Pattern of hair cell loss and delayed peripheral neuron degeneration in inner ear by a high-dose intratympanic gentamicin

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Abstract

To gain insights into the ototoxic effects of aminoglycoside antibiotics (AmAn) and delayed peripheral ganglion neuron death in the inner ear, experimental animal models were widely used with several different approaches including AmAn systemic injections, combination treatment of AmAn and diuretics, or local application of AmAn. In these approaches, systemic AmAn treatment alone usually causes incomplete damage to hair cells in the inner ear. Co-administration of diuretic and AmAn can completely destroy the cochlear hair cells, but it is impossible to damage the vestibular system. Only the approach of AmAn local application can selectively eliminate most sensory hair cells in the inner ear. Therefore, AmAn local application is more suitable for studies for complete hair cell destructions in cochlear and vestibular system and the following delayed peripheral ganglion neuron death. In current studies, guinea pigs were unilaterally treated with a high concentration of gentamicin (GM, 40 mg/ml) through the tympanic membrane into the middle ear cavity. Auditory functions and vestibular functions were measured before and after GM treatment. The loss of hair cells and delayed degeneration of ganglion neurons in both cochlear and vestibular system were quantified 30 days or 60 days after treatment. The results showed that both auditory and vestibular functions were completely abolished after GM treatment. The sensory hair cells were totally missing in the cochlea, and severely destroyed in vestibular end-organs. The delayed spiral ganglion neuron death 60 days after the deafening procedure was over 50%. However, no obvious pathological changes were observed in vestibular ganglion neurons 60 days post-treatment. These results indicated that a high concentration of gentamycin delivered to the middle ear cavity can destroy most sensory hair cells in the inner ear that subsequently causes the delayed spiral ganglion neuron degeneration. This model might be useful for studies of hair cell regenerations, delayed degeneration of peripheral auditory neurons, and/or vestibular compensation. In addition, a potential problem of ABR recording for unilateral deafness and issues about vestibular compensation are also discussed.

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1. Introduction

Loss of sensory hair cells and their connecting neurons in the inner ear often leads to permanent hearing loss and/or vestibular disorders. Although permanent hearing loss and vestibular dysfunction are not life threatening, the quality of life will be heavily influenced by the permanent disability. Since the destruction of sensory hair cells and peripheral ganglion neurons in the inner ear are unrepairable because the
injured hair cells and peripheral neurons cannot naturally regenerate, it is difficult to rescue the sensorineural hearing loss and balance dysfunction. The cochlear implant is widely applied in clinic for profound deafness patients to bypass the dysfunctional cochlea and to substitute for the missing hair cells. The cochlear implant provides electrical signals to spiral ganglion neurons and their axons projecting to the cochlear nucleus. Therefore, sufficient spiral ganglion neuron survival is a fundamental prerequisite for efficient work of cochlear implant (Shibata et al., 2011; Pfingst et al., 2011). Thus, there is a strong clinical need for establishing ways of promoting spiral ganglion neuron survival following hair cell death. Kanamycin or gentamicin selectively destroys sensory hair cells in the inner ear followed by a slow progress of secondary damage to spiral ganglion neurons due to the lack of electrical stimulation and neurotrophic factors. This secondary degeneration of spiral ganglion neurons is referred to as “delayed spiral ganglion neuron death” (Ding and Salvi, 2005; McFadden et al., 2004; Ding et al., 2010a). In order to better understand the interdependence of sensory hair cells and peripheral ganglion neurons in the inner ear, as well as the hair cell death pathways, hair cell regeneration, or delayed spiral ganglion neuron death, numerous animal deafing models have been studied in the field of hearing research. The studies would benefit by the use of animal models with well-defined cell damage in the inner ear.

