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Short communication

Protective effect of edaravone against streptomycin-induced vestibulotoxicity in the guinea pig

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Abstract

This study investigated alleviation of streptomycin-induced vestibulotoxicity by edaravone in guinea pigs. Edaravone, a free radical scavenger, has potent free radical quenching action and is used in clinical practice to treat cerebral infarction. Streptomycin was administered to the inner ear by osmotic pump for 24 h, and edaravone ($n=8$) or saline ($n=6$) was intraperitoneally injected once a day for 7 days. We observed horizontal vestibulo-ocular reflex as a marker of postoperative vestibular function. Animals injected with saline showed statistically smaller gains than those injected with edaravone. These results suggest that edaravone suppresses streptomycin-induced vestibulotoxicity.

Keywords: Edaravone; Free radical scavenger; Horizontal vestibulo-ocular reflex; Osmotic pump; Streptomycin; (Guinea pig)

1. Introduction

Many drugs are known to be toxic to the inner ear. Aminoglycoside antibiotics enjoy widespread clinical use as highly effective antimicrobial agents. However, a serious limitation to the use of these drugs is their side effect of ototoxicity (loss of hearing or balance). Streptomycin causes preferential damage to the vestibular system with little auditory involvement (Song et al., 1998; Sugasawa et al., 2001; Wanamaker et al., 1999). Free radical scavengers have been shown to protect against aminoglycoside-induced ototoxicity (Nakagawa et al., 1999; Sha and Schacht, 2000; Song et al., 1998; Takumida and Anniko, 2002). Studies show that edaravone is a free radical scavenger and has potent free radical quenching action (Watanabe et al., 1998, 1994; Yamamoto et al., 1997). In the present study, we investigated the protective action of edaravone against vestibulotoxicity from streptomycin.

2. Materials and methods

2.1. Animals

Experiments were performed on 14 male Hartley guinea pigs (380–565 g) with normal Preyer's reflexes and tympanic membranes. The experimental protocol was approved by the Committee for Ethics in Animal Experiments of the Yamaguchi University School of Medicine. Experiments were carried out under the Guidelines for Animal Experiments of the Yamaguchi University School of Medicine and the Law and Notification of the Government of Japan.

2.2. Evaluation of vestibular function

Before osmotic pump implantation, we calculated horizontal vestibulo-ocular reflex gains using our method (Horiike et al., 2002). For the purpose of immobilizing the guinea pig, a cage designed to hold the animal still during experiments was mounted on top of a turntable apparatus (Daiichi Medical, Tokyo, Japan). The cage was of two parts, one for the head and the other for the body, and the two parts were joined. The two-part construction

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was intended to remove as much influence of the animal's body movements during sinusoidal rotation as possible. The animal's head was fixed firmly with both auricles held between sponge-covered plates that held both acoustic meati horizontally such that the midpoint of a straight line joining the lateral semicircular canals was located on the rotation axis of the turntable. We set up the infrared CCD camera (Nagashima Medical, Tokyo, Japan) perpendicular to the sagittal plane of the guinea pig's head and in a plane parallel to the rotational plane of the turntable apparatus. By opening an aperture on the left side of the head cage, eye movements of guinea pigs were videotaped (Hi8 format, Sony, Tokyo, Japan) in the dark with the infrared CCD camera. We stored the video images on a computer (Power Mac G4, Apple Computer, CA, USA). Each image was converted to an image file with QuickTime 4.0 optional (Apple Computer). For automatic analysis of guinea pig eye movement, we created a macro for use with the National Institutes of Health (NIH) Image analysis software (http://rsb.info.nih.gov/nih-image/). Our macro is available at http://www.cc.yamaguchi-u.ac.jp/~ent/gan-kyu3d/ikeda.html. After capturing eye movement on the computer with this macro, we removed unnecessary portions of the images, and set the threshold to provide for clear outlines of the pupil. The $X-Y$ center of the pupil was analyzed, and the horizontal and vertical components of eye movements were calculated. We calculated slow-phase velocities, found the maximum slow-phase velocity, and calculated the horizontal vestibulo-ocular reflex gain by dividing the maximum slow-phase velocity by the peak angular velocity.

2.3. Pump implantation

Under pentobarbital anesthesia (28 mg/kg, i.p.), 1.5 ml of lidocaine HCl was injected into the right postauricular region of each guinea pig, and the mastoid bulla was opened by a postauricular incision to allow visualization of the round window under a surgical microscope. A tiny hole was made adjacent to the round window with a perforating burr. A catheter filled with streptomycin (30%, dissolved in saline; Meiji Seika Kaisha, Tokyo, Japan) and connected to an osmotic pump (Model 2002, Alza, Palo Alto, CA, USA) was then inserted. The pump was placed under the skin on the back. The pump ran continuously for 24 h, injecting 0.5 μl/h; next, 3.6 μg of streptomycin was injected over 24 h into the inner ear. After the wound was washed with saline, a small amount of piperacillin sodium was introduced. After wound closure, piperacillin sodium at a dose of 40 mg/kg was injected i.m., and oxytetracycline HCl ointment was applied to the wound. During the operation and for 24 h following the operation, each animal was kept warm with an electric blanket. In our previous study, vestibular function after the implantation of an osmotic pump and the infusion of saline was within the preoperative range (Shimogori and Yamashita, 2000; Shimogori et al., 1999), and auditory function was in the same way (Sugahara et al., 2001).

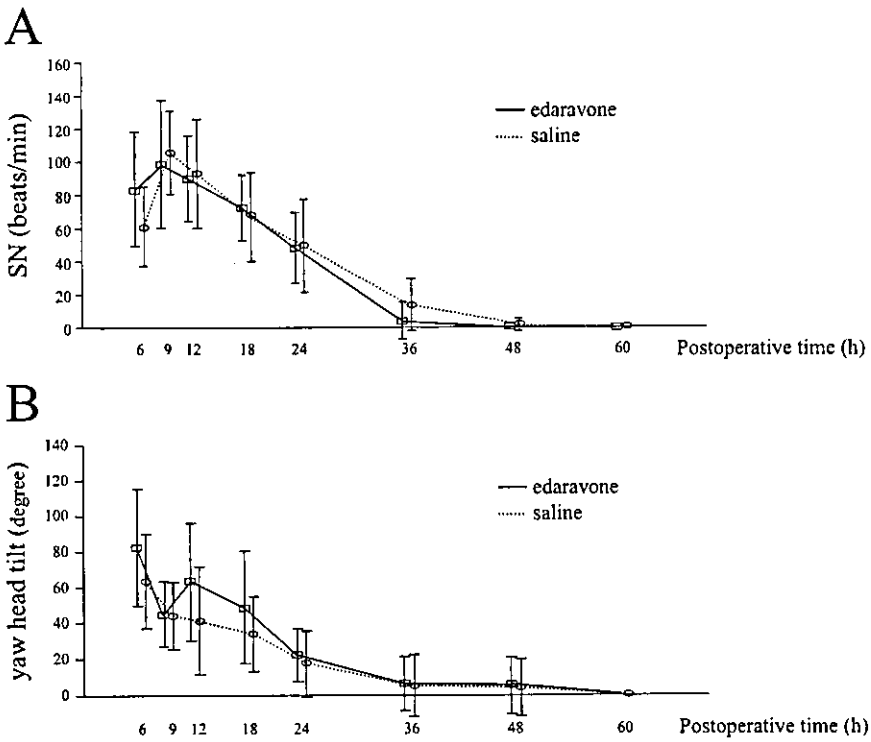

Fig. 1. (A) Spontaneous nystagmus (SN) after the operation. (B) Yaw head tilt after the operation. Error bar, ± 1 S.D.

2.4. Drug administration

These animals were divided into two groups. Eight animals of the fourteen received edaravone (Mitsubishi Pharma, Tokyo, Japan) at a dose of 3 mg/kg i.p. once a day for 7 days after the operation (edaravone group). Edaravone was dissolved in 1 N NaOH, and the pH was adjusted to 7.0 with 1 N HCl. The remaining six animals received the same amount of saline i.p. in the same manner (control group).

2.5. Statistical analysis

At 6, 9, 12, 18, 24, 36, 48 and 60 h after the operation, we measured spontaneous nystagmus beats per min and yaw head tilt (Curthoys et al., 1988). We measured the gains with sinusoidal rotation at 0.1 Hz and a peak angular velocity of 60°/s before the operation and at 3 and 7 days postoperatively. We calculated the gain ratio by dividing the postoperative gain by the preoperative gain. The Mann–Whitney *U*-test was used to assess differences between the two groups with significance set at $P<0.05$. All data are the means ± S.D. for the two groups.

3. Results

3.1. Spontaneous nystagmus and yaw head tilt

No statistical difference in postoperative spontaneous nystagmus or yaw head tilt was found between the two groups. In both groups, the peak of spontaneous nystagmus was observed at 9 h after the operation, and spontaneous nystagmus disappeared within 60 h after the operation, additionally, yaw head tilt also disappeared within 60 h after the operation (Fig. 1A,B).

3.2. The gain ratio

In the edaravone group, gains on the intact side preoperatively, 3 days postoperatively and 7 days postoperatively were 0.383 ± 0.081 (mean ± S.D.), 0.252 ± 0.09 and 0.241 ± 0.067, respectively, and those on the lesioned side were 0.378 ± 0.08, 0.204 ± 0.085 and 0.216 ± 0.053, respectively. In the control group, gains on the intact side were 0.377 ± 0.073, 0.177 ± 0.06 and 0.211 ± 0.048, respectively, and those on the lesioned side were 0.443 ± 0.066, 0.09 ± 0.045 and 0.137 ± 0.059, respectively. At 3 and 7 days after the operation, statistical differences in the gain ratio on the lesioned side were found between the edaravone and control groups (day 3, 0.575 ± 0.333 vs. 0.202 ± 0.086, $P<0.01$; day 7, 0.589 ± 0.149 vs. 0.308 ± 0.124, $P<0.05$) (Fig. 2).

