y, Watanabe K, Yamanaka H, Gotoh S, Nishi-Takeshima M, Sugimoto T, Kikuchi T, Takasaka T, Noda T. 1999. Altered cochlear fibrocytes in a mouse model of DFN3 nonsyndromic deafness. Science 285:1408–1411.

Nakazawa K, Spicer SS, Schulte BA. 1995. Ultrastructural localization of Na,K-ATPase in the gerbil cochlea. J Histochem Cytochem 43:981–991.

Okamoto Y, Hoya N, Kamiya K, Fujii M, Ogawa K, Matsunaga T. 2005. Permanent threshold shift caused by acute cochlear mitochondrial dysfunction is primarily mediated by degeneration of the lateral wall of the cochlea. Audiol Neurootol 10:220–233.

Pickles JO. 2004. Mutation in mitochondrial DNA as a cause of presbyacusis. Audiol Neurootol 9:23–33.

Plontke SK, Wood AW, Salt AN. 2002. Analysis of gentamicin kinetics in fluids of the inner ear with round window administration. Otol Neurotol 23:967–974.

Salt A. 2001. Dynamics of the inner ear fluids. In: Santos-Sacchi J, Jahn AF, editors. Physiology of the ear. 2nd ed. San Diego, CA: Singlar, Thompson Learning. p 333–355.

Salt AN, Ma Y. 2001. Quantification of solute entry into cochlear perilymph through the round window membrane. Hear Res 154:88–97.

Schrantz N, Blanchard DA, Auffredou MT, Sharma S, Leca G, Vazquez A. 1999. Role of caspases and possible involvement of retinoblastoma protein during TGFbeta-mediated apoptosis of human B lymphocytes. Oncogene 18:3511–3519.

Seidman MD, Quirk WS, Shirwany NA. 1999. Mechanisms of alterations in the microcirculation of the cochlea. Ann N Y Acad Sci 884:226–232.

Spicer SS, Schulte BA. 1996. The fine structure of spiral ligament cells relates to ion return to the stria and varies with place-frequency. Hear Res 100:80–100.

Thornberry NA, Lazebnik Y. 1998. Caspases: enemies within. Science 281:1312–1316.

Van De Water TR, Lallemend F, Eshraghi AA, Ahsan S, He J, Guzman J, Polak M, Malgrange B, Lefebvre PP, Staecker H, Balkany TJ. 2004. Caspases, the enemy within, and their role in oxidative stress-induced apoptosis of inner ear sensory cells. Otol Neurotol 25:627–632.

Vaux DL, Strasser A. 1996. The molecular biology of apoptosis. Proc Natl Acad Sci U S A 93:2239–2244.

Wangemann P. 1995. Comparison of ion transport mechanisms between vestibular dark cells and strial marginal cells. Hear Res 90:149–157.

Wangemann P, Schacht J. 1996. Homeostatic mechanisms in the cochlea. In: Dallos P, Popper AN, Fay RR, editors. The Cochlea. New York: Springer. p 130–185.

Wu R, Hoshino T. 1999. Changes in off-lesion endocochlear potential following localized lesion in the lateral wall. Acta Otolaryngol 119:550–554.

Xia AP, Kikuchi T, Minowa O, Katori Y, Oshima T, Noda T, Ikeda K. 2002. Late-onset hearing loss in a mouse model of DFN3 non-syndromic deafness: morphologic and immunohistochemical analyses. Hear Res 166:150–158.

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Otoacoustic emissions, ear fullness and tinnitus in the recovery course of sudden deafness

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Abstract

Objective: This study aimed to investigate how the symptoms of ear fullness, tinnitus and otoacoustic emissions (OAE) change in relation to the recovery course of pure tone audiometry thresholds (PTA) in sudden deafness (SD).

Methods: This study analyzed follow-up data on ear fullness, tinnitus and otoacoustic emissions of eight SD patients with good hearing improvement (Group A) and eight SD patients with poor hearing improvement (Group B) in an attempt to elucidate the behavior of these symptoms in their recovery course. This study was done until there was no change in the PTA for more than 1 week and hearing recovery was no longer expected.

Results: All patients from both groups had ear fullness and tinnitus in association with the onset of SD. However, these symptoms improved only in Group A. showing a significant relationship between PTA recovery and the improvement of ear fullness annoyance ($P < 0.05$), presence of tinnitus ($P < 0.01$), improvement in tinnitus loudness ($P < 0.01$) and in tinnitus annoyance ($P < 0.01$). No patients (Group A or B) had OAE responses at their first examination. In Group A, OAE responses appeared simultaneously with improvement of hearing levels in five patients (63%) and it appeared later than hearing levels improvement in the other three patients (37%) from Group A. No patient from Group B showed OAE response on follow-up.

Conclusion: SD patients with good hearing improvement (Group A) tended to have OAE responses and the sensations of the ear fullness and tinnitus improved almost simultaneously with hearing level improvement. Their PTA improvement occurred primarily in the low to mid frequencies, with high frequencies showing less recovery. When hearing recovery was not full, OAEs did not reappear for these frequencies. Patients with poor hearing improvement tended to have absent OAEs and persistent ear fullness and tinnitus.

