Potential of noise-induced hearing loss by amikacin in guinea pigs

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Abstract

Noise and aminoglycosides initially attack cochlear outer hair cells (OHCs). Distortion product otoacoustic emissions (DPOAEs) are used for the early diagnosis of damage to OHCs. The effects of sub-damaging doses of amikacin, an aminoglycoside antibiotic agent, on noise-induced hearing loss (NIHL) were examined in guinea pigs. Animals were grouped by gender and exposed to broadband noise at 105 dB SPL for 12 h and/or injected i.m. with either amikacin (100 mg/kg/day) or saline for 10 days. Auditory brainstem response (ABR) thresholds, along with DPOAE amplitudes, were measured serially before and after noise exposure. DPOAE amplitudes decreased and ABR thresholds elevated immediately after noise exposure and then gradually recovered. At all frequencies, the emission amplitudes recovered completely to pre-exposure baseline values by 4 days after noise exposure. There was no effect of amikacin on either the ABR threshold or DPOAE amplitudes, in animals treated with amikacin only. However, amikacin significantly prolonged the effect of noise exposure on DPOAE amplitude but not on the noise-induced temporary threshold shift (TTS) of the ABR. In animals treated with a combination of noise and amikacin, significant changes in DPOAE amplitudes were still observed at 4 weeks after cessation of noise exposure. No gender difference in the responses to noise and/or amikacin could be demonstrated. The present findings indicate that even sub-damaging dosages of amikacin might impair recovery from NIHL in guinea pigs. © 2001 Elsevier Science B.V. All rights reserved.

Key words: Distortion product otoacoustic emission; Noise; Amikacin; Outer hair cell; Guinea pig

1. Introduction

Cochlear outer hair cells (OHCs) are known to be the

primary targets of both the ototoxic effects of aminoglycoside antibiotics (Huizing and de Groot, 1987; Go-varts et al., 1990; Wang and Li, 2000) and excessively intense sound (Lim, 1966; Cody and Russell, 1985; Canlon and Fransson, 1995; Shoji et al., 2000a). Otoacoustic emissions (OAEs), originally described by Kemp (1978), are considered to reflect normal cochlear function related to the integrity of the OHC system (Brownell et al., 1985). Moderate noise exposure that gives rise to a temporary threshold shift (TTS) has been shown to alter the amplitude of distortion product otoacoustic emissions (DPOAEs) (Schmiedt, 1986; Sutton et al., 1994). The ototoxic effects of aminoglycoside antibiotics result in initial depression of electrophysiological and audiological measures in the high frequencies and then progression of auditory impairment toward the lower frequencies. It has been demonstrated that $2f_1$ (the lower pure primary tone used for DPOAE

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Abbreviations: OHCs, outer hair cells; DPOAE, distortion product otoacoustic emission; ABR, auditory brainstem response; TTS, temporary threshold shift; NIHL, noise-induced hearing loss; OAE, otoacoustic emission; f_1 , the lower pure primary tone used for DPOAE measurements; f_2 , the higher pure primary tone used for DPOAE measurements; DP-gram, a graph of DPOAE amplitude (in dB SPL) as a function of stimulus frequency (f_2 in this study); SPL, sound pressure level; DSP, digital signal processing; MN1, male guinea pigs treated with noise plus normal saline; FN1, female guinea pigs treated with noise plus normal saline; MA10, male guinea pigs treated with amikacin; FA10, female guinea pigs treated with amikacin; MN1A10, male guinea pigs treated with noise plus amikacin; FN1A10, female guinea pigs treated with noise plus amikacin; GDNF, glial cell line-derived neurotrophic factor

measurements)— f_2 (the higher pure primary tone used for DPOAE measurements) DPOAE testing is preferable to click-evoked auditory brainstem response (ABR) testing for early detection of gentamicin toxicity of the cochlea (Shi and Martin, 1997). In this study, a wider frequency range of DPOAEs was used for investigating OHCs of different turns of the cochlea.