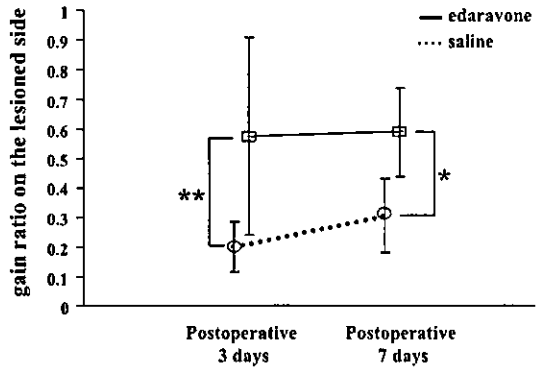

Fig. 2. The gain ratios on the lesioned side at 3 and 7 days after the operation. Error bar, ± 1 S.D., $^{**}P<0.01$, $^{*}P<0.05$.

4. Discussion

The gain ratios on the lesioned side in the control group in this study were equivalent to those in the hemilabyrinthectomized guinea pigs in the study by Vibert et al. (1993). Additionally, the S.D. of the gain ratio on the lesioned side at 3 days in the control group was small, indicating that our method may produce stable vestibular disorder (Fig. 2). The S.D. of the gain ratios on the lesioned side at 7 days after the operation were almost equal between the groups, which might be thought that central vestibular compensation led to the correction of vestibular imbalance in all animals (Smith and Curthoys, 1989).

Evidence is accumulating that generation of reactive oxygen species is an important factor in inner ear damage (Nakagawa et al., 1999; Sha and Schacht, 2000; Song et al., 1998; Tabuchi et al., 2001; Takumida and Anniko, 2002). The hydroxyl radical is one of reactive oxygen species and an extremely powerful oxidant that is indiscriminately reactive with almost all biological substances (Tabuchi et al., 2001). It is therefore suspected that the tissue damage induced by reactive oxygen species is mainly due to hydroxyl radicals. It has been reported that edaravone is capable of directly trapping a variety of free radical species and reacts with hydroxyl or peroxyl radicals to form some oxidized compounds (Watanabe et al., 1998, 1994; Yamamoto et al., 1997). As stated earlier, inner ear damage caused by aminoglycoside is ameliorated by the presence of free radical scavengers (Sha and Schacht, 2000; Sinswat et al., 2000; Song et al., 1998; Takumida and Anniko, 2002). However, we cannot find any report about the effects of edaravone on the inner ear in vivo and in vitro. In this study, the gain ratios of the edaravone group on the lesioned side, which were significantly larger than those of the control group at 3 and 7 days after the operation (Fig. 2). From this evidence, it appears that the free radical scavenging action of edaravone may contribute to its protective effect against streptomycin-induced vestibulotoxicity in the guinea pig.

Edaravone showed no effect on reducing spontaneous nystagmus or yaw head tilt. Concerning this, we cannot find any previous report showing the correlation between spon-

taneous nystagmus, yaw head tilt and horizontal vestibulo-ocular reflex after vestibular disorder. However, we expect that the gain may be a good indicator to evaluate vestibular function, rather than spontaneous nystagmus or yaw head tilt that is static symptom. Horizontal vestibulo-ocular reflex is induced by rotation stimulation, indicating that this reflex is dynamic and more physiological phenomenon after vestibular disorder (Curthoys et al., 1988).

We conclude that edaravone may improve streptomycin-induced balance loss. We expect further studies to evaluate the efficacy of edaravone against cochleotoxicity and vestibulotoxicity caused by many drugs, including aminoglycoside antibiotics, and edaravone be useful in vestibular disease therapy.

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Effect of Edaravone on Streptomycin-Induced Vestibulotoxicity in the Guinea Pig

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Objectives/Hypothesis: **The effect of topical administration of edaravone to the inner ear was investigated in guinea pigs with streptomycin-induced vestibulotoxicity.** ***Methods:*** **Vestibulotoxicity was induced in 20 animals by delivery of streptomycin into the inner ear through osmotic pump for 24 hours. Edaravone (n = 8, systemic administration group) or saline (n = 6, control group) was injected intraperitoneally once a day for 7 days or edaravone-soaked Gelfoam was placed on the round window before wound closure (n = 6, topical administration group).** ***Results:*** **Yaw head tilt and spontaneous nystagmus were observed in all animals after the operation. The number of spontaneous nystagmus beats in the topical administration group was statistically less than that in other two groups at 12, 18, and 24 hours after the operation.** ***Conclusion:*** **The study results suggest that topical administration of edaravone better suppresses streptomycin-induced vestibulotoxicity than systemic administration.** ***Key Words:*** **Edaravone, topical administration, spontaneous nystagmus, osmotic pump, streptomycin, guinea pig.**

Laryngoscope, **114:1630–1632, 2004**

INTRODUCTION

Aminoglycoside antibiotics are highly effective antimicrobial agents and have achieved widespread clinical use. However, a serious limitation of the use of these drugs is their ototoxicity. Free radical scavengers have been shown to protect against aminoglycoside-induced ototoxicity.[1–6] Edaravone, a free radical scavenger and neuroprotective agent, has potent free radical scavenging and antioxidant activity[7,8] and is used in clinical practice to treat cerebral infarction. From a previous study, we reported the protective effect of systemic administration (intraperitoneal injection) of edaravone against streptomycin-induced vestibulotoxicity in the guinea pig.[1] A transtympanic drug delivery system is currently used clinically for the treatment of vertigo.[9] With this system, we can accurately administer therapeutic and test compounds to the inner ear. We have considered use of edaravone in conjunction with this system; thus, we investigated the effect of topical administration of edaravone in animals with streptomycin-induced vestibulotoxicity.

MATERIALS AND METHODS

Experiments were performed in 20 male Hartley guinea pigs (weight, 380–590 g) with normal Preyer's reflexes and tympanic membranes. Under pentobarbital anesthesia (28 mg/kg given intraperitoneally), 1.5 mL of lidocaine HCl was injected into the right-side postauricular region of each guinea pig, and the mastoid bulla was opened by postauricular incision to allow visualization of the round window under a surgical microscope. A tiny hole was made adjacent to the round window with a perforating burr. A catheter filled with 30% streptomycin dissolved in saline (Meiji Seika Kaisha Ltd., Tokyo, Japan) and connected to an osmotic pump (volume, 200 μL) (model 2002, Alza Co., Palo Alto, CA) was then inserted. The pump, which was placed under the skin on the animal's back, ran continuously, injecting 3.6 μg streptomycin at 0.5 μL/h into the inner ear for 24 hours. After the wound was washed with saline, a small amount of piperacillin sodium was introduced. After wound closure, 40 mg/kg piperacillin sodium was injected intramuscularly, and oxytetracycline HCl ointment was applied to the wound. During the operation and for 24 hours after the operation, each animal was kept warm with an electric blanket.

The 20 animals were divided into three groups. Eight animals received 3 mg/kg edaravone intraperitoneally (Mitsubishi Pharma Co., Tokyo, Japan). The first intraperitoneal administration was performed just after pump implantation surgery, and the administration was performed once daily for 7 days (systemic administration group). Six animals received a 2 × 2-mm piece of Gelfoam (Pfizer Inc., New York, NY) soaked in edaravone (at a density of 3 mg/mL) that was placed on the round window just before wound closure (topical administration group). The edaravone, which was dissolved in 1 N NaOH with pH adjusted to 7.0 with 1 N HCl, was prepared just before the operation. The remaining six animals received 1 mL/kg saline intraperitoneally once daily for 7 days after the operation (control group).

At 6, 9, 12, 18,24, 36, 48, and 60 hours after the operation, we observed yaw head tilt and spontaneous nystagmus. We measured the degree of yaw head tilt according to the method Curthoys et al.[10] reported previously. We measured the frequency of spontaneous nystagmus in each animal in the light as the number of quick-phase beats per minute after surgery. The measurement was obtained three times at each observation time, and the mean nystagmus beat number was calculated for each time. Data are shown as mean ± SD for each group. The statistical significance of differences between the three groups was assessed by ANOVA with Stat View 5.0 (SAS Institute, Inc., Cary, NC). A P of less than .05 was considered significant.

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The experimental protocol was approved by the Committee for Ethics in Animal Experiments of Yamaguchi University School of Medicine (Yamaguchi, Japan). Experiments were carried out under the Guidelines for Animal Experiments of the Yamaguchi University School of Medicine and the Law and Notification of the Government of Japan.

RESULTS

No statistical differences in yaw head tilt were found between groups after the operation (Fig. 1). Statistical differences in spontaneous nystagmus beats were found after the operation at 12, 18, and 24 hours. Respectively, the numbers of beats per minute were 42.222 ± 29.079, 30.0 ± 27.752, and 13.5 ± 24.607 in the topical administration group, 89.625 ± 25.773, 72.125 ± 19.766, and 47.875 ± 21.310 in the systemic administration group, and 92.833 ± 32.842, 66.5 ± 26.861, and 49.167 ± 27.867 in the control group (at 12 h, $F(2,17) = 5.973$ [$P = .011$]; at 18 h, $F(2,17) = 5.614$ [$P = .013$]; and at 24 h, $F(2,17) = 4.322$ [$P = .03$]) (Fig. 2). Both yaw head tilt and spontaneous nystagmus disappeared in all three groups within 60 hours after the operation.

DISCUSSION

Aminoglycosides, including streptomycin, are capable of stimulating the formation of reactive oxygen species, and evidence is accumulating that generation of reactive oxygen species is an important factor in inner ear damage.[1-6,11,12] It has been shown that aminoglycoside itself is not cytotoxic; rather, the toxicity is due to a metabolic by-product, an aminoglycoside-iron complex. Once the complex is formed, it can continuously catalyze the formation of superoxide radicals from molecular oxygen and an electron donor. The H_2O_2 formed by production of superoxide radicals suggests that further reactive oxygen species are produced in the reaction cascade.[3,11] Subsequent to H_2O_2 generation, hydroxyl radicals may be formed in a Fenton-type reaction.[13,14] The hydroxyl radical is an extremely powerful oxidant that is indiscriminately reactive with almost all biological substances.[12] Therefore, it is suspected that the tissue damage induced by reactive

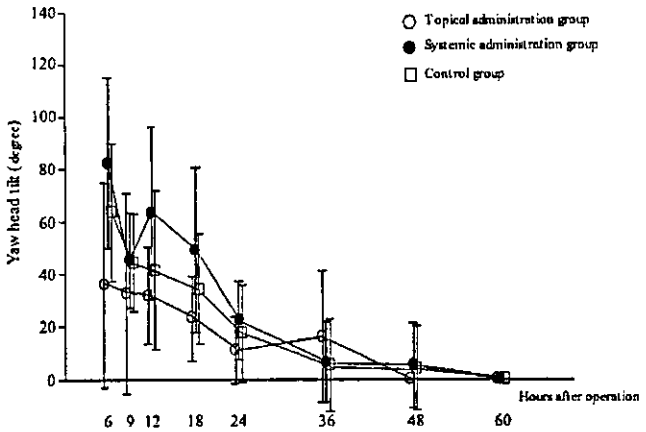

Fig. 1. Yaw head tilt after surgery in the three study groups. No statistical differences were found in values between groups. Error bar, ±1 SD.