Cochlear and vestibular hair cell damage can be induced by numerous factors, such as acoustic trauma, aging, ototoxic chemicals, blast wave, irradiation, infection, heavy metals, pesticide, herbicide, organic solvent, and gene mutations, etc (Ding and Salvi, 2005; Ding et al., 2012, 2011a, 2011b, 2004, 2010b, 2007; Ding et al., 1999a; Ding et al., 2002a, 2013, 2011c, 1999b; McFadden et al., 1999; Qi et al., 2008; Wang et al., 1999; Wu et al., 2011a, 2011b; Someya et al., 2009; Nicotera et al., 2004; Kane et al., 2012; Guan, 2011; Federspil et al., 1976). These various aetiologies have led to many attempts to develop appropriate animal models for different study purposes. Since the ototoxic effects and neurotoxic effects are presented together in most toxic chemicals, such as platinum-based antineoplastic drugs, antimalarial drugs, and heavy metals, those chemicals should not be used for the study of delayed neuron death. However, aminoglycoside antibiotics such as kanamycin or gentamicin directly destroy sensory hair cells while preserving the ganglion neurons in the inner ear during its injury, although the spiral ganglion neurons finally will indirectly afterwards. Therefore, kanamycin or gentamicin-induced direct injury to sensory hair cells in the inner ear is considered an ideal experimental models of delayed spiral ganglion neuron death.

In order to establish the experimental model of sensory hair cell damage and delayed neuron death, several strategies or approaches were attempted. One approach is systemic treatment of kanamycin or gentamicin using consecutive daily doses, because a single dose failed to induce inner ear damage due to obstruction of the blood-labyrinth barrier, except in the case of acute renal failure or genetic defects (Guan, 2011; Federspil et al., 1976). Continued kanamycin or gentamicin systemic treatment for several days often causes severe but incomplete damage to the cochlear hair cells and vestibular hair cells (Ding and Salvi, 2005). To overcome the obstruction of the blood-labyrinth barrier and enhance the entry of ototoxic drugs into the inner ear, loop-inhibiting diuretics, such as ethacrynic acid or furosemide, which can abolish the blood flow in vessels supplying the lateral wall of the cochlea and damage the stria vascularis, temporarily open the blood—cochlear barrier thereby enhancing the entry of ototoxic drugs into the cochlea (Ding and Salvi, 2005; McFadden et al., 2004; Ding et al., 2010a, 2011a, 2004, 2010b, 2007, 2002a; Ding et al., 2003; Li et al., 2011; Liu et al., 2011; McFadden et al., 2002). Co-administering loop diuretics with kanamycin or gentamicin can result in a complete hair cell loss in the cochlea, but cannot injure the vestibular hair cells (Ding and Salvi, 2005; Ding et al., 2010a, 2004; Liu et al., 2011; McFadden et al., 2002; Ding et al., 1995). Therefore, this approach is only able to wipe out all hair cells and allowing for the study of delayed spiral ganglion neurons death in the cochlea. To destroy the sensory hair cells in both the cochlear and vestibular system while bypassing the blood-labyrinth barrier, an alternative approach for gentamicin ototoxicity is via the round window membrane which is permeable to many agents (Ding and Salvi, 2005; Ding et al., 2011a; He et al., 2009), or direct delivery into the cochlear or vestibular cavity (Li et al., 2004; Swan et al., 2008; Wagner et al., 2005). Since the local application of gentamicin by tympanic injection is simple and effective to damage both cochlear and vestibular sensory hair cells, we applied gentamicin to one ear to create a unilateral loss of sensory hair cell loss in both the cochlear and vestibular system. The functional and pathological changes in the cochlear and vestibular system were observed after gentamicin treatment, and the delayed peripheral ganglion neurons degeneration was also evaluated.

2. Materials and methods

2.1. Animal treatment

Twenty 250—300 g adult pigmented guinea pigs with normal Preyer's reflexes were used in the present study. Animals were anesthetized with a mixture of ketamine (30 mg/kg i.p.) and chlorpromazine (15 mg/kg, i.p.), and placed on a temperature-controlled heating pad with a rectal probe to maintain core temperature at 37°C. Guinea pigs were placed in a right lateral position, and the left meatus of the external ear was sterilized by filling with 70% ethanol for 5 min. The meatus of the external ear was then dried by aseptic cotton swab. Under a surgical microscope and sterile conditions, approximate 200 µl gentamicin sulfate dissolved in normal saline at a concentration of 40 mg/ml in 1 ml syringe was penetrated through tympanic membrane, and slowly injected into the middle ear cavity until the solution completely filled the middle ear cavity and overflowed into the external ear canal as described previously (He et al., 2009).
After gentamicin tympanic injection, animals were placed in a lateral position with the treated ear upward, until animals wakened from the anesthesia so that any air bubbles in the irregularly shaped lacunae in the middle ear cavity would float out of the round window niche. In each animal, the left ear was administrated with gentamicin and the right ear was injected with saline as self-control ear. The functional and morphological analysis in both cochlear and vestibular systems were investigated, and the delayed ganglion neuron death in both cochlear and vestibular system was also assessed 30 days and/or 60 days after GM treatment respectively.