Keywords: Sudden sensorineural hearing loss; Follow-up; Tinnitus; Ear fullness; Otoacoustic emissions; Recovery course

1. Introduction

Tinnitus and ear fullness are some of the controversial prognostic factors reported in the literature [1–3]. However, to our knowledge, how these symptoms change chronologically in the recovery course of sudden deafness (SD) has not been described.

Otoacoustic emission (OAE) is an objective and sensitive testing of the cochlear outer hair cell function [4]. It has been reported to have a direct relationship to hearing threshold sensitivity, being effective as an early detector of ototoxicity [5], noise-induced hearing loss [6] or as a prognostic predictor of SD in cases that OAE responses appear before changes in the pure tone audiometry (PTA) [7]. Distortion product OAE (DPOAE) is a frequency-specific OAE that allows the cochlear status assessment at various frequencies being absent in sensorineural hearing loss exceeding 50 dB HL [8].

The present study aimed to describe the changes in DPOAE, ear fullness and tinnitus in the SD recovery course.

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2. Patients and methods

2.1. Patients

According to the criteria of the Ministry of Health and Welfare of Japan [9], recovery was ranked as follows: no change (improvement in hearing of less than 10 dB on average); slight improvement (improvement in hearing of 10 dB or more but less than 30 dB on average); marked improvement (improvement in hearing of 30 dB or more on average); complete recovery (all 5 frequencies of final audiogram were 20 dB or better, or improvement to the same degree of hearing as in the contralateral ear). The average hearing level on these criteria was calculated as the mean of the hearing levels measured at 250, 500, 1000, 2000 and 4000 Hz.

Between June 2003 and December 2005, 119 SD patients received adenosine tri-phosphate (ATP) and vitamin B as a basic treatment in our university hospital. Among these 119 SD patients, 8 patients with complete hearing recovery or marked improvement were selected with no concern of their initial hearing levels. This group of patients was named "Group A" (patients 1–8 in Figs. 2–4), composed of 5 men and 3 women from 18 to 58 years old (overall mean age 39.6 ± 17.9 years). Another group of SD patients, named "Group B", was formed of the selection of eight patients with slight improvement or no change in their hearing levels that was severe or profound. They matched the patients from Group A in age and sex (5 men and 3 women from 35 to 57 years old, overall mean age 48.0 ± 8.4 years) (Group B, patients 9–16 in Figs. 2 and 3). For the reasons that, not only this study was based on SD clinic examines performed on Wednesdays, but also that our university hospital has been a reference for SD patients who had already received previous treatment elsewhere with no success, reducing the chances of a positive prognosis with late treatment, the number of SD patients with recovery and enough follow-up data was relatively small in this study.

The criterion of SD in this study was defined as patients being able to describe the onset day of SD but for whom the cause of SD was unknown. Before the onset of SD, hearing loss was not noted. This study excluded patients with progressive, fluctuating, chronic or bilateral hearing loss, previous otological or audiological history, altered tympanogram or abnormal magnetic resonance imaging result.

All of the patients received audiological examination (PTA, DPOAE and answered a questionnaire about tinnitus and ear fullness) (Fig. 1) on the same day, on weekly basis, from the day they came to our hospital until their hearing levels stabilized.

2.2. Pure tone audiometry

The same audiometer (Rion, Model AA-79S, Rion, Tokyo, Japan) was used in a sound-insulated chamber throughout the study to evaluate hearing levels. The average