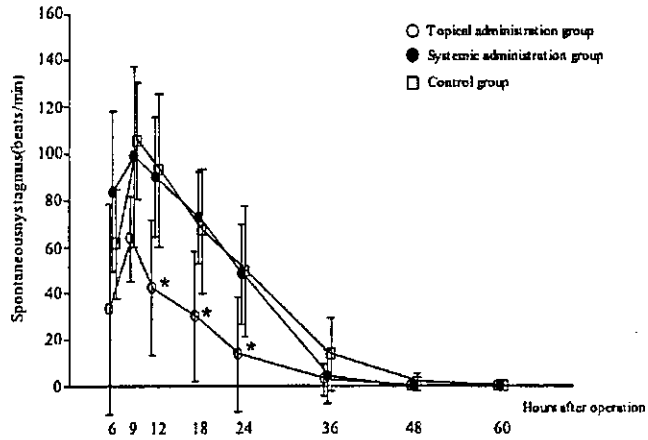

Fig. 2. Spontaneous nystagmus beats per minute after surgery in the three study groups. Statistical differences were found between three groups at 12, 18, and 24 hours after the operation. Error bar, ±1 SD; *$P < .05$.

oxygen species is due mainly to hydroxyl radicals. Edaravone is capable of trapping a variety of free radical species, and it reacts with hydroxyl or peroxyl radicals to form particular oxidized compounds.[7,8] Inner ear damage caused by aminoglycoside is ameliorated by the presence of free radical scavengers.[1-6,11]

In our previous study,[15-18] vestibular and auditory function after the implantation of an osmotic pump and the infusion of saline was within the preoperative range. In the present study, we observed changes in static symptoms using spontaneous nystagmus beats and degrees of yaw head tilt as markers of vestibular function. Spontaneous nystagmus beats in the systemic administration group were equal in number to those in the control group after the operation. The effect of systemic administration of edaravone against streptomycin-induced vestibulotoxicity was established previously on the basis of dynamic symptoms marked by horizontal vestibulo-ocular reflex gains at 3 and 7 days after the operation.[1] In the present study, the number of spontaneous nystagmus beats was significantly less after the operation in the topical administration group than in the control and the systemic administration groups. Thus, it appears that the free radical scavenging action of edaravone may contribute to its protective effect against streptomycin-induced vestibulotoxicity and that topical administration of edaravone may be more effective than systemic administration against streptomycin-induced vestibulotoxicity. Systemic administration of edaravone may reduce the adverse effects of treatment because of the reduced dose.

With respect to static symptoms, significant differences were not found between the three groups. It is possible that yaw head tilt served only as a rough indicator and that it is not suitable for evaluating the effect of edaravone against streptomycin-induced vestibulotoxicity.

CONCLUSION

Edaravone may contribute a protective effect against streptomycin-induced vestibulotoxicity and topical admin-

istration better suppress streptomycin-induced vestibulotoxicity than systemic administration.

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Peripheral vestibular disorder induced by (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA)

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Abstract

An intracochlear infusion of (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) was done in guinea pigs with a syringe pump and peripheral vestibular disorder was induced. Spontaneous nystagmus toward the intact side reached a peak 9 h after the infusion and disappeared within 18 h. As a control, artificial perilymph was infused and animals had no nystagmus. The nystagmus frequency was decreased by simultaneous infusion of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in a dose-dependent manner. In the animals treated with AMPA or AMPA + CNQX, caloric tests performed 1 week after treatment revealed a partial dysfunction of vestibular periphery. These results indicate that the nystagmus observed is induced by AMPA via AMPA receptors and that AMPA-induced vestibular disorder is partial. This animal model may be a candidate for pharmacological study of inner ear diseases induced by glutamate excitotoxicity.

Keywords: AMPA; CNQX; Vestibular periphery; Spontaneous nystagmus; Caloric test

Development of drug delivery systems for the inner ear has given a new turn to the treatment of inner ear diseases [7,16]. Topical application therapy for inner ear diseases is now of great interest. The benefits of topical application therapy are that it is possible to administer drugs at a high effective dose without systemic side effects, and that even drugs which cannot pass through the blood-inner ear barrier can be applied. A new inner ear pathologic model is needed that enables evaluation of the effects of agents, which are candidates for topical application therapy.

Unilateral labyrinthectomy is a popular method for study of the vestibular system [2]. Animals with unilateral labyrinthectomy have a dead labyrinth, and their peripheral vestibular function does not recover. A primary response of the vestibular system to unilateral labyrinthectomy is that of vestibular compensation [1,10]. Clinically, most people suffering from vertigo have partial vestibular dysfunction rather than a dead labyrinth. The labyrinthectomy model does not always reflect the clinical pathology of vertigo.

The excitatory amino acid in the peripheral vestibular system is thought to be glutamate [17], and (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid (AMPA) receptors are of primary importance in neuronal transmission in the vestibular periphery [3,4,11,17]. An experimental AMPA application induced a transient, reversible disorder in inner ear hair cells and caused ischemia-like histological changes in the cochlea [13]. It is well known that glutamate causes excitotoxicity in neuronal cells in brain ischemia [9]. Furthermore, evidence for a correlation between glutamate and reactive oxygen species in the inner ear is accumulating [8,19,20]. We hypothesized that an animal model with an AMPA-induced vestibular disorder would be useful for the pharmaceutical study of drugs such as free radical scavengers.

The aim of this study was to make an animal model by intracochlear administration of AMPA that has partial and reversible peripheral vestibular disorder.

We used 49 male Hartley guinea pigs with normal Preyer reflexes and tympanic membranes in this study. The study protocol was reviewed by the Committee for Ethics in Animal Experiments of Yamaguchi University School of Medicine. The study was carried out in accordance with the Guidelines

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for Animal Experiments of the Yamaguchi University School of Medicine and the Law (No. 105) and Notification No. 6 of the Government of Japan.

We administered xylazine (16 mg/kg, ip)-ketamine (16 mg/kg, ip) anesthesia. Then 1.5 ml lidocaine HCl was injected into the right postauricular region for local anesthesia. The mastoid bulla was opened by postauricular incision to allow visualization of the round window with the surgical microscope. A tiny hole was made with a perforating burr (0.5 mm in diameter) adjacent to the round window. A polyethylene catheter filled with agent and connected to a syringe filled with the same agent was inserted into the hole. The syringe was set on a syringe pump (SP-70, Nipro, Osaka, Japan) and infusion was done at 0.6 ml/h for 5 min. After drug infusion, the hole was covered with a small piece of muscle and sealed with fibrin glue (Bolheal, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan). After the wound was washed with saline, a small amount of piperacillin sodium (PIPC) was introduced to prevent infection. After closure, PIPC (40 mg/kg) was injected intramuscularly, and oxytetracycline HCl ointment was applied to the wound. During the operation, the animal's body temperature was maintained at 37 °C. Each animal was kept warm with an electric blanket for 3 h following the operation.

Fifteen animals were treated with 10 mM AMPA (Sigma–Aldrich Co., S. Louis, MO, USA) (AMPA group). Seven animals were treated with 10 mM AMPA + 10 mM 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt (CNQX; Sigma–Aldrich Co., a potent, competitive AMPA receptor antagonist) (AMPA + 10 mM CNQX group). Ten animals were treated with 10 mM AMPA + 20 mM CNQX (AMPA + 20 mM CNQX group). Six animals were treated with 20 mM CNQX only (CNQX group). Eleven animals were treated with artificial perilymph (113.5 mM NaCl, 5.4 mM KCl, 2.0 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10.0 mM glucose, 10.0 mM *N*-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid) as a control group (perilymph group). AMPA and CNQX were dissolved in the artificial perilymph, and each solution was prepared.

After surgery, we determined how many animals had spontaneous nystagmus in each group. Fisher's exact method or a χ^2-test was used to evaluate differences between groups with significance set at $p < 0.05$. We measured the frequency of spontaneous nystagmus in each animal in 80 cm^2 free field in the light as the number of quick phase beats per minute at 6, 9, 12, 15, and 18 h after surgery. The measurement was done three times in each observation time, and the mean nystagmus beat number was calculated in each time. In each group, the mean nystagmus beat number was calculated, and differences between groups were evaluated by one-way ANOVA with significance set at $p < 0.05$. We performed caloric tests on the animals of each group 1 week after surgery. Each animal was placed in a transparent box designed to fix and hold the head and body without covering the nose or mouth. The box was positioned in a plane tilted in the 50° head-up condition. The caloric test was done by irrigation of the external auditory meatus with 5 ml of ice water for 10 s in the dark. Nystagmus was recorded on videotape with an infrared charge-coupled device camera, and the caloric response time was measured. We calculated the ratio of the treated side response time (right) to the untreated side response time (left). Differences between groups were evaluated by one-way ANOVA with significance set at $p < 0.05$. After physiological evaluation, each animal was first deeply anesthetized with pentobarbital. Transperilymphatic perfusion through the cochlear hole was done with 4% paraformaldehyde in 0.1 M phosphate buffer, and then the animal was decapitated immediately. After the temporal bone was dissected, the ampulla of the lateral semicircular canal was excised and soaked in the same fixative for 1 h. The specimen was then decalcified in 10% ethylenediamine-tetraacetic acid for 1 h, rinsed in 0.01 M phosphate buffer saline, dehydrated in a series of ethanol steps, and embedded in water-soluble resin (JB-4, Polysciences, Inc., Warrington, PA, USA). Sections were cut 2 μm-thick, stained with hematoxylin and eosin, and were visualized with a 100-x oil immersion objective with a microscope (Nikon Corp. Tokyo, Japan).