All animals were purchased from Experimental Animal Center of Xiangya Medical College, Central South University and all experimental procedures regarding animal care and use were approved by the Institutional Animal Care and Use Committee of Xiangya Medical College.

2.2. Evaluation of hearing function

The procedures for measuring the auditory brainstem response (ABR) and cochlear compound action potential (CAP) have been described in our previous publications (Ding et al., 2002a, 1999b; Wu et al., 2011a; Kane et al., 2012; Ding et al., 2003; Li et al., 2011; Ding and Jin, 1998; Ding et al., 1993, 1996; Wang et al., 1996; Ding and Zhang, 1995). Each animal was tested before drug treatment and on the day of termination. Animals were anesthetized as described above and the body temperature was maintained at 37 °C using a heating pad. Testing was performed in a sound-isolated and electrically-shielded booth. For ABR measurement, three recording electrodes were inserted subcutaneously at the vertex and at both mastoids respectively. The responses were recorded and processed by TDT system III (TDT, USA). The ABR was elicited with tone bursts at 1, 2, 4, 8, 12, 16 kHz. Stimuli were generated digitally with 3 ms duration, 0.5 ms rise/fall time, alternating phase, repeated at 21/s, and fed to a magnetic speaker (MFI, TDT, USA) located 10 cm in front of the test ear. Evoked potentials were filtered between 100 and 3000 Hz, and averaged 512 times. The thresholds were obtained by reducing the sound intensity in 5 dB increments from 90 dB SPL to below the intensity where the ABR disappeared. The ABR threshold was defined as the lowest intensity capable of eliciting a replicable, visually detectable response. The CAP was recorded from facial canal by a thin recording electrode. As described previously (Ding and Jin, 1998; Ding et al., 1993, 1996; Wang et al., 1996; Ding and Zhang, 1995), in brief, a surgical incision was made at the postauditory process to expose the facial nerve. After removing the facial nerve from the facial canal, a Teflon-coated silver recording electrode was gradually insert into the facial canal until the horizontal segment of facial canal, which is just separated from cochlea by a very thin bony wall. Afterwards, the facial canal was closed with dental cement and the incision was sutured. The sound stimuli used to elicit the CAP were basically the same as for the ABR recording, except the CAP was averaged 100 times. The CAP were basically the same as for the ABR recording, except the CAP was averaged 100 times.

2.3. Evaluation of vestibular function

The procedures for measuring the vestibular nystagmus response (VNR) and the swimming test (ST) have been described in our previous publications (Ding et al., 1999b, 1997; Li et al., 2004; Zhou et al., 2009). To evaluate the duration of the VNR, the guinea pig was restrained in a custom holder. After irrigating the external ear canal with 0.5 ml of 0 °C water, the head of guinea pig was fixed in a position in which the lateral semicircular canal was oriented vertically to the ground. The VNR duration was recorded visually with a stopwatch. In addition, animal balance behavior was also observed during the ST. Animals were placed into a pool filled with warm water for about one minutes to determine if the animal could swim or roll over in the water (Zhou et al., 2009).