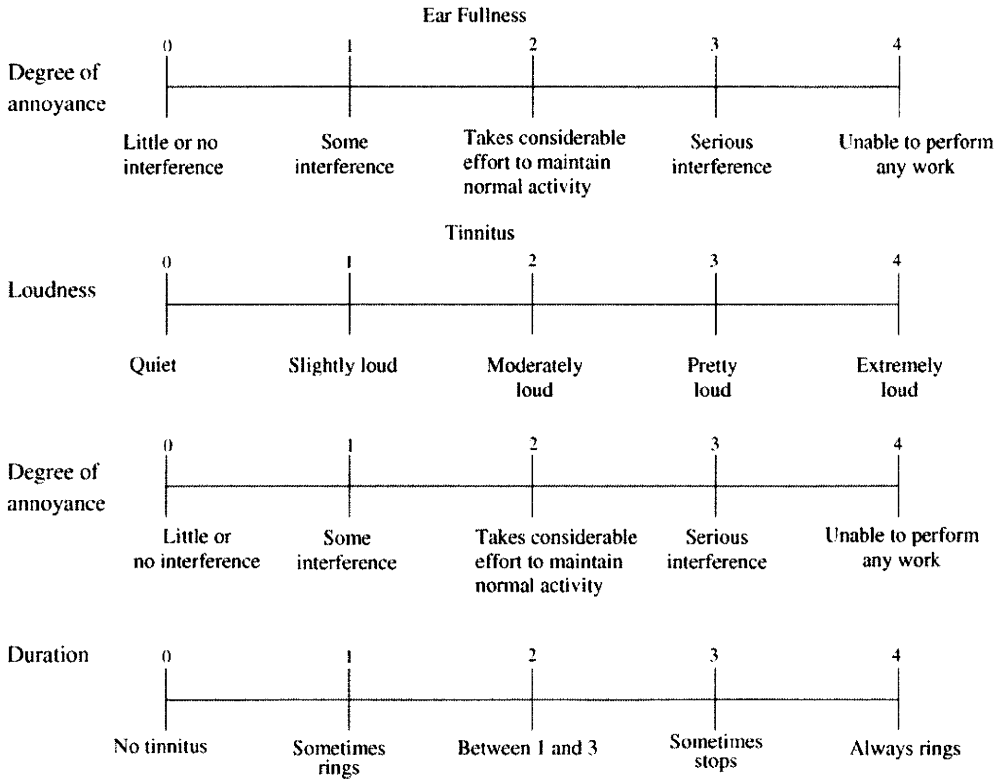

Fig. 1. Questionnaire about tinnitus and ear fullness. Rating scales for tinnitus loudness, duration and the degree of annoyance of tinnitus were rated on a five-point scale.

hearing level was expressed as the average score at five frequencies (250, 500, 1000, 2000 and 4000 Hz). If the patients did not respond to the maximum sound level produced by the audiometer, the threshold was defined as 5 dB beyond the maximum intensity level presented.

Hearing improvement level was evaluated as the degree of recovery when no additional hearing recovery could be expected, using the criteria of the Ministry of Health and Welfare of Japan.

2.3. Distortion product otoacoustic emissions (DPOAE)

The DPOAE ($2f_1$–f_2) were collected bilaterally using Otodynamics ILO 92 version 2.04. They were measured at three points per octave, according to the following parameters: $1 \leq f_2 \leq 6$ kHz, where f_2 was the higher frequency primary tone; $f_2/f_1 = 1.22$; the level for f_1 was 65 dB spl and the level for f_2 was 50 dB spl. All DPOAE data were plotted as a function of f_2.

DPOAE was assessed weekly together with pure tone audiometry, and the questionnaire about ear fullness and tinnitus.

2.4. Tinnitus and ear fullness assessment

A written questionnaire was assessed weekly regarding the presence/absence, laterality, loudness, duration and annoyance level of tinnitus as well as the presence/absence and annoyance level of ear fullness (Fig. 1).

2.5. Statistical analysis

Hearing level status (initial and final) and group difference were compared in pairs to tinnitus features (annoyance, loudness, duration), presence of vertigo and ear fullness.

Paired statistical analysis was performed with Mann-Whitney U-test. A P-value of less than 0.05 was considered statistically significant.

3. Results

Figs. 2 and 3 show the recovery course of tinnitus loudness and tinnitus annoyance, respectively. Patients in Group A are numbered from 1 to 8 (Figs. 2–4). Patients in Group B are numbered from 9 to 16 (Figs. 2 and 3).

Ear fullness was present in four patients from Group A (patient nos. 4, 5, 7 and 8) and in five patients from Group B (patient nos. 9, 10, 11, 12 and 15) at the first visit. One patient from Group A (patient no. 8 in Figs. 2–4) had complete hearing recovery. After the hearing levels stabilized, ear fullness was still present in two patients from Group A (patient nos. 5 and 7). At the first examination, five patients from Group B (patient nos. 9, 10, 11, 12 and 15) had ear fullness. On follow-up, ear fullness was still present in all of these five patients and in two additional patients from this group who

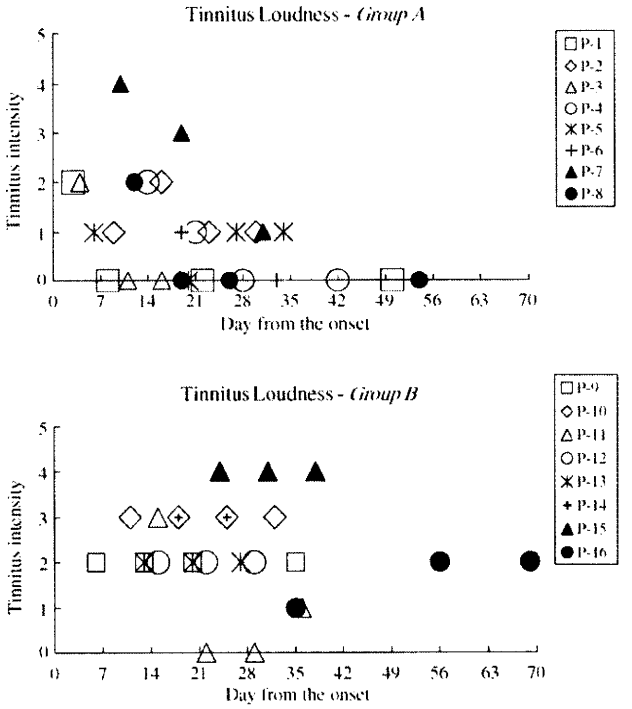

Fig. 2. Recovery course of tinnitus loudness in Group A (sudden deafness (SD) with good hearing improvement) and in Group B (SD with poor hearing improvement). Group A had a reduction in their tinnitus loudness, while most of patients in Group B kept the same tinnitus loudness level on follow-up visits. P: Patient. The onset day was "Day 0".