The direction of nystagmus after surgery was all toward the intact side. Thirteen of 15 animals in the AMPA group had spontaneous nystagmus after surgery. In the perilymph group, no spontaneous nystagmus was observed. The difference in nystagmus frequency between these two groups was statistically significant ($p < 0.0001$, χ^2-test). In the AMPA + 10 mM CNQX group, 6 of 7 animals had spontaneous nystagmus. In the AMPA + 20 mM CNQX group, 2 of 10 animals had spontaneous nystagmus. A statistically significant difference in nystagmus frequency was found between these two groups ($p = 0.0152$, Fisher's exact method) (Table 1). The spontaneous nystagmus frequency in the AMPA group reached a peak 9 h after surgery, it decreased gradually thereafter, and it disappeared within 18 h. In the animals of the CNQX group, no spontaneous nystagmus was observed. Simultaneous administration of AMPA and CNQX resulted in suppression of the nystagmus frequency in a CNQX dose-dependent manner. Significant differences in nystagmus frequency were found at 9 and 12 h in the AMPA + 20 mM CNQX group, and at 12 h in the AMPA + 10 mM CNQX group (Fig. 1).

Table 1
Spontaneous nystagmus in each treatment group after surgery

Group	Nystagmus		
	(+)	(−)	
AMPA ($n = 15$)	13	2	$p < 0.0001$
Perilymph ($n = 11$)	0	11	
20 mM CNQX ($n = 6$)	0	6	
AMPA + 10 mM CNQX ($n = 7$)	6	1	$p = 0.0152$
AMPA + 20 mM CNQX ($n = 10$)	2	8	

Number of animals with (+) and without (−) spontaneous nystagumus are listed for each treatment group. Significant group differences are noted with brackets. AMPA: (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid; CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione.

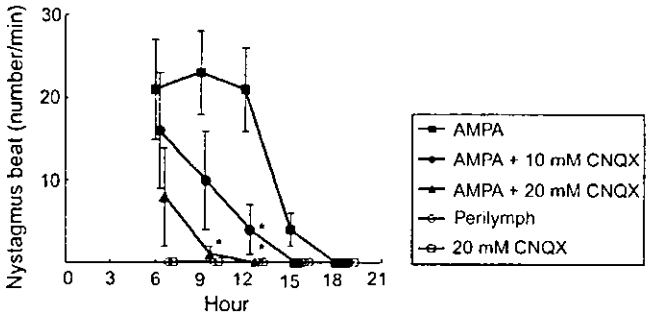

Fig. 1. Spontaneous nystagmus beats per minute after surgery. Group mean ± S.E.M. are plotted. Animals in the perilymph or 20 mM CNQX group have no nystagmus. Among the other three groups, significant differences are found 9 and 12 h after surgery in the AMPA + 20 mM CNQX group and 12 h after surgery in the AMPA + 10 mM CNQX group ($^*p = 0.0245$). AMPA: (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid. CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione.

In each animal in the CNQX and perilymph groups, the caloric response time on the treated side was almost equal to that on the untreated side. The mean time ratios were 1.161 ± 0.071 and 1.104 ± 0.062, respectively. The mean time ratios in the AMPA and the two AMPA + CNQX groups were less than those of the CNQX and perilymph groups 1 week after surgery (Fig. 2). These differences were statistically significant. There were no statistically significant differences between the AMPA, AMPA + 10 mM CNQX, and AMPA + 20 mM CNQX groups.

Histopathological examinations were done with a light microscope 1 week after surgery. In the AMPA + 10 mM CNQX (Fig. 3A), AMPA + 20 mM CNQX (Fig. 3B), and AMPA (Fig. 3C) groups, hyperlucent lesions were observed around hair cells. In the AMPA and AMPA + 20 mM CNQX groups, the nuclei of hair cells showed clear arrangement. In the CNQX (data not shown) and perilymph (Fig. 3D) groups, sensory epithelia had a normal appearance.

It has been confirmed that our surgical procedure, especially the cochleotomy, does not cause inner ear damage

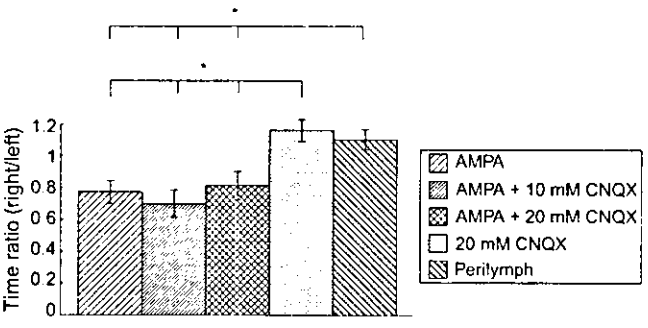

Fig. 2. Time ratio of each group 1 week after surgery. The time ratio is the ratio of the caloric response time on the treated side (right) to the caloric response time on the untreated side (left). Group mean ± S.E.M. are plotted. The time ratios of the AMPA group, the AMPA + 10 mM CNQX group, and the AMPA + 20 mM CNQX group are significantly smaller than those of the CNQX and perilymph groups ($^*p = 0.0003$). AMPA: (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid. CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione.

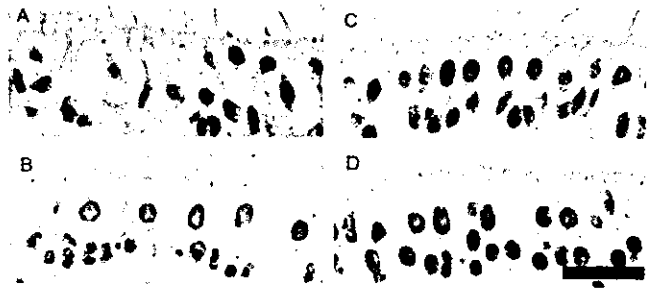

Fig. 3. Light micrographs of histopathological specimens of ampullae of the lateral semicircular canals on the treated side. (A) 1 week after AMPA + 10 mM CNQX infusion; (B) 1 week after AMPA + 20 mM CNQX infusion; (C) 1 week after AMPA infusion; (D) 1 week after artificial perilymph infusion. Bar indicates 20 μm. AMPA: (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid; CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione.

[14,18]. Spontaneous nystagmus was observed in the AMPA group and not in the perilymph group, which means that the observed nystagmus was induced by AMPA. The nystagmus frequencies observed were lower than those observed in chemically or surgically labyrinthectomized animals [15]. Therefore, the AMPA-induced vestibular disorder in the present study was not as severe as that in the labyrinthectomized animals. Hstopathological data was consistent with this fact. In our preliminary study, animals with AMPA infusion under the same condition showed remarkable swelling of the nerve chalice in hair cells of the ampullae of the lateral semicircular canals 40 min after the infusion. In this study, 1 week after surgery, swelling of the nerve chalice remained but decreased, indicating that cellular damages induced by AMPA is reversible. It has been reported that application of CNQX, a potent, competitive antagonist of the AMPA receptor, to the unilateral medial vestibular nucleus induced no effect on eye movement or posture in guinea pigs [5]. In this study, intracochlear infusion of CNQX caused no spontaneous nystagmus. The AMPA-induced nystagmus was suppressed by the simultaneous infusion of CNQX in a dose-dependent manner, which indicates that the infusion of AMPA may induce the nystagmus mainly via AMPA receptors. When excessive amounts of AMPA bind to the postsynaptic AMPA receptors in hair cells, postsynaptic dendrites swelling occur due to the entry of excessive amounts of sodium and chloride ions, together with water, followed by overwhelming influx of Ca^{2+} ions into the postsynaptic dendrites [8,9]. These may lead to an imbalance of peripheral vestibular input and cause nystagmus. But after the AMPA injury, synaptic repair may occur, which may lead to the functional recovery [13]. It is well known that glutamate excitotoxicity occurs in neurons during ischemia [6,9]. Using experimental glutamate excitotoxicity, it may be possible to make a new model, which represents an ischemia-like disorder in the vestibular periphery. This model may be useful to evaluate efficacy in topical application of drugs on peripheral vestibular functional recovery. But central compensation always accompanies functional recovery procedure. Prudent evaluation is needed.

In our preliminary work, we confirmed that ink infused through a cochlear hole under the same conditions as we used in this work (0.6 ml/h for 5 min) spread neither to the contralateral inner ear nor to the subarachnoidal space. Higher infusion rates induced ink spreading to those regions. AMPA concentration is also important. The concentration we used in this study was relatively high compared with previous report [13]. But low concentrations cause no obvious static symptoms such as spontaneous nystagmus. It is essential that the AMPA concentration should be 10 mM and the infusion condition at 0.6 ml/h for 5 min.

We administered AMPA and CNQX simultaneously to examine AMPA-induced vestibular pathology, and we have found an interesting fact. Simultaneous CNQX infusion suppressed spontaneous nystagmus in a dose-dependent manner, whereas no statistically significant difference was found in caloric response times between the AMPA, AMPA + 10 mM CNQX, and AMPA + 20 mM CNQX groups 1 week after surgery. Furthermore, histopathological findings in the AMPA group were similar to those in the AMPA + 20 mM CNQX group. It is certain that CNQX infusion contributed to the decrease of spontaneous nystagmus in the acute phase. However, CNQX infusion may not lead to sufficient functional or structural recovery 1 week after surgery. The reason of the delayed-structural recovery in the AMPA + 10 mM CNQX group is unclear. Efficacy of an experimental inner ear therapy with the glutamate antagonists memantine and caroverine was reported [12]. Our data indicate that further investigation is needed to evaluate CNQX efficacy as a candidate for the topical application therapy for inner ear disease.

In conclusion, our investigation provides direct evidence that intracochlear AMPA infusion induces via AMPA receptors partial peripheral vestibular disorder and that this animal model may be a candidate for pharmacological study of inner ear diseases induced by glutamate excitotoxicity.