2.4. Histological analysis

2.4.1. Cochlear and vestibular hair cell evaluation

After animals were anesthetized and decapitated, cochlea were removed immediately from temporal bones. The inner ear cavity was opened and the otolithic membrane in the saccule and utricle were removed. Then cochlea were perfused with 0.5% silver nitrate solution and then fixed with 4% formaldehyde overnight at 4 °C. Following fixation, the basilar membrane and the end organs of the vestibular system including the macula of the utricle and saccule, ampullary cristae of the three semicircular canals were carefully dissected out under an anatomical microscope and processed for flat surface preparation (Ding et al., 1999b, 2003; Li et al., 2004; Ding et al., 1989, 2001, 1981, 1986, 1987). To quantify hair cell loss, surface preparations of the cochlear basilar membrane were examined with a light microscope (Zeiss, Germany). Hair cells were counted over a 0.24 mm interval along the entire organ of Corti as described previously (Ding and Salvi, 2005; Ding et al., 2011a, 2011b, 2013, 1999b; McFadden et al., 1999; Qi et al., 2008; Wang et al., 1999; Kane et al., 2012; Ding et al., 2003; Li et al., 2011; Liu et al., 2011; McFadden et al., 2002). The cochleogram showing the percent hair cell loss as a function of the percent distance from cochlear apex was constructed using custom cochleogram software. A laboratory norm of hair cell number for young normal guinea pigs was used as a reference to calculate percent of hair cell loss. To compare the data, individual cochleograms were aggregated to produce a mean cochleogram for each group. The flat surface preparations of the vestibular end organs were also observed under the light microscope and photographed. To quantify the level of hair cell loss, the number of hair cell in each vestibular end-organ was counted in several regions of each sample. The hair cell density was obtained and stored in a personal computer for further analysis using Image J software (NIH, USA). Hair cell density measurements were made from three to five regions of each specimen and a mean value was computed for each specimen as described previously (Ding and Salvi, 2005; Ding et al., 2011a, 2011b, 2013, 1999b; McFadden et al., 1999; Qi
et al., 2008; Wang et al., 1999; Kane et al., 2012; Ding et al., 2003; Li et al., 2011; Liu et al., 2011; McFadden et al., 2002).

2.5. Quantification of cochlear and vestibular ganglion neurons

The temporal bone was obtained as described above and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) overnight at 4 °C. The temporal bone was decalcified in 3% hydrochloric acid at room temperature for 3 days. Afterwards, specimens were further fixed with 2% osmium for 2 h and then dehydrated with gradient alcohol and acetone series. Afterwards, specimens were immersed in acetone and Epon 812 mixture at a ratio of 1:1 overnight. Finally specimens were embedded in Epon 812 at 60 °C for polymerization. The specimens were then sectioned serially at a thickness of 4 μm in the horizontal plane parallel to the cochlear modiolus using a microtome. Sections were collected on glass slides, and stained with toluidine blue. All sections were examined under the light microscope (Zeiss, Germany) and photographed (McFadden et al., 2004; Someya et al., 2009; Kane et al., 2012; Li et al., 2011; Ding et al., 1993, 1997, 2002b, 1998). The densities of spiral ganglion neurons (SGN) in each turn of the cochlea and the superior and posterior vestibular ganglion neurons (VGN) were quantified using image J software as described previously (Ding et al., 1998, 2002c). The boundaries of Rosenthal's canal were outlined and its cross-sectional area was calculated. The number of SGN in each canal was counted, and the density of SGN was computed by dividing the number of SGN by the canal cross-sectional area. The pathological changes and the density of superior and inferior VGN were also evaluated. To avoid double counting, every fifth section was selected for cell counts. Only SGN and VGN with a clear nucleus and nucleolus were counted.

2.6. Statistical analysis

All statistical analyses were carried out by t-test and one-way ANOVA followed by Newman–Keuls post hoc analyses using the GraphPad Prism5 software. All tests were two-sided with the threshold for statistical significance set at $P < 0.05$.

3. Results

3.1. Changes of hearing

Prior to the transtympanic application of gentamicin or saline, tone bursts at 1, 4, 8, 16 kHz elicited ABR thresholds were in a range of 20–40 dB SPL across all tested frequencies. There was no significant difference between the left ear and the right ear in the pretesting ($t$ test, $P > 0.05$). On the day of termination, auditory function was tested again by ABR and CAP respectively. The ABR responses for both ears (right ears were saline control, left ear were treated by GM) were all detectable, and only small threshold elevations were observed in the GM treated ear due to bone conduction cross over to control ear (Fig 1 A and B). In contrast, the results obtained from CAP were different to those obtained from ABR measurement. The CAP thresholds in saline control ear were very similar to the ABR thresholds in all guinea pigs ($t$ test, $P > 0.05$). However, CAP responses were undetectable in GM treated ear, unlike wave forms of ABR which may suggest the ABR reflections from contralateral ear, the saline control ear (Fig. 1C, D, E).