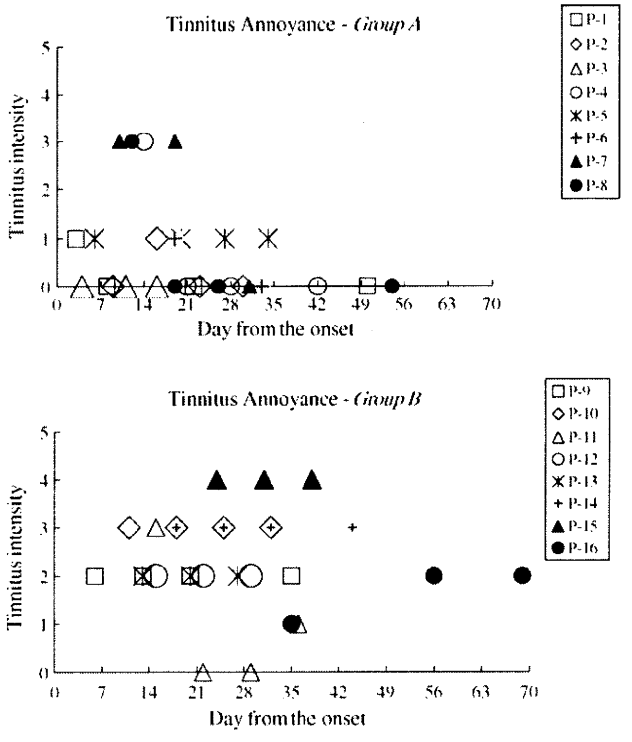

Fig. 3. Recovery course of tinnitus annoyance in Group A (SD with good hearing improvement) and in Group B (SD with poor hearing improvement). Group A had a reduction in their tinnitus annoyance, while most of patients in Group B kept the same degree of annoyance on follow-up visits. P: Patient. The onset day was "Day 0".

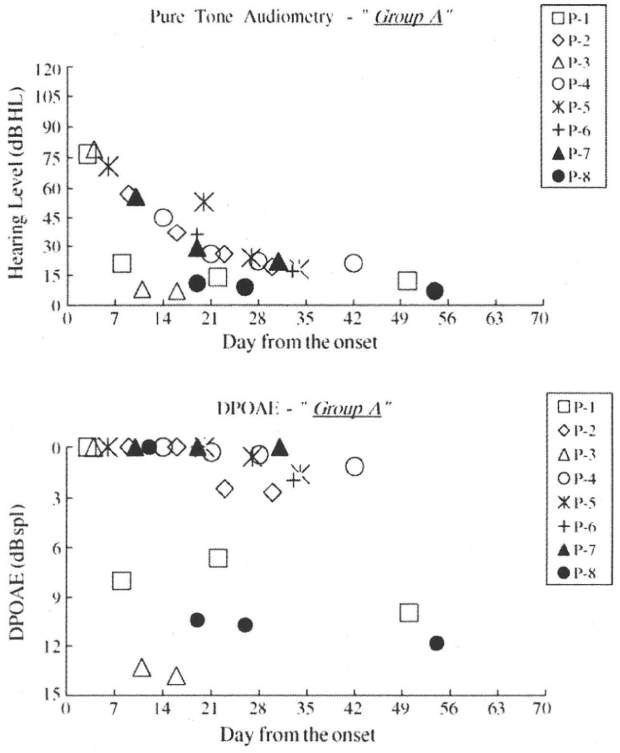

Fig. 4. Comparison of PTA and DPOAE in Group A's recovery course. Patient nos. 1, 3 and 8 had fast recovery, while patient nos. 2, 4, 5, 6 and 7 had gradual delayed recovery. P: Patient. The onset day was "Day 0".

had no previous complaint of ear fullness (patient nos. 13 and 14). Even though the initial presence of ear fullness was not related to hearing recovery, its improvement on follow-up was closely related to the hearing recovery ($P < 0.05$).

Tinnitus was present in all patients at their first visit. On follow-up, tinnitus disappeared in four patients from Group A (patient nos. 1, 3, 4 and 8) but remained in all patients from Group B. Disappearance of tinnitus on follow-up was related to hearing improvement ($P < 0.01$).

Tinnitus duration was not related to hearing improvement. If present on follow-up, it remained continuous until the hearing level stabilization in all patients from both groups, except for patient nos. 11 and 13 that had intermittent tinnitus on follow-up. Tinnitus loudness improved in two patients from Group A (patient nos. 2 and 7) and, even though it remained the same in other two patients (nos. 5 and 6), it was not considered annoying to any of them. On the other hand, tinnitus loudness remained the same level in six patients from Group B, improved in one patient (no. 11) and worsened in one other patient (no. 16). It was still considered annoying to all of the patients from Group B after their hearing level stabilized. Fig. 2 shows that final tinnitus loudness was statistically better in Group A compared to final tinnitus loudness in Group B ($P < 0.01$). Tinnitus annoyance at the end of the follow-up was also significantly better in Group A than in Group B ($P < 0.001$). The annoyance level was also rated significantly higher in patients with worse initial hearing level ($P < 0.05$) and worse final hearing level ($P < 0.01$) (Fig. 3).