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Inner Ear Changes With Intracochlear Gentamicin Administration in Guinea Pigs

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Objectives/Hypothesis: **Transtympanic administration of gentamicin is reported to be a useful treatment for vertigo in such conditions as Meniere's disease, and determining appropriate clinical dosage of gentamicin is difficult. The authors examined the relation between gentamicin dosages and inner ear function in guinea pigs.** ***Study Design:*** **This study is a basic science project designed to examine cochlear and vestibular function in animal models.** ***Methods:*** **Various concentrations of gentamicin solution were infused into the right inner ear of guinea pigs by osmotic pumps. Caloric nystagmus as a marker of vestibular function and the change in auditory brainstem response (ABR) threshold as a marker of cochlear function were observed.** ***Results:*** **After 14 days of treatment, high gentamicin concentrations of 40 mg/mL caused canal paralysis and a rapid shift in ABR threshold. Animals exposed to low gentamicin concentrations of 4 mg/mL showed no obvious change in either vestibular or cochlear function. Animals exposed to moderate gentamicin concentrations of 12 mg/mL showed a moderate shift in ABR threshold and caloric malfunction. Histopathological examination revealed that after 14 days of treatment with 40 mg/mL gentamicin, severe cytoplasmic damage occurred in both vestibular and cochlear end organs. In animals treated with 12 mg/mL gentamicin, hair cells remained in the cochlear third turn and ampulla of the lateral semicircular canal.** ***Conclusion:*** **The authors established an animal model that showed the moderate damage of inner ear with moderate-dose gentamicin. The study results indicated that the appropriate administration of gentamicin could establish a stable effect on the inner ear. It may be important to select the protocol that delivers a stable dosage of gentamicin to treat patients with Meniere's disease safely and effectively.** ***Key Words:*** **Gentamicin, ototoxicity, vertigo, auditory brainstem response, caloric response.**

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INTRODUCTION

Ototoxicity can be a major complication in patients treated with aminoglycosides. Park and Cohen[1] reported that, whereas kanamycin and amikacin are toxic mainly to the cochlea, streptomycin and gentamicin are toxic mainly to the vestibular organ. Nevertheless, aminoglycosides continue to be used to treat vertigo. In 1948, Fower[2] first used the vestibulotoxic effects of aminoglycosides to treat patients with vertigo, and in 1956, Schuknecht[3] administrated intratympanic aminoglycosides in patients with unilateral Meniere's disease. Recently, several authors have reported gentamicin administration into the tympanic space of patients with Meniere's disease.[4–8] A variety of protocols (a variety of agent concentrations, administration frequencies, and dosages) is used to treat Meniere's disease by transtympanic gentamicin infusion. The effects and side effects of gentamicin vary widely, and the best dosage range used to control vertigo remains unknown. If the precise relation between dosage and effect can be found, treatment with gentamicin may become both safe and efficacious. Thus, the aim of our study was to evaluate the effects of gentamicin on the inner ear using an exact dosage infusion through osmotic pump.

MATERIALS AND METHODS

We used 22 Hartley guinea pigs (weight, 400–500 g) with normal Preyer reflexes and normal tympanic membranes. The animals were divided into three groups, and an osmotic pump was implanted in the right ear of each animal. Sixteen animals initially received saline in the cochlea through the osmotic pump. Six animals were used to standardize the caloric response. Auditory brainstem response (ABR) thresholds were assessed 7 days after pump implantation, and only animals with no more than a 10-dB difference between the right ear (operated side) and the left ear (nonoperated side) were used in the study. After the first ABR examination, the pumps were replaced with new osmotic pumps filled with gentamicin solution (Schering-Plough KK, Kenilworth, NJ) as follows: for group I (n = 6), 40 mg/mL; for group II (n = 5), 12 mg/mL; and for group III (n = 5), 4 mg/mL. The 40-mg/mL gentamicin solution was mixed with saline to produce the dilute gentamicin solutions for groups II and III. The day of pump implantation was taken as day 0. ABR thresholds were measured on days 4, 7, and 14 after pump exchange (APE), and caloric testing was performed on APE day 14. All animals were killed after the last ABR examination, and their temporal bones were removed and fixed in 4% paraformaldehyde.

Osmotic Pump Implantation

We used an Alzet miniosmotic pump (model 2002, Direct Co., Cupertino, CA) and a flow rate of 0.5 μL/h. The pump was connected to a 10-cm polyethylene catheter (inner diameter, 0.28 mm; outer diameter, 0.61 mm), and both the pump and catheter were filled with saline. After induction of anesthesia with 28 mg/kg intraperitoneal pentobarbital and administration of local anesthetic (1.5 mL 1% lidocaine HCl), the temporal bone was exposed through a postauricular incision. The mastoid bulla was opened with a 4-mm diamond burr to create a 4-mm-round viewing window. A smaller second hole was made with a perforating burr at a distance of 1 mm from the viewing window. The catheter tip was inserted into the hole, and saline was infused into the perilymphatic space of the cochlea. The polyethylene catheter was fixed to the mastoid bulla with dental cement (GC Fuji Co., Tokyo, Japan). A small amount of piperacillin sodium was introduced to prevent infection, and after wound closure, 40 mg/kg piperacillin sodium was also injected intramuscularly. Body temperature was maintained at 37°C during and for 24 hours after the operation; each animal was kept warm with an electric blanket.

Hearing Assessment

After the operation, we determined the ABR threshold of each animal under anesthesia induced by 32 mg/kg intraperitoneal pentobarbital. Responses were recorded between subcutaneous stainless steel electrodes at the vertex (positive) and the antinion (negative); the animal's lower back served as the ground. The sound stimuli consisted of 8-millisecond tone bursts with a rise-fall time of 2 milliseconds at a frequency of 8000 Hz. Stimuli were presented through a 10-cm tube connecting an earphone to the external auditory canal. The stimulus intensity was evaluated with a sound level meter (NA-60, Rion, Tokyo, Japan) adjacent to the tip of tube. Responses to 500 stimuli were recorded with a signal processor (Synax 1100, NEC Co., Tokyo, Japan). Auditory brainstem response thresholds were defined as the lowest stimulus intensity that produced reliable peak III or V ABR waveforms. Data were analyzed with Stat View, version 4.5 J, for Macintosh (Abacus Concepts, Berkley, CA). The ABR threshold values in each group were compared with the Fisher protected least significant difference (PLSD) test, and $P < .05$ was accepted as statistically significant.

Assessment of Vestibular Function

We performed the caloric test 7 days after pump implantation and just before the last ABR examination (on APE day 14). Each animal was placed in a transparent box designed to hold its head and body without covering the nose or mouth. The box was fixed to a metal board and tilted at 50° with the animal's head in an upright position. The caloric test was performed by irrigating the animal's right ear with 5 mL of ice water for 10 seconds. The duration of the nystagmus response was then recorded on videotape in the darkened room with an infrared charge-coupled device camera.

Histological Examination

After the last ABR examination, each animal was killed by means of an overdose of pentobarbital anesthesia, and the right-side temporal bones were removed from each animal. Each cochlea was opened at the apex, base, and oval window and perfused gently for 30 minutes with a fixative of 4% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4), then rinsed in 0.01 mol/L phosphate-buffered saline (PBS) (pH 7.4). The organ of Corti was removed from the temporal bone preparation. The specimens were permeabilized with 0.3% Triton X-100 for 10 minutes, then incubated with fluorescein isothiocyanate-conjugated phalloidin (1:50 dilution) (Sigma Chemical Company, St. Louis, MO) at room temperature for 1 hour. After being rinsed in PBS, specimens were mounted with use of Slow Fade Light Antifade Kit (Molecular Probes, Inc., Eugene, OR), and surface structures were observed under a fluorescence microscope.

After removal of the cochlea, the temporal bones were decalcified, dehydrated, and embedded in water-soluble resin (JB-4, Polysciences, Inc., Warrington, PA). The samples were sliced at a thickness of 2 μm and stained with H&E, and the semicircular canals were observed under a light microscope.

The experimental protocol was reviewed by the Committee for Ethics on Animal Experiments of Yamaguchi University School of Medicine (Yamaguchi, Japan). Experiments were carried out in accordance with the university research guidelines, Japanese Federal Law 105, and Notification 6 of the Japanese government.

RESULTS

Auditory Brainstem Response Thresholds

Changes in ABR thresholds are shown according to group in Figure 1. The ABR thresholds were elevated in all groups after the gentamicin infusion. The ABR thresholds in group I were significantly increased on APE day 4 compared with those of other groups. All animals showed an ABR response. The mean ABR threshold value was increased to 56.7 dB on APE day 14 in group I. Changes in ABR threshold in group II were distinctive on APE day 7. Changes in ABR thresholds were smallest in group III and increased to only approximately 10 dB by APE day 14.

Caloric Responses

The duration of nystagmus in the nonoperated guinea pigs was 55 ± 10 seconds. Therefore, we used these animals (n = 6) in the first caloric test. The caloric responses of all animals are shown in Figure 2. On APE day 14, no group I animals showed a normal caloric response, but in group II, 5 animals showed normal caloric nystagmus. All group III animals showed normal caloric nystagmus.

Histological Changes

Typical specimens on APE day 14 are shown in Figures 3 and 4. In group I, the organ of Corti showed com-

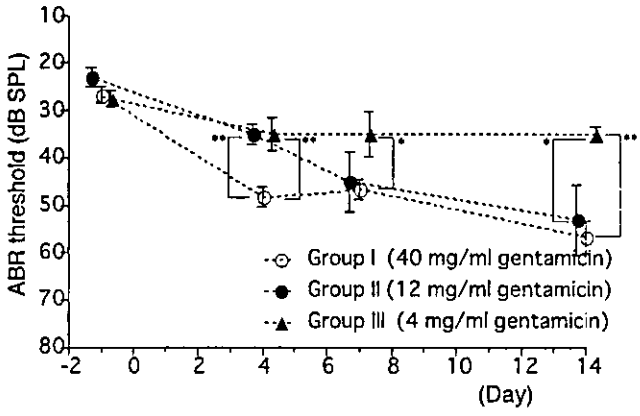

Fig. 1. Changes in auditory brainstem response (ABR) thresholds (in decibels sound pressure level [dB SPL]). The ABR threshold value increased more quickly in group I than in group II or III, and the ABR threshold value was significantly greater than in groups II and III on days 4, 7, and 14. The ABR threshold in group III was not significantly increased. Error bar = ±1 SD; $^{**}P < .01$; $^{*}P < .05$.