3.2. Cochlear hair cell missing

Typical surface views of the basilar membrane and mean cochleogram from saline control ears and GM treated ears are shown in Fig. 2. 30 days after treatment, both outer hair cells and inner hair cells in GM treated ears were completely missing along the entire basilar membrane, which is consistent with the CAP absence. In contrast, all hair cells in control ears were intact (Fig. 2A, B, C). Cochlear sections similarly show that the organ of Corti in each turn was completely collapsed in GM treated ears. However, the organ of Corti was normal in all saline control ears (Fig. 3). In a total of 14 examined GM treated ears, only a very inner hair cells were detected in the apical turn in two animals, while all hair cells through the entire length of the cochlea were totally absent in the remaining ears treated with GM.

3.3. Balance dysfunction

To estimate the changes of vestibular function, the duration of the VNR was examined before and 30 days after GM
treatment. Fig. 4A shows the average duration of the VNR before and 30 days after GM administration. In pretesting, the duration of the VNR was 48.80 ± 11.81 s. However, the VNR response completely disappeared 30 days after GM treatment. In addition, we also found that guinea pigs exhibited obvious head tilt in the direction of GM treated ear about 2–3 days after treatment (Fig. 4B). However, a progressive recovery of head position was observed afterwards. The head position was nearly normal 10–15 days after treatment. Guinea pig maintained normal body balance in the water in pretesting. However, after GM local application, animals showed obvious balance dysfunction that involved rolled over in the water (Fig. 4C, D).

Fig. 2. Cochlear hair cell loss. (A) Representative surface preparation of cochlear basilar membrane from saline treated ear. Note all inner hair cells and outer hair cells were intact. (B) Representative surface preparation of cochlear basilar membrane from GM treated ears 30 days after treatment. Note all sensory hair cells were completely destroyed. (C) Mean cochleogram shows nearly 100% hair cells were missing in GM treated ears along the enter length of the cochlea. In contrast, no cochlear hair cell missing in saline treated ears (N = 5 for each condition).

Fig. 3. Representative images of cochlear sections for a saline treated ear and a GM treated ear respectively. (A–D) The organ of Corti in each turn and spiral ganglion neurons and auditory nerve fibers in Rosenthal's canal were normal in saline treated ear. (E–H) All hair cell were missing and the density of spiral ganglion neurons and nerve fibers in Rosenthal's canal were also diminished in GM treated ear. (A and E: apical turn, B and F: upper middle turn, C and G: lower middle turn, D and H: basal turn).
3.4. Vestibular hair cell loss

Vestibular hair cell damage was assessed 30 days after treatment. A typical surface preparation view from the macula of the utricle in a saline control ear and a GM treated ear were as shown in Fig. 5A and B respectively. The cuticular plate of hair cells was clearly visible with high cell density in the saline treated ear, but most hair cells were missing in the GM treated ear (Fig. 5A, B). Quantitative analysis of hair cell density in the macula of the saccule, macula of the utricle and the three crista ampullaris showed that the hair cell density in GM treated ears was greatly reduced in comparison with saline control ears. Significant difference in hair cell density of vestibular end-organs was detected between the saline control ears and GM treated ears (one-way ANOVA, P < 0.01) (Fig. 5C, D).

3.5. Delayed SGN destruction

The density of SGN in Rosenthal’s canal in each turn was counted and compared between saline treated ears and GM treated ears 30 days and 60 days after treatment respectively. In saline treated control ears, SGN and auditory nerve fibers in Rosenthal’s canal were densely packed, while in GM treated ears, SGN and auditory nerve fibers were greatly reduced, especially 60 days post-GM (Fig 6). Compared with control cochleae, the density of SGN began to decrease about 20% across the entire cochlea in each turn 30 days after GM treatment (Newman–Keuls test, P < 0.01). In contrast, more than 50% of SGNs were missing 60 days after GM treatment (Newman–Keuls test, P < 0.01) (Fig 7).

3.6. Delayed VGN destruction

Whether it was 30 days or 60 days after treatment, the histology and morphology of VGN in both saline treated ears and GM treated ears were still present and normal (Fig. 8A, B). There was no significant statistical difference in VGN density between saline treated ears and GM treated ears (Fig. 8).