Fig. 4 shows the recovery course of PTA and DPOAE of the patients from Group A. No patients had OAE responses at the first examination, and no OAE responses appeared before the improvement in PTA levels. OAE responses did not appear in any of the patients from Group B until the end of the follow-up. In patients from Group A, OAEs was not be observed in the frequencies that had not reached sufficient recovery. Patients 2, 4, 5, 6 and 7 still had some degree of hearing loss in

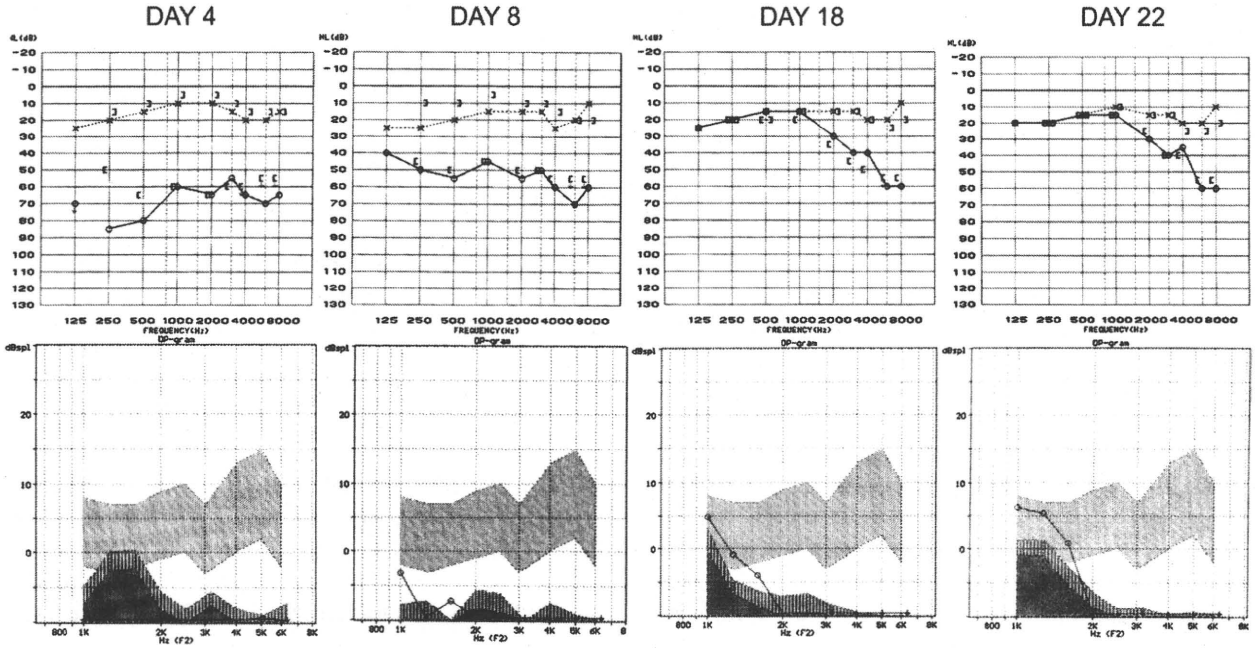

Fig. 5. Audiometry and DPOAE in the recovery course of patient no. 5 (51 years old, woman). No DPOAE response on follow-up from 2 kHz even with PTA levels better than 50 dB HL. Day 4, Day 8, Day 18 and Day 22 mean that the period between the onset and the examination was 4, 8, 18 and 22 days, respectively.

the high frequencies and, in these respective frequencies, OAEs were not observed. Fig. 5 illustrates this situation.

4. Discussion

The major finding of this study is that OAE responses, ear fullness and tinnitus follow similar patterns to the hearing levels recovery in SD. Good hearing improvement tend to have ear fullness and tinnitus improved almost simultaneously with hearing level improvement, while in poor hearing improvement these symptoms tend to persist. Furthermore, our study revealed that a more detailed analysis of Group A's recovery course showed two different recovery patterns: fast recovery (patient nos. 1, 3 and 8) and delayed recovery (patient nos. 2, 4–7). The fast-recovery patients initially had tinnitus, no ear fullness (except for patient no. 8, who only complained of it at the 1st. visit), and no DPOAE responses. On the following examination (about a week later or less) all responses were normal (normal hearing levels, normal DPOAEs, absence of tinnitus and ear fullness). Ear fullness was only present initially in patient no. 8, and only at the first visit. The delayed-recovery patients initially had tinnitus, ear fullness and no DPOAE. They showed a gradual hearing level improvement (Fig. 4) followed by a gradual complete or partial DPOAE recovery and gradual improvement of ear fullness and tinnitus.

As long as we observed, the delayed-recovery patients also had the symptoms of ear fullness and tinnitus following a similar gradual pattern of the hearing levels improvement. However, in some patients (nos. 4, 5 and 7) the DPOAE responses came later than expected.