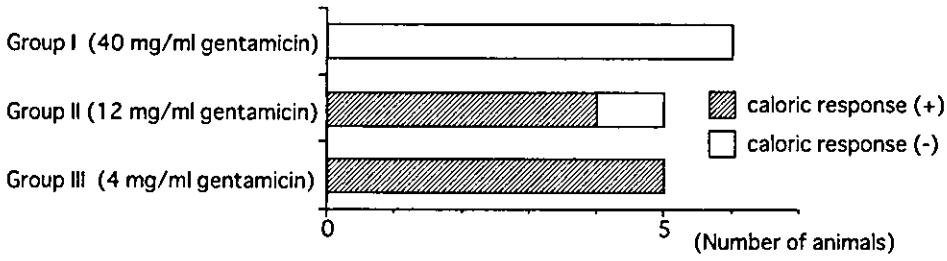

Fig. 2. Positive (+) and negative (−) caloric test responses 14 days after the administration of gentamicin. No group I animals showed normal caloric test responses, whereas all group III animals showed normal responses.

plete loss of outer hair cells in all three rows in the second and third turns. Group II animals showed severe loss of outer hair cells in the second turn, and residual outer hair cells were observed in the third turn (Fig. 3). In group III, almost normal outer hair cells were visible in the second and third turns of the cochlea (data not shown). Inner hair cells were not damaged in any group.

In group I, the sections of ampullae in the lateral semicircular canal showed severe cytoplasmic damage and decreases in the thickness of the sensory epithelium. In group II, the thickness of the sensory epithelium was normal and sensory cells were seen (Fig. 4). No significant change was observed in vestibular end organs of animals in group III (data not shown).

DISCUSSION

There have been many reports describing the ototoxicity of aminoglycosides. Conlee et al.[9] reported that cochlear function evaluated by cochlear microphonic thresholds in albino and pigmented guinea pigs deteriorated after systemic administration of 100 mg/kg gentamicin, but vestibular function was not assessed. The rate of hair cell damage increased in a dose-dependent manner. With respect to topical administration of aminoglycosides, Conlon and Smith[10] reported that continuous infusion of 5% neomycin solution to the intracochlear space caused an elevation in compound action potential thresholds and that topical treatment with aminoglycoside caused cochlear dysfunction. Polgar et al.[11] reported morphological changes in the gerbil posterior crista ampullaris following transtympanic administration of gentamicin and streptomycin. They injected 50 mg/mL gentamicin or 350 mg/mL streptomycin mixed with gelfoam® powder. Vestibular hair cells were damaged by both gentamicin and streptomycin. Wanamaker et al.[12] showed dose-dependent cochlear and vestibular damage by intratympanic gentamicin injection, and the amount of damage was consistent in both cochlear and vestibular hair cells from the same animal. However, in these reports, the exact amount of aminoglycosides infused into the inner ear was unclear because the systemically administered agent can be eliminated through the excretory system and the agent infused into the tympanic space can flow out from the eustachian tube.

Therefore, we developed a method of cochlear cannulation that enabled us to infuse drugs into the animal's inner ear directly and precisely, with minimal invasion. Our animal model did not show an ABR threshold shift after 14 days of saline infusion,[13] nor did it show vestibular impairment.[14] We confirmed that thresholds were not increased 7 days after pump implantation and before the start of drug infusion following the exchange of osmotic pumps. Animals showing a difference in ABR threshold between the right ear (operated side) and the left ear (nonoperated side) were removed from the study. Thus, the effect of gentamicin on the inner ear could be clearly observed.

The ABR threshold values gradually increased after infusion of gentamicin; these changes were dose dependent. Group I animals received high concentrations of gentamicin and their thresholds increased more rapidly than those of group III animals, which received low concentrations of gentamicin. The changes in vestibular function showed much the same pattern. The caloric responses in group I animals disappeared after 14 days, whereas those in group III animals were almost normal.

We examined morphological changes in the surface structures of the cochlea and the sections of the ampulla of the lateral semicircular canal. The group III specimens ap-

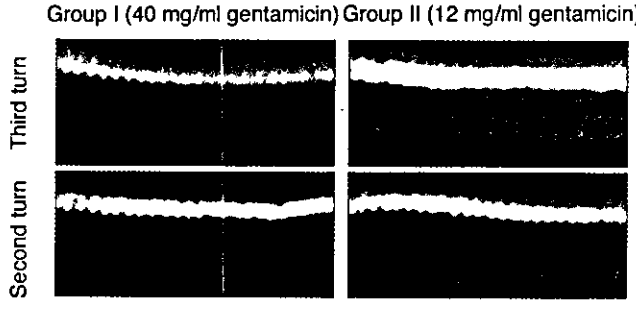

Fig. 3. Surface structure of the organ of Corti. In group I (**A** and **C**), complete loss of outer hair cells was observed in the second and third turns, whereas in group II (**B** and **D**), residual outer hair cells were seen in the third turn. No inner hair cells were damaged in any group. Bar = 20 μm.

Fig. 4. Hematoxylin and eosin (H&E)–stained sections of ampulla of the lateral semicircular canal. In group I (**A**), severe cytoplasmic damages and a decrease in the thickness of the sensory epithelium were seen. In group II (**B**), sensory cells were seen. Bar = 10 μm.

peared normal. The cochlear surface structures and ampullae were damaged more severely in group I than in group II animals. This showed that the ototoxicity of gentamicin infused into the cochlea occurred in a dose-dependent manner in both the cochlea and semicircular canal.

Aminoglycosides have been used to treat vertigo for many years. The aim of these treatments is the loss of vestibular function. Transtympanic administration of gentamicin has been reported recently to be a useful treatment for vertigo in Meniere's disease.[4-8] The aim of the recent treatments is the restoration or maintenance of vestibular function. Harner et al.[4] reported that the dark cells in the labyrinth are most vulnerable to gentamicin and that their destruction probably reduces endolymph production. The optimal concentration of gentamicin should damage only the dark cells, not the sensory hair cells, and should not deactivate cochlear and vestibular function, but it is difficult to determine the appropriate clinical concentration of gentamicin. Curative effects of gentamicin were reported, along with their side effects. Blackley[15] reported the relation between total dose and the effects of gentamicin to be irregular and that many different protocols in the transtympanic gentamicin therapy have been used. The concentration of administrated gentamicin varied from 25 to 40 mg/mL, the frequencies of administration varied from 4 times daily to 6 times weekly, and total dose varied from 10 to 720 mg. In addition, the rate of hearing loss as a side effect of gentamicin therapy was irregular, and it was not the rule that high concentrations caused high rates of hearing loss. These variations in side effects could be the result of the various sensitivities toward gentamicin among individuals and various absorptions of gentamicin during transtympanic administration. Our results show that a high concentration of gentamicin could pose a high risk of hearing loss in guinea pigs and suggest that stable administration could stabilize ototoxicity.

Thomsen et al.[16] reported a new delivery system (microcatheter in the round window niche and electronic micropump) for infusion of gentamicin into the inner ear in patients with Meniere's disease. In their experiments, continuous infusion of gentamicin was effective in controlling vertigo, and they concluded that a new delivery system might be able to reduce the risk of hearing loss after gentamicin administration because such a delivery system would allow the inner ear to be exposed to a consistent amount of gentamicin. Continuous exposure to gentamicin can control the total dose of gentamicin and reduce individual differences because the effects of the drug appear gradually. A new delivery system may be better than transtympanic administration in that administration of gentamicin could be canceled when side effects are observed. We established an animal model in which gentamicin damaged the cochlea and vestibular organ equally. However, we could not find a concentration of gentamicin that yielded only vestibular or only cochlear dysfunction. This means that hearing loss cannot be prevented by control of the total dose of gentamicin. In addition, it was known that the ototoxic effects of gentamicin appeared after a delay of days.[17] Therefore, the safe treatment for Meniere's disease may be slow, controlled administration of gentamicin into the inner ear and immediate cancellation when side effects occur.

CONCLUSION

We examined the relation between gentamicin concentrations and effects on inner ear function. Auditory brainstem response thresholds were increased in a dose-dependent manner. Continuous drug administration in animal model could establish a stable effect on the inner ear. Our results suggest the possibility that gentamicin at an appropriate concentration may act as an effective agent in the treatment of vertigo.

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Prediction of Progression from Atypical to Definite Ménière's Disease using Electrocochleography and Glycerol and Furosemide Tests

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Kimura H, Aso S, Watanabe Y. *Prediction of progression from atypical to definite Ménière's disease using electrocochleography and glycerol and furosemide tests.* Acta Otolaryngol 2003; 123: 388–395.

Objective—To investigate whether electrocochleography (ECochG) and glycerol and furosemide tests could predict progression from atypical to definite Ménière's disease (MD).

Material and methods—ECochG and glycerol and furosemide tests were performed in 1569 patients with various cochleovestibular diseases, including definite MD, atypical MD, syphilitic labyrinthitis, delayed endolymphatic hydrops, sudden hearing loss, cochleovestibulopathy and sensorineural hearing loss. Patients with atypical MD were divided into five categories based on their symptoms.

Results—A total of 115/118 patients (97%) with definite MD who underwent all 3 tests showed a positive result in at least 1 test. Ninety-nine patients who did not satisfy the diagnostic criteria of definite MD but had vertigo and/or hearing loss at the first visit subsequently progressed to definite MD. It was retrospectively found that 92% of patients showed at least 1 positive finding in these 3 tests at the initial stage. In those patients who showed a negative test result in either ECochG or the glycerol test, the possibility of progression to definite MD was low.

Conclusion—The combination of ECochG and the glycerol and furosemide tests was helpful in diagnosing endolymphatic hydrops (ELH). ECochG and the glycerol test were effective tools for predicting the progression to definite MD in patients with atypical MD, sudden hearing loss and other cochleovestibular diseases. Our test results also indicated that the pathological state of atypical MD included both non-ELH and ELH. *Key words: cochlear Ménière's disease, endolymphatic hydrops, vestibular Ménière's disease.*

INTRODUCTION

Otologists believe that the underlying pathological state of Ménière's disease (MD) is idiopathic endolymphatic hydrops (ELH) (1). ELH is characterized by repetitive vertiginous attacks and fluctuating hearing loss (1). The management of ELH differs from that of other cochleovestibular diseases, i.e. it requires the administration of diuretics, salt restriction and surgical treatment for intractable patients (2). Patients with vertigo and/or hearing loss often ask otologists about restrictions of their everyday activities and the possibility of recurrence. Thus, it is important to differentiate ELH from non-ELH.