Synergistic toxic effects of noise and aminoglycoside antibiotics on auditory function have been reported by other investigators (Jauhiainen et al., 1972; Brown et al., 1978, 1985; Brummett et al., 1992), with the dosage of aminoglycoside antibiotic administered alone being large enough to produce a small amount of measurable permanent damage, and the sound alone also intense enough to do so. It is difficult to determine if an interaction might occur and result in small or even no permanent ototoxic effects when lower doses of an ototoxic drug and moderate acoustic trauma are administered. In addition, under clinical conditions, patients who need to receive aminoglycoside antibiotics for treatment of their infections usually are not exposed to loud noises during the therapeutic period. It is thus important to know whether low doses of aminoglycoside antibiotics are harmful to patients who have been previously exposed to noise. Therefore, in this study, we attempted to examine the effect of typical clinical uses of amikacin on moderate, reversible, noise-induced hearing loss (NIHL) in guinea pigs.

A previous study in rats showed that there is a gender difference in the susceptibility to aminoglycoside-induced ototoxicity (Mills et al., 1999). Treatment with kanamycin (400 mg/kg/day) for 13 consecutive days caused changes in all test frequencies of DPOAEs in male rats. However, significant changes were found in female rats only after treatment with the same daily dose of kanamycin for 22 consecutive days. Considering that the usual clinical uses of aminoglycoside antibiotics are at lower dosages and that the duration of therapy lasts for 10–14 days, we investigated whether a lower dose (100 mg/kg/day) and a shorter duration (10 days) of aminoglycosides would have a measurable effect on DPOAEs and whether there is a gender difference. In addition, the gender effect on the ototraumatic interaction between amikacin and acoustic exposure was also studied.

The objectives of this study were to investigate and compare: (1) the effect of noise or a lower dose of amikacin on DPOAEs; (2) whether amikacin modifies the temporary effects of noise on cochlear function measured as TTS of ABR and changes in DPOAE amplitude; and (3) whether there is a gender difference in the ototoxic effects caused by amikacin alone or in combination with noise.

The main findings of this study indicate that amika-

cin at sub-damaging doses impaired the recovery of NIHL, thus converting reversible into irreversible changes of DPOAE amplitude. This suggests that physicians need to be cautious when using aminoglycoside antibiotics to treat patients who have previously been exposed to noise.

2. Materials and methods

The use and care of animals reported in this study were approved by National Taiwan University's Animal Care and Use Committee.

2.1. Animals

Thirty-six guinea pigs weighing 250–300 g with Preyer's reflexes and normal ABR thresholds (<25 dB sound pressure level (SPL)) were used in this experiment. The animals were free from both illness and middle ear infections at the time of the study.

Animals were grouped by gender and treatment as follows: (1) male guinea pigs treated with noise plus normal saline (MN1), (2) female guinea pigs treated with noise plus normal saline (FN1), (3) male guinea pigs treated with amikacin (MA10), (4) female guinea pigs treated with amikacin (FA10), (5) male guinea pigs treated with noise plus amikacin (MN1A10), and (6) female guinea pigs treated with noise plus amikacin (FN1A10), with $n=6$ for each group.

2.2. DPOAE measurements

Measurements were performed in a double-walled sound booth (Shing Ho Acoustic Technical Engineering, Taipei, Taiwan). DPOAEs were elicited by two continuous, pure primary tones at frequencies f_1 and f_2 ($f_2:f_1$ was fixed at 1.22), generated by two separate transducers (ER2; Etymotic Research, Elk Grove Village, IL, USA) connected to a digital signal processing (DSP) board (SmartOAE DSP Board for Windows; Intelligent Hearing Systems, Miami, FL, USA). Two short plastic tubes connected the transducer outputs to the OAE probe (ER10B+; Etymotic Research), which also contained a miniature low-noise microphone for emission detection. The tapered end of the probe was extended with a short, soft silicon tip (2 mm long); it was deeply and tightly inserted into the external ear canal. The microphone of the probe system detected the overall acoustic signal in the external ear canal during tone stimulation. Its response was preamplified (+40 dB) then analyzed by the SmartOAE DSP board. The entire stimulation and detection process was automatically controlled using a PC computer driven