Concerning the aspects of tinnitus, the aspects of loudness and annoyance were closely related to each other, showing almost the same recovery patterns. On the other hand, the duration of tinnitus showed a different pattern in the recovery course. Tinnitus tended to persist continuously even after the other audiological aspects had shown improvement, and the next step was its completely disappearance, instead of behaving intermittently during the recovery process.

Sakata and Kato [1] have reported that the presence of ear fullness at the first medical examination is not related to auditory function. However, its disappearance on follow-up is associated with good hearing prognosis. Ear fullness was initially present in four patients from Group A. Even though ear fullness has improved, it was still present on follow-up in two additional patients (nos. 5 and 7), respectively the patients with worse HL average on follow-up. Five patients from Group B (patient nos. 9, 10, 11, 12 and 15) had ear fullness at the first examination and, not only it remained in all of these patients, but ear fullness appeared on follow-up in two more patients from this group (patient nos. 13 and 14). Tinnitus was present in all patients from both groups at the initial examination and, on follow-up, it had disappeared in four patients from Group A. Three of these patients (nos. 1, 3 and 8) had complete hearing recovery. Ear fullness recovery course followed a similar pattern to the improvement of tinnitus annoyance and loudness. Patients with fast hearing recovery also had fast improvement of tinnitus loudness, tinnitus annoyance and ear fullness. On the other hand, patients with remaining tinnitus annoyance and loudness during the follow-up, tended to have persistent ear fullness with a similar rated annoyance level (Fig. 1).

Ben-David et al. [10] described tinnitus as a favorable prognostic manifestation in SD suggesting that, as tinnitus frequently precedes the SD onset, it may indicate activity in the living hair cells and their recovery potential. We also expected this theory to be shown as OAE responses previously to the audiometric thresholds recovery, as Lalaki et al. [7] observed in 60% of the recovered patients. However, it did not happen in any of our patients. Conversely, the DPOAE responses had either appeared simultaneously to the audiometric thresholds recovery (patient nos. 1, 2, 3, 6 and 8) or later (patient nos. 4, 5 and 7) (Fig. 4 shows patient no. 5). On the other hand, similarly to our findings, it has been reported that DPOAE recovery in SD is later than PTA [11] and these authors concluded that DPOAE is a sensitive and direct tool to evaluate the cochlear function considering that outer hair cells may not have recovered completely even after improvement in pure tone thresholds. DPOAE improvement was not related to any of the tinnitus aspects or ear fullness.

Our study revealed that neither the absence of DPOAE in the first visit, the presence/absence of ear fullness or tinnitus were predictor factors of hearing recovery in SD, as changes in these symptoms were not seen before PTA improvement. Further studies with a longer follow-up period may be useful for a better understanding of the relationship between the hearing loss in SD and the its related symptoms.

5. Conclusion

The study showed that ear fullness and tinnitus follow similar recovery patterns to the hearing level improvement. Patients with fast recovery of their hearing levels also have almost simultaneous appearance of OAE responses, fast improvement of ear fullness and of tinnitus. Patients with delayed hearing levels improvement also have gradual appearance of OAE responses and gradual improvement of ear fullness and tinnitus. Tinnitus loudness was closely related to tinnitus annoyance, showing almost the same improvement pattern.

As far as we examined, in SD patients with poor hearing improvement, symptoms as, tinnitus and ear fullness tend to persist for up to 3 months from the onset of SD. Long-term follow-up needs to be done regarding tinnitus and ear fullness in patients with SD.

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References

[1] Sakata T, Kato T. Feeling of ear fullness in acute sensorineural hearing loss. Acta Otolaryngol 2006;126(8):828–33.

[2] Nakashima T, Yanagita N. Outcome of sudden deafness with and without vertigo. Laryngoscope 1993;103(10):1145–9.

[3] Mamak A, Yilmaz S, Cansiz H, Inci E, Guclu E, Derekoylu L. A study of prognostic factors in sudden hearing loss. Ear Nose Throat J 2005;84(10):641–4.

[4] Kemp DT. Otoacoustic emissions, their origin in cochlear function, and use. Br Med Bull 2002;63:223–41.

[5] Stavroulaki P, Vossinakis IC, Dinopoulou D, Doudounakis S, Adamopoulos G, Apostolopoulos N. Otoacoustic emissions for monitoring aminoglycoside-induced ototoxicity in children with cystic fibrosis. Arch Otolaryngol Head Neck Surg 2002;128 (2):150–5.

[6] Seixas NS, Kujawa SG, Norton S, Sheppard L, Neitzel R, Slee A. Predictors of hearing threshold levels and distortion product otoacoustic emissions among noise exposed young adults. Occup Environ Med 2004;61(11):899–907.

[7] Lalaki P, Markou K, Tsalighopoulos MG, Daniilidis I. Transiently evoked otoacoustic emissions as a prognostic indicator in idiopathic sudden hearing loss. Scand Audiol Suppl 2001;52:141–5.

[8] Gorga MP, Neely ST, Ohlrich B, Hoover B, Redner J, Peters J. From laboratory to clinic: a large scale study of distortion product otoacoustic emissions in ears with normal hearing and ears with hearing loss. Ear Hear 1997;18(6):440–55.