Otologists have difficulty diagnosing MD as the auditory and vestibular symptoms may not always occur simultaneously and not all of the symptoms occur in many patients (3). One type of MD shows some, but not all, of the features of MD and is termed "atypical MD" (2). In order to solve the aforementioned diagnostic problems, electrocochleography (ECochG) and glycerol and furosemide tests have been used to objectively diagnose ELH (2, 4). ECochG and the glycerol test can detect cochlear hydrops, while the furosemide test detects vestibular hydrops (5). In order to assess the prognosis of MD, we performed these three tests in patients at the time of their first visit. We investigated whether these tests could provide important information, especially that required for predicting progression from atypical to definite MD, using a large amount of data obtained in our outpatient clinic. In addition, the pathological state of atypical MD was investigated.

MATERIAL AND METHODS

The subjects of the study were 1569 patients (702 males, 867 females; mean age 50 years; age range 8–77 years) with various cochleovestibular diseases (Table I). An outline of the diagnoses made based on patients' cochleovestibular symptoms and signs is shown in Fig. 1. ECochG was performed in 1413 patients, the glycerol test in 672 and the furosemide test in 335.

For the diagnosis of MD, the criteria proposed by The Meniere's Disease Research Committee of Japan were applied (6). In these criteria, patients with the triad of recurrent episodic vertigo, fluctuating hearing loss and unknown causes of these two symptoms are diagnosed as having "definite MD". Patients with bilateral MD were excluded from this study. Hearing loss was confirmed by pure-tone audiometry using AA-61BN, AA-75 and AA-76 instruments (Rion Ltd., Japan). Patients with age-related impairment of hearing level determined based on Oda's (7) report on changes of sensorineural hearing levels in elderly Japanese subjects were also excluded from the study. Patients were confirmed to have fluctuating hearing loss when pure-tone audiometry showed an improvement of ≥ 15 dB in at least 2 frequencies and then hearing loss worsened again.

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Table I. *Results of ECochG and the glycerol and furosemide tests. Statistical comparisons were made between patients with definite MD and those with other diseases*

Disease	No. of patients	ECochG Nos. of positive cases/total cases (%)	*p*	Glycerol test Nos. of positive cases/total cases (%)	*p*	Furosemide test Nos. of positive cases/total cases (%)	*p*
Definite MD	352	235/326 (72)	–	144/260 (55)	–	75/141 (53)	–
Atypical MD	319	107/300 (36)	< 0.01	58/155 (37)	< 0.01	34/73 (47)	NS
Syphilitic labyrinthitis	33	17/30 (57)	NS	7/12 (58)	NS	7/11 (64)	NS
DEH							
Ipsilateral type	20	1/2 (50)	NS	1/1 (100)	NC	9/18 (50)	NS
Contralateral type	46	25/40 (63)	NS	15/32 (47)	NS	10/29 (35)	NS
Sudden hearing loss	322	89/318 (28)	< 0.01	16/61 (26)	< 0.01	4/17 (24)	< 0.05
Cochleovestibulopathy	150	28/139 (20)	< 0.01	14/55 (25)	< 0.01	10/33 (30)	< 0.05
SNHL	327	52/258 (20)	< 0.01	17/96 (18)	< 0.01	1/13 (8)	< 0.01
Total	1569	554/1413 (39)		272/672 (40)		150/335 (45)	

NC = not calculated.

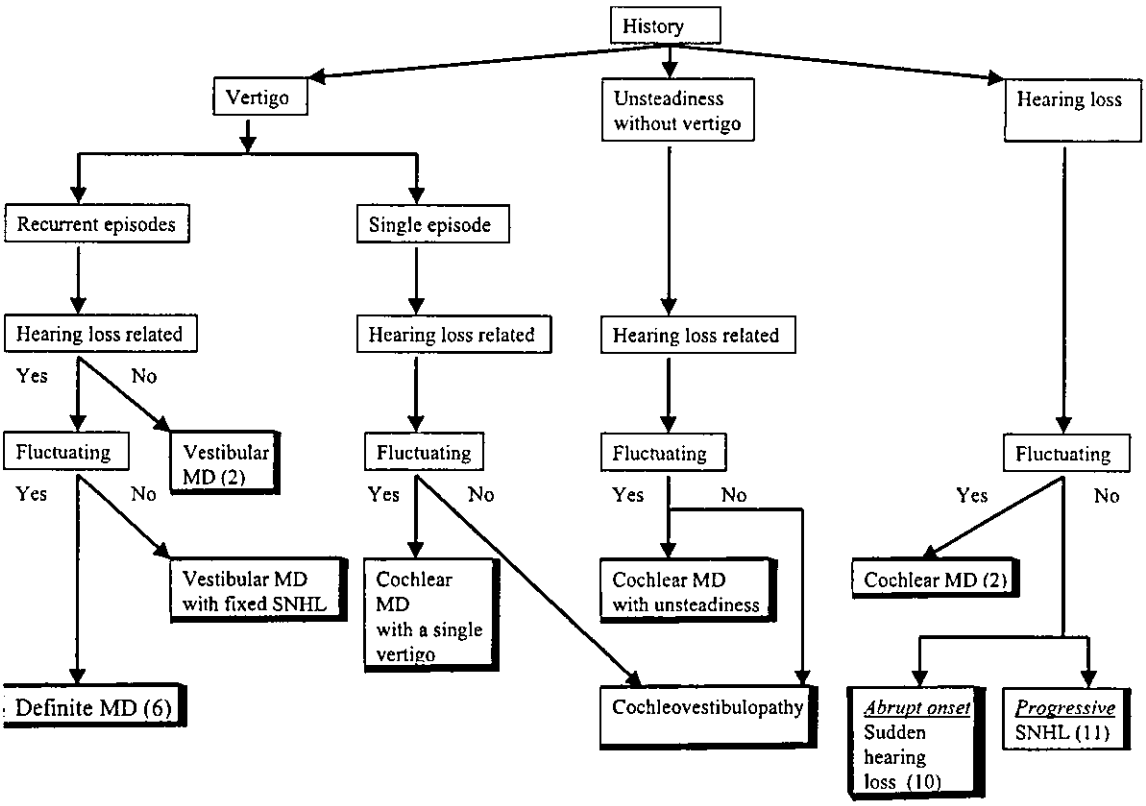

Fig. 1. An outline of differential diagnoses based on patients' cochleovestibular signs and symptoms. The numbers in parentheses indicate references.

When patients showed some but not all features of definite MD they were regarded as having "atypical MD". Patients with atypical MD were divided into five categories based on the characteristics of vertigo and hearing loss as follows (Fig. 2). Patients with recurrent episodic vertigo, who belonged to the vestibular MD group, were sub-divided into two groups of hearing loss type (patients with complicating fixed sensorineural hearing loss) and normal hearing type (those with normal hearing). Patients with fluctuating hearing loss, who belonged to the cochlear MD group, were sub-divided into three groups of

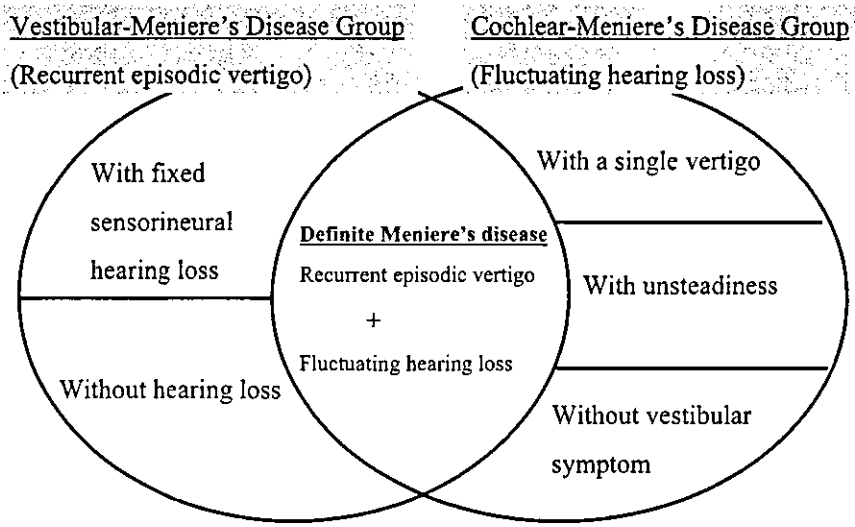

Fig. 2. Classification of atypical MD.

single vertigo type (patients with a single episode of vertigo), unsteady type (those without vertigo but with unsteadiness) and no vestibular symptom type (those entirely without vestibular symptoms).

The diagnosis of syphilitic labyrinthitis was made based on the following criteria proposed by Huang and Lin (8): recurrent attacks of episodic vertigo; fluctuating or progressive hearing loss; and positive fluorescent treponemal antibody absorption test. Delayed endolymphatic hydrops (DEH) was diagnosed according to the method proposed by Aso and Watanabe (9). Their diagnostic criteria of ipsilateral DEH were profound unilateral sensorineural hearing loss (≥ 90 dB) in 1 ear discovered in childhood and the onset of episodic vertigo from the ear with hearing loss after a long period of time. Their definition of contralateral DEH was a profound unilateral sensorineural hearing loss in 1 ear (≥ 90 dB) and the development of fluctuating hearing loss and/or episodic vertigo in the opposite ear after a number of years. Sudden hearing loss was defined as the acute onset of idiopathic sensorineural hearing loss with or without vertigo (10). Other cochleovestibular disorders that did not belong to the above disease entity were defined as "cochleovestibulopathy". This category included inner ear disorders with non-fluctuating hearing loss, a single episode of vertigo and/or unsteadiness of a varying degree and abnormal inner ear findings on electronystagmography. "Sensorineural hearing loss" (SNHL) indicated slowly progressive hearing loss and had no identifiable etiology, especially with regard to certain origins such as vestibular schwannoma (11).