4. Discussion

4.1. Complete hair cell damage by GM local application

Studies with the aim of understanding the mechanisms of hearing loss and vestibular dysfunction can be greatly aided by animal models in which cells lesion occurs in well-defined patterns. In the current study, we found that intratympanic delivery of a single high dose of GM resulted in a permanent hearing loss and severe vestibular dysfunction which were confirmed by almost total sensory hair cell loss in both the cochlear and vestibular system. The results demonstrate that GM effectively infiltrated into the inner ear via the round window membrane which successfully avoided the obstacles of the blood-labyrinth barrier. Usually there are two ways to deliver chemicals on the round window membrane. One approach is to place a small amount of chemical solution about 20 μl on the round window niche; however, with this method it is difficult to cause complete destruction of all sensory hair cells in the inner ear (He et al., 2009; Ding et al., 1989). Another approach is intratympanic injection to fill up the middle ear cavity with GM solution which is not only simple, but also very effective, because the large GM solution is present for a longer period, enough to destroy most hair cells in the inner ear. This is consistent with previous reports showing that a single intratympanic dose of GM can induce a rapid GM accumulation with a high peak value in the fluid of the inner ear that last for 24 h at least, which is sufficient to induce a massive hair cell loss (He et al., 2009; Ding et al., 1989). In addition, intratympanic GM enters the cochlea by diffusion through the round window membrane that enters into

Fig. 4. Vestibular dysfunction. (A) Mean duration of VNR in pretesting was about 50 s, while 30 days after GM treatment, VNR was completely absent (mean ± SD, n = 10 ears per group). (B) Experimental guinea pigs showed obvious head tilt in the direction of GM treated ear (left) 2 days after GM treatment in comparison with normal animal (right). (C) In swimming test, normal orientation was detected in normal animals before the experiment. (D) Swimming orientation was severely disrupted in unilateral ear damage 30 days post-GM.
the scala tympani first, subsequently the scala vestibule via the helicotrema at the apex, and then into the vestibular cavity. This long diffusion pathway causes more hair cell lesions in the cochlea than the vestibular system. Therefore, longer exposure of GM via round window membrane is needed to establish all hair cell damage in the vestibular system (Li et al., 2004; Wanamaker et al., 1998).

Fig. 5. Density of hair cells in vestibule-end organs. (A) Surface review of macula of utricle showing sensory hair cell survival in saline treated ear. (B) Massive hair cell loss was found in the macula of utricle from GM treated ear. (C) The hair cell density in macula of sacculus and utricle in saline treated ears were compared with GM treated ears. Note that the hair cell densities in macula of sacculus and utricle were greatly reduced by GM treatment. (D) The hair cell density in crista of ampulla in three semicircular canals in saline treated ears was compared with GM treated ears. Note that the hair cell densities in all crista of the ampulla was significantly reduced. Data are expressed as mean ± SD, n = 6 ears per group. * Significant difference from control (P < 0.01).

Fig. 6. Representative images showing the density of spiral ganglion neurons in Rosenthal's canal. (A–E) 60 days after saline treatment, spiral ganglions at each portion of the cochlea are present and normal. (F–J) 60 days after GM treatment the density of spiral ganglion neurons in Rosenthal's canal was greatly reduced.
rotrophins from an unknown source which may be different.

The reason is that VGN may receive electric stimulations or neurotrophic factors to VGN, which may be barely adequate to maintain the vestibular neurons survival. Another reason is the incomplete destruction of sensory hair cells in vestibular end-organs. The remained few sensory hair cells can still supply signal stimulation in experimental conditions, a complete hair cell missing has been shown to prevent SGN degeneration. To better understand the process of delayed spiral ganglion neuron degeneration in experimental conditions, a complete hair cell missing is needed as an ideal animal model. In current study, a single-dose intratympanic GM successfully destroyed 100% cochlear hair cells. After disappearance of cochlear hair cells, some SGNs were missing 30 days post-GM treatment, and continue progressive SGN degeneration was observed 60 days after deafening procedure. These results are basically consistent with previous studies showing that delayed SGN neuron death is in a time-dependent manner (Ding and Salvi, 2005; McFadden et al., 2004; Ding et al., 2010a). Since the cochlear implants electrically stimulate residual SGNs to provide auditory signals to the auditory center, therefore the functional ganglion neurons are crucial to the successful use of cochlear implants. According to reports in the literature, providing an exogenous supply of neurotrophins and/or chronic intracochlear electrical stimulation has been shown to prevent SGN degeneration. To better understand the process of delayed spiral ganglion neuron degeneration in experimental conditions, a complete hair cell missing is needed as an ideal animal model. In current study, a single-dose intratympanic GM successfully destroyed 100% cochlear hair cells. After disappearance of cochlear hair cells, some SGNs were missing 30 days post-GM treatment, and continue progressive SGN degeneration was observed 60 days after deafening procedure. These results are basically consistent with previous studies showing that delayed SGN neuron death is in a time-dependent manner (Ding and Salvi, 2005; McFadden et al., 2004; Ding et al., 2010a; Versnel et al., 2007). As to vestibular ganglion neurons, however, no obvious degeneration was found by 60 days post-treatment in current study, which is consistent with previous studies that the VGN can be survival for a long period without vestibular hair cells (Dupont et al., 1993). Once again, our findings indicated that VGN can really survive longer than SGN after sensory hair cell loss. In current study, at least two reasons account for the VGN survival. One is the incomplete destruction of sensory hair cells in vestibular end-organs. The remained few survival vestibular hair cells can still supply signal stimulations and neurotrophic factors to VGN, which may be barely adequate to maintain the vestibular neurons survival. Another reason is that VGN may receive electric stimulations or neurotrophins from an unknown source which may be different from those of SGN. This unknown region definitely needs to be further studied.