[9] Sugiura M, Naganawa S, Teranishi M, Nakashima T. Three-dimensional fluid-attenuated inversion recovery magnetic resonance imaging findings in patients with sudden sensorineural hearing loss. Laryngoscope 2006;116(8):1451–4.

[10] Ben-David J, Luntz M, Magamsa I, Fradis M, Sabo E, Podoshin L. Tinnitus as a prognostic sign in idiopathic sudden sensorineural hearing loss. Int Tinnitus J 2001;7(1):62–4.

[11] Zhang Q, Deng Y, Xing G, Cheng Z. Observation of distortion product otoacoustic emissions in recovery course of sudden deafness. Lin Chuang Er Bi Yan Hou Ke Za Zhi 1999;13(10):443–5 [in Chinese].

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Three-Dimensional Fluid-Attenuated Inversion Recovery Magnetic Resonance Imaging Findings and Prognosis in Sudden Sensorineural Hearing Loss

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Objectives/Hypothesis: Three-dimensional fluid-attenuated inversion recovery (3D-FLAIR) magnetic resonance imaging (MRI) has recently been developed to detect high concentrations of protein or hemorrhage. We have previously reported that 50% of patients with sudden sensorineural hearing loss (SNHL) show high signals in the affected inner ear on 3D-FLAIR MRI. However, the relationship between 3D-FLAIR findings and hearing prognosis is unclear. Our objective was to evaluate the relationship between the results of 3D-FLAIR MRI at 3 Tesla and prognosis in sudden SNHL.

Study Design and Methods: We used 3D-FLAIR at 3 Tesla with and without gadolinium enhancement to evaluate the pathologic conditions in the inner ears of 48 patients with sudden SNHL.

Results: Thirty-one of 48 patients with sudden SNHL showed high signals in the affected inner ear on precontrast 3D-FLAIR. Hearing improvement in patients with high signals in the affected inner ear on precontrast 3D-FLAIR (25 ± 19 dB) was significantly worse than that in patients with no signal (45 ± 27 dB; $P < .05$). Our analysis suggests that high signals in the affected inner ear on precontrast 3D-FLAIR MRI is a new prognostic factor for sudden SNHL.

Conclusions: 3D-FLAIR findings show that high signals in the cochlea on precontrast 3D-FLAIR are related to a poor hearing prognosis. These signals may reflect minor hemorrhage or an increased concentration of protein in the inner ear, which has passed through blood vessels with increased permeability or has originated in disrupted cells in the inner ear.

Key Words: Fluid-attenuated inversion recovery, sudden sensorineural hearing loss, magnetic resonance imaging, prognosis.

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INTRODUCTION

The pathology of sudden sensorineural hearing loss (SNHL) remains unclear. The hypothesized pathologies include viral infection,[1] vascular compromise,[2] disruption of cochlear membranes,[2] inner ear anomaly,[3] and immunologic diseases.[1] However, no cause is found in most cases.

We have previously reported that four of eight patients with sudden SNHL showed high precontrast signals in the inner ear on three-dimensional fluid-attenuated inversion recovery (3D-FLAIR), and one of these four patients showed gadolinium (Gd) enhancement in the affected inner ear on 3D-FLAIR.[4] Furthermore, high signals in the affected inner ear are present in other inner ear diseases.[5–8] These signals may reflect minor hemorrhage or an increased concentration of protein in the inner ear, which has passed through blood vessels with increased permeability.[4] However, the relationship between 3D-FLAIR findings and clinical signs has not been clarified. We cannot make a prognosis for patients with sudden SNHL before treatment, although these patients wish to know the prognosis for sudden SNHL. In this study, we investigated the correlation between 3D-FLAIR findings and SNHL prognosis and between 3D-FLAIR findings and other clinical signs.

MATERIALS AND METHODS

Subjects

We evaluated 48 patients (24 men and 24 women; mean age ± standard deviation, 50.0 ± 16.4 yr) with unilateral sudden SNHL who visited Nagoya University Hospital between December 2005

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and July 2007. The criteria for sudden SNHL used in this study were that the patient could describe the day of onset of sudden SNHL, which had no obvious cause, and that no HL had been noted before the onset of SNHL. We excluded patients with fluctuating HL or progressive HL. All these patients were examined with T1- and T2-weighted magnetic resonance imaging (MRI) and 3D-FLAIR MRI before and after Gd enhancement. We also evaluated 11 patients at second and third examinations more than 3 months after onset using 3D-FLAIR MRI in a follow-up study. Eight patients, who had been reported in our previous study,[3] were included in this study. In all patients, hydrocortisone was administered intravenously 200 mg per day for 4 days and then 100 mg for 3 days with adenosine triphosphate (60 mg per day).

Audiologic Findings

Hearing levels were evaluated using an audiometer (Model AA-79S; Rion, Tokyo, Japan) in a sound-insulated chamber. The initial audiograms were obtained at the first visit, and the final audiograms were taken after 2 months had elapsed since the onset of deafness, except for patients who recovered completely within this period. Serial audiograms were compared with tympanograms and speech discrimination scores, when available. The average hearing level was expressed as the average score at three frequencies (500, 1,000, and 2,000 Hz). If the patient did not respond to the maximum sound level produced by the audiometer, we defined the threshold as 5 dB added to the maximum level.