The results of ECochG and the glycerol and furosemide tests were investigated. In particular, the test results of patients who were not diagnosed as having definite MD at the first visit but who eventually satisfied the criteria for definite MD were retrospectively investigated. Patients who were confirmed to have definite MD within 1 month after the onset of symptoms were excluded from the analysis. Statistical analyses were performed using a χ^2 test and $p < 0.05$ was considered significant.

Transtympanic ECochG was performed in each patient at the early stage of disease. A stainless-steel needle electrode was placed in the promontory wall through the anesthetized tympanic membrane. Reference and ground electrodes were placed on the ipsilateral ear lobe and forehead, respectively. Clicks and tone bursts were used as sound stimuli and were repeated 250 times. The signal was directed to a preamplifier with a time constant of 0.03 s and using a high-cut filter at 3000 Hz in click stimulation. The sampling rate and the sampling point in the signal processor were 100 Hz and 1024 points, respectively. The amplitudes of the action potential (AP) and summating potential (SP) were determined using a computer. The upper 99% confidence limit of the SP/AP ratio in response to a 100 dB sound pressure limit click was 0.37 in 29 volunteers with normal hearing. If the SP/AP ratio was > 0.37, this was regarded as a positive result (12). In the glycerol test, i.v. administration of the glycerol solution was performed at our clinic to avoid severe side-effects such as headache and nausea due to oral glycerol intake (13). Standard audiometric techniques were used to obtain pure-tone thresholds using an ascending intensity technique. The test result was judged to be positive if an improvement of ≥ 10 dB at ≥ 2 audiometric frequencies was observed.

The furosemide vestibulo-ocular reflex (VOR) test was performed in each patient at the early stage of

the disease. In this test, the slow phase velocity generated during harmonic sinusoidal rotation was measured before and 30, 60 and 90 min after an i.v. administration of 20 mg furosemide, as described previously (14). An increase or decrease of $\geq 10\%$ in directional preponderance was regarded as a positive result.

RESULTS

ECochG and glycerol and furosemide tests

Table II shows the overall results of ECochG and the glycerol and furosemide tests. The frequency of positive results of both ECochG and the glycerol test in definite MD was not statistically different from that in syphilitic labyrinthitis or contralateral DEH. The frequency of positive results of the furosemide test in definite MD was not statistically different from that in syphilitic labyrinthitis, DEH or atypical MD.

Fig. 3 shows the results of the battery of all three tests and of combinations of two tests. In the combination of ECochG and the glycerol test, 215/245 patients (88%) with definite MD showed positive results in either test. In the combination of ECochG and the furosemide test, 109/123 patients (89%) with definite MD showed positive results in either test. In the combination of the glycerol and furosemide tests, 100/133 patients (75%) with definite MD showed

Table II. *Results of ECochG and the glycerol and furosemide tests in patients with five types of atypical MD. Statistical comparisons were made between patients with definite MD and those with other diseases*

Disease	No. of patients	ECochG Nos. of positive cases/total cases (%)	*p*	Glycerol test Nos. of positive cases/total cases (%)	*p*	Furosemide test Nos. of positive cases/total cases (%)	*p*
Vestibular MD group							
Vestibular MD with fixed SNHL	141	38/134 (28)	<0.01	19/60 (32)	<0.01	19/34 (56)	NS
Vestibular MD without hearing loss	24	1/20 (5)	<0.01	0/1 (0)	NC	3/6 (50)	NS
Cochlear MD group							
Cochlear MD with single vertigo	29	17/29 (59)	NS	9/20 (45)	NS	3/7 (43)	NS
Cochlear MD with unsteadiness	48	16/42 (38)	<0.01	14/32 (44)	NS	7/16 (44)	NS
Cochlear MD without vestibular symptoms	77	35/75 (47)	<0.01	16/42 (38)	<0.05	2/10 (20)	<0.05

NC = not calculated.

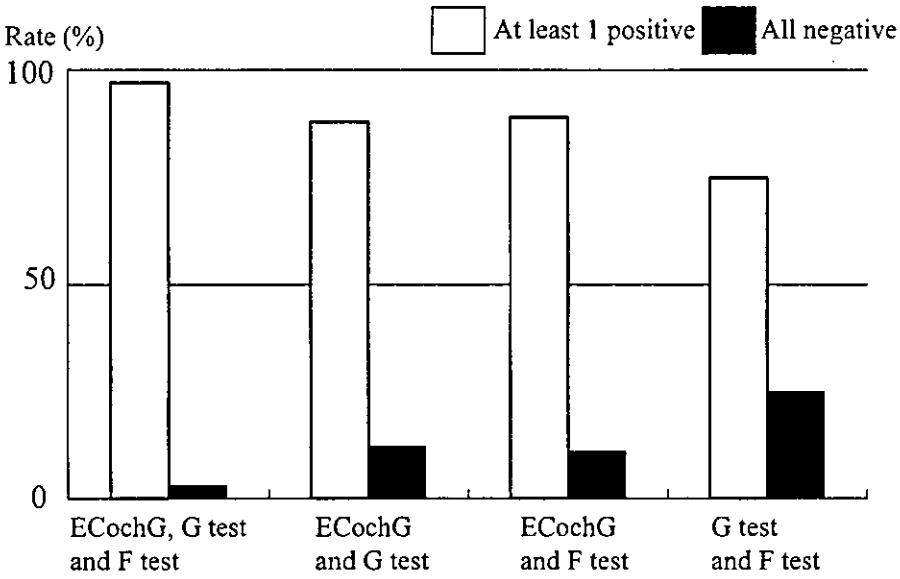

Fig. 3. Results of the battery of three tests and of combinations of two tests. G = glycerol; F = furosemide.

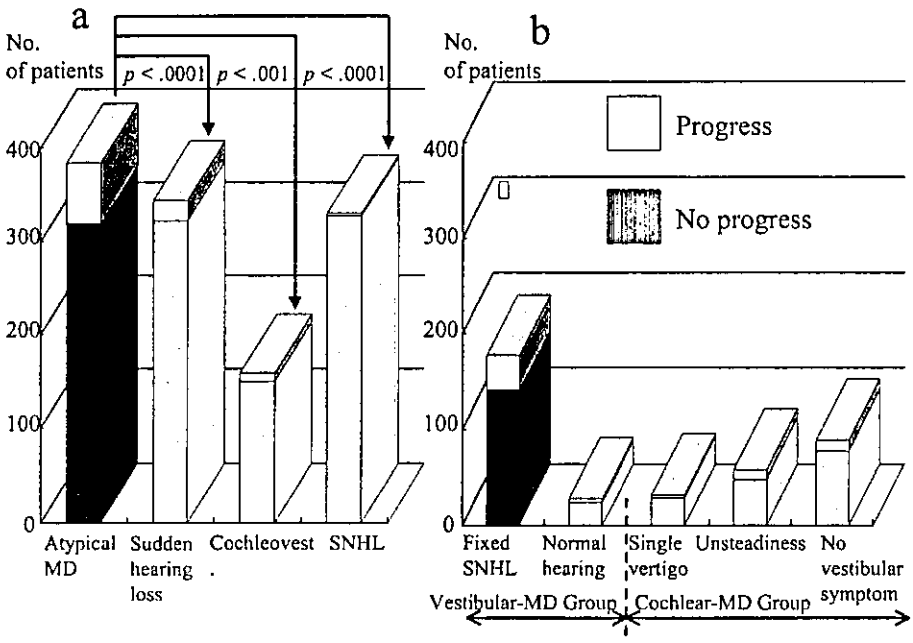

Fig. 4. (*a*) The incidence of progression from atypical MD, sudden hearing loss, cochleovestibulopathy and SNHL to definite MD. (*b*) The incidence of progression from the five types of atypical MD to definite MD.

positive results in either test. In the battery of all 3 tests, 115/118 patients (97%) with definite MD showed at least 1 positive result in the 3 tests.

Atypical MD

Table II shows the results of the three tests in patients with five types of atypical MD. In ECochG, the frequency of positive results in patients with the single vertigo type in the cochlear MD group was not statistically different from that of patients with definite MD. In the glycerol test, the frequency of positive results in patients with the single vertigo type and the unsteady type in the cochlear MD group was not statistically different from that of patients with definite MD. In the furosemide test, the frequency of positive results in patients with the no vestibular symptom type in the cochlear MD group was statistically different from that of patients with definite MD. The results of all three tests in patients with the single vertigo type in the cochlear MD group were not statistically different from those of patients with definite MD.

Progression to definite MD

Of 352 patients who had the final diagnosis of definite MD, 65 (18%) had initially been diagnosed as having atypical MD, 22 (6%) as having sudden hearing loss, 9 (3%) as having cochleovestibulopathy and 3 (0.9%) as having SNHL. Of 65 patients with atypical MD that progressed to definite MD, 40 belonged to the vestibular MD group (36 with hearing loss type and 4 with normal hearing type) and 25 belonged to the cochlear MD group (3 with single vertigo type, 10 with unsteady type and 12 with no vestibular symptom type). In total, 99 patients (28%) who were initially diagnosed as not having definite MD were subsequently confirmed to have it.

The incidences of progression from atypical MD, sudden hearing loss, cochleovestibulopathy and SNHL to definite MD were 17%, 6%, 6% and 0.9%, respectively (Fig. 4*a*). The incidences of progression from sudden hearing loss, cochleovestibulopathy and SNHL to definite MD were significantly lower than that from atypical MD to definite MD. The incidences of progression from hearing loss type and normal hearing type in the vestibular MD group to definite MD were 20% and 14%, respectively (Fig. 4*b*). The incidences of progression from single vertigo type, unsteady type and no vestibular symptom type in the cochlear MD group to definite MD were 9%, 17% and 13%, respectively.

Prediction of progression to definite MD

A total of 99 patients were subsequently confirmed to have definite MD and underwent some of the three tests: ECochG ($n = 94$); the glycerol test ($n = 78$); and the furosemide test ($n = 44$). Of these 99 patients, 91 (92%) showed at least 1 positive result.

In ECochG, the frequency of positive results was significantly different between cases with and without progression to definite MD in the patient population with atypical MD, except for the single vertigo type in the cochlear MD group and those with sudden hearing loss, cochleovestibulopathy and SNHL (Figs. 5 and 6). In the patients who progressed to definite MD, only 20% (19/94) of the patients showed a negative test result in ECochG.