4.3. Potential problems from ABR recording

Auditory brainstem response (ABR) is an auditory evoked potential, widely used to test the hearing function in clinic and experimental animal models. It can be recorded via electrodes placed on the scalp by the computer averaging technology (Wu et al., 2011a; Kane et al., 2012; Wang et al., 1996; Jewett et al., 1970; Starr and Achor, 1975). ABR is usually detected in the first 10 ms following acoustic stimuli with several typical repeated waves. The wave I of ABR is generated by auditory nerve, and the following waves II, III, IV, and V are generated by cochlear nucleus, superior olivary complex, lateral lemniscus, and inferior colliculus, respectively. Therefore, wave III and the following waves are actually reflecting the activity in central components of the auditory pathway by hearing signal input from both ears. Although the sound stimuli can be towards one ear for ABR testing, the sound can be listened with both ears. In clinic measurement of hearing by means of an audiometer, if there is a 30 dB hearing difference between the two ears, a sound masking is needed to cover up the normal ear, otherwise the real hearing response from deaf ear will be difficult to evaluate. In the experimental animal, such as guinea pig which has a small head that the sound is even easier to conduct to contralateral ear. In our previous study, we have demonstrated that the ABR threshold was similar between the two ears from unilateral deaf guinea pigs (Wang et al., 1996). Consistently, all experimental animals in current study have complete deafness and hair cell loss in one ear. However, ABR can still be detected from these completely deafened ears (Fig. 1). These findings may indicate that ABR results can be confused by signals from the contralateral normal ear in unilateral deafened animal. Another interesting finding from current study is that the CAP recording from the deaf ear actually elicited the ABR wave without wave I and wave II. This suggests strongly that the electrode in the facial canal can receive hearing signals from auditory pathway in brain stem by opposite normal ear, if the testing ear was deaf. It once again proves that the ABR is inapplicable to unilateral deafness unless the contralateral normal ear was covered up by sound masking.

4.4. About vestibular compensation

Unilateral destruction of vestibular system always leads to a syndrome of postural and oculomotor disorders in rodents due to the disruption of central vestibulo-ocular and vestibulo-spinal pathways (Zhou et al., 2009, 2013; Deveze et al., 2014). However, many of these deficits gradually subside in a process of behavioural recovery with the passage of time, which is described as “vestibular compensation” (Zhou et al., 2013; Darlington et al., 1991). In the present study, we also noted this phenomenon in unilateral vestibular destruction in guinea pigs. As mentioned in the results, around 2–3 days after GM treatment, animals exhibited obvious head tilt in the direction of non-functioning ear. Then, animal head gradually recovered to the normal position by 10–15 days after treatment. Although the balance function of
these animals with unilateral vestibular dysfunction appears normal on the ground, however, when animals were placed into the water under buoyancy environment for swim test, all tested guinea pigs had significant balance dysfunction in water which under buoyancy environment for swim test, all tested guinea pigs had significant balance dysfunction in water which was detected in VGN density between saline and GM treated ears (mean ± SD, n = 6 ears per group).

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