by SmartOAE software (Intelligent Hearing Systems, version 3.72). This software, based on Fourier transformation calculation, generated the stimulations through two independent channels, checked the actual levels of stimulating tones, and computed the complex response at frequency $2f_1-f_2$. The spectral resolution of each frequency data point was 4.88 Hz. The stimulus parameters were chosen according to published data about DPOAEs (Probst et al., 1991). The testing frequency range of f_2 was 1.1–17.7 kHz with four frequencies per octave. The two primaries, L_1 and L_2 , were set 65 and 55 dB SPL. Amplitudes of DPOAEs were measured at selected frequencies. A graph of DPOAE amplitude (in dB SPL) as a function of stimulus frequency (f_2 in this study) (DP-gram), was used for reporting DPOAE findings. Data are described with respect to f_2 frequency since the generator site of the $2f_1-f_2$ distortion product has been most closely correlated with the f_2 frequency place in the cochlea (Brown and Kemp, 1984).

2.3. ABR measurements

The hearing status of all animals was evaluated with click-evoked ABR as described previously (Hsu et al., 2000). In brief, an evoked potential system (Nicolet, Spirit, Madison, WI, USA) was used to measure the thresholds of the ABR. The response signals were recorded by subcutaneous needle electrodes. The active electrodes were placed in the vertex and the ipsilateral retro-auricular region with a ground electrode on the neck of the animal. Click sounds (57.7/s) were used to evoke an ABR. The intensity of the stimulus was varied in 5-dB stepwise increments. Threshold was defined as the lowest intensity level at which a response was still visible. At threshold intensity, at least two sequences of recordings were made to confirm response reproducibility.

2.4. Noise exposure

Noise-exposed animals were exposed to broadband noise at around 105 dB SPL for 12 h. During noise exposure, animals were kept in a cage inside a sound-proof room. The broadband noise was produced by an audiometer (Grason-Stadler, model GSI-10, Milford, NH, USA) and presented by a set of loudspeakers on both sides of the cage. The frequency ranges were from 32 Hz to 20 kHz. The SPL of the noise was measured with a sound level meter (Nion, model NA-24, Tokyo, Japan) situated on the cage at the onset and upon completion of noise exposure. The sound intensity varied by 3 dB throughout the exposure field and was measured as 105 dB at the center of the chamber where subjects were placed.

2.5. Experimental procedures

Noise exposure was presented during the first 12 h of day 0. Amikacin (amikacin sulfate, Banyu, Tokyo, Japan), 100 mg/kg/day, or an equivalent volume of saline was administered i.m. to animals starting from day 0 for 10 consecutive days. The control groups were not exposed to noise.

ABR thresholds in response to clicks as well as amplitudes of DPOAEs were obtained in anesthetized animals before noise exposure and/or amikacin administration and on days 0, 1, 2, 4, 7, 9, 14, and 21, as well as on the final 28th day.

2.6. Statistical analysis

Mean DPOAE measurements were calculated for each group and separated by frequency levels. Comparisons among the same test group were made by one-way repeated measures analysis of variance followed by

Fig. 1. Changes in amplitudes of DPOAEs after noise exposure (105 dB SPL, 12 h) in male (A) and female (B) guinea pigs. Stimulus levels were $L_1=65$ and $L_2=55$ dB SPL. Error bars, representing ± 1 S.E.M., are included only for the baseline and day 0 (immediate post-exposure curves in the plots). The dotted line at the bottom of each graph represents the noise floor of the emission recording system. # $P<0.05$ (indicates a statistically significant difference from the baseline for the first 3 days after noise exposure, as explained in the text).

Dunnett's test when appropriate. A P value of less than 0.05 was considered statistically significant. For results shown in Figs. 1–3, error bars, representing ± 1 S.E.M., are only shown for the baseline and day 0 (immediate post-exposure curves in the plots). For results shown in Fig. 4, data are presented as mean $\pm$ S.E.M. For results shown in Fig. 5, error bars are not included. These presentations of results are for the purpose of clarity.