The outcome of sudden SNHL was evaluated using the criteria of the Ministry of Health and Welfare in Japan.[9] The average hearing level on these criteria was calculated as the mean of the hearing levels measured at 250, 500, 1,000, 2,000, and 4,000 Hz. Recovery was ranked as follows:[10] no change (improvement in hearing of less than 10 dB on average); slight improvement (improvement in hearing of 10 dB or more but less than 30 dB on average); remarkable improvement (improvement in hearing of 30 dB or more on average); and complete recovery (all 5 frequencies on the final audiogram were 20 dB or less or improvement to the same degree of hearing as in the contralateral ear). The prognosis score was assigned as follows: 0 = no change; 1 = slight improvement; and 2 = remarkable improvement or complete recovery.

The periods between the onset of HL and the MRI study were compared between patients with and without high signals on 3D-FLAIR. The average prognosis scores were compared between patients with and without high signals on 3D-FLAIR. All statistical analyses were performed using the Mann-Whitney U test or the χ^2 test.

A multivariate regression analysis was used to identify the prognostic factors that were related to the final audiograms. The following factors were examined as explanatory variables: age, sex, presence of vertigo at the onset of sudden SNHL, the period from onset of sudden SNHL to first visit, precontrast high signals in the inner ear on 3D-FLAIR, and the initial audiogram. The data were analyzed by multivariate regression using the SPSS 8.0 statistical package (SPSS, Inc., Chicago, IL).

MRI

All scans were performed at 3 Tesla MRI (Trio; Siemens, Erlangen, Germany) using a receive-only eight-channel phased-array coil. Before and after the intravenous administration of a single dose of Gd–diethylenetriaminepentaacetic acid–bis methylamide (Gd–DTPA–BMA; Omniscan; Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) at 0.1 mmol/kg, T1-weighted 3D fast low-angle shot (FLASH) imaging and 3D-FLAIR imaging were performed. Heavily T2-weighted 3D constructive interference in the steady state imaging was performed only before the contrast material was administered to delineate the anatomy of the cerebrospinal fluid space. 3D-FLAIR and 3D-FLASH images were obtained before and after the administration of a single dose of Gd–DTPA–BMA. After the contrast was administered, 3D-FLASH was scanned first. Then, contrast-enhanced 3D-FLAIR was initiated 7 minutes after the Gd was administered so that the contrast 3D-FLAIR images were determined approximately 10 minutes after the administration of the Gd. These methods have been described in detail in previous reports.[4,10–12]

MRI Findings

The MRI findings for the inner ear in patients with sudden SNHL were evaluated using our own criteria. MRI findings were ranked as follows: none (no signal in the affected inner ear); faint (the signals in the affected inner ear were higher than those of the cerebrospinal fluid but lower than those of the cerebellar white matter); moderately high (the signals in the affected inner ear were as high as those of the cerebellar white matter); and very high (the signals in affected inner ear were higher than those in the cerebellar white matter).

RESULTS

The characteristics of 48 patients with sudden SNHL are summarized in Table I. Thirty-one of the 48 patients with sudden SNHL showed high signals in the affected inner ear on precontrast 3D-FLAIR. Sixteen of these 31 patients also showed Gd enhancement on 3D-FLAIR in the affected inner ear. The high-signal areas observed on 3D-FLAIR were not detected by T1- or heavily T2-weighted MRI in any of these patients. All patients had no signals in the unaffected inner ear. We could perform follow-up MRI study in 11 of 48 patients. In these patients, high signals in affected inner ears on precontrast or postcontrast 3D-FLAIR had disappeared after approximately 90 to 150 days from onset of sudden SNHL. Figure 1 shows a follow-up MRI finding of a patient with right sudden SNHL.

The average initial hearing level was 80 ± 17 dB in the group with high signals on precontrast 3D-FLAIR and 75 ± 19 dB in the group with no signals. There was no statistically significant difference in the initial hearing levels of the two groups (Mann-Whitney U test). However, hearing improvement in the high-signal group (25 ± 19 dB) was significantly worse than that in the no-signal group (45 ± 27 dB; $P < .01$; Mann-Whitney U test) (Fig. 2) (Table I).

A multivariate regression analysis of the audiologic assessment was made for all patients, which included age [a positive value of the β (normalized regression) coefficient indicates a better hearing prognosis in young patients], the presence of vertigo at the onset of sudden SNHL (a negative value of the β coefficient indicates that a lack of vertigo entails a better hearing prognosis than that of patients with vertigo), precontrast high signals in the inner ear on 3D-FLAIR (a positive value of the β coefficient indicates that a lack of high signals reflects a better hearing prognosis than the prognosis in the presence of high signals), the period from the onset of sudden SNHL to the first visit (a positive value of the β coefficient indicates a poorer hearing prognosis in patients who visited late), and initial audiogram (a positive value of the β coefficient indicates a poorer hearing prognosis in patients with a high level of initial HL). This analysis revealed that