3. Results

Fig. 1 shows the effect of 12-h noise exposure on DPOAE amplitudes in male (the MN1 group) and female (the FN1 group) animals. As shown, both the MN1 and FN1 groups displayed a maximal depression of DPOAE amplitudes on day 0 immediately after noise exposure which gradually returned to the baseline in 4 days. Statistical analyses across all test frequencies of DPOAEs between the baseline and day 4 recovery data indicate no statistical differences in DPOAE amplitudes for most of the frequencies tested. Depressions of emissions for f_2 were noted for the first three post-

Fig. 2. Changes in amplitudes of DPOAEs induced by amikacin (100 mg/kg, i.m., for 10 consecutive days) in male (A) and female (B) guinea pigs. Stimulus levels, error bars, and the dotted line are explained in the legend for Fig. 1.

Fig. 3. Changes in amplitudes of DPOAEs induced by a combination of noise and amikacin in male (A) and female (B) guinea pigs. Stimulus levels, error bars, and the dotted line are explained in the legend for Fig. 1. * $P < 0.05$ (indicates a statistically significant difference from the baseline for all tested days after noise exposure, as explained in the text).

exposure days from 6.3 to 17.7 kHz in the MN1 group and from 3.7 to 17.7 kHz in the FN1 group.

Fig. 2 shows the effect of amikacin on DPOAE amplitudes in male (the MA10 group) and female (the FA10 group) animals. As shown, amikacin, at the doses used in this study, caused no significant changes in DPOAE amplitudes for f_2 in the frequency range tested in either the MA10 or FA10 groups.

Fig. 3 shows the effect of a combination of noise exposure and amikacin on DPOAE amplitudes in male (the MN1A10 group) and female (the FN1A10 group) animals. As shown, the same noise exposure also caused immediate and significant depressions in DPOAE amplitudes for f_2 on day 0 in both the MN1A10 and FN1A10 groups. However, unlike the recovery found in animals receiving noise exposure only, depressions of DPOAE amplitudes persisted until the end of the 28-day observation period in both study groups. Consequently, depressions of emissions for f_2 were observed from 3.7 to 17.7 kHz in the MN1A10 group and from 4.4 to 17.7 kHz in the FN1A10 group for all tested days.

Fig. 4. Changes of DPOAE amplitudes for f_2 at 8.8, 12.5, and 17.7 kHz at the baseline (A), and on days 0 (B), 14 (C), and 28 (D). * $P < 0.05$ (indicates a statistically significant difference between male and female animals).

Fig. 4 compares gender differences in the effect of noise exposure alone, amikacin alone, or their combination on DPOAE amplitudes for f_2 at 8.8, 12.5, and 17.7 kHz at the baseline, and on days 0, 14, and 28. These frequencies and testing days were chosen for comparison because they were in the range of prominent effects induced by these three experimental interventions. As shown, differences between male and female animals in DPOAE amplitudes for f_2 at any selected frequency on any given day (except 17.7 kHz of noise alone group on day 0) did not reach levels of statistical significance.

Unlike the effects on DPOAE amplitudes, these experimental interventions had different influences on the threshold shift of ABR, another test commonly used for auditory functions. Fig. 5 shows the threshold shift of ABR in various study groups over time. As shown, the threshold shift of ABR was observed in all noise exposure groups (the MN1, FN1, MA10N1, and FA10N1 groups), but not in non-exposure groups (MA10, FA10). The ABR thresholds significantly increased after noise exposure on day 0 and gradually recovered to their baselines within 4 days. Although the ABR thresholds on days 1 and 2 in the noise exposure groups seemed to be higher than their baselines, no statistical significance could be detected. Amikacin alone caused

no significant changes in ABR thresholds for the entire 28-day observation period after the first dose in the MA10 and FA10 groups.

Fig. 5. ABR threshold shifts in guinea pigs after noise exposure and/or amikacin administration. For noise and/or amikacin groups (MN1, FN1, MA10N1, FA10N1), ABR thresholds significantly increased on the day of noise exposure. * $P < 0.05$ (indicates a statistically significant difference from the baseline).

4. Discussion

Results of this study clearly demonstrate that DPOAEs are more sensitive than click-ABR in detecting NIHL at higher frequencies. An ideal test for identifying shifts in cochlear function should be highly repeatable and sensitive to minor damage. A previous study concluded that various OAE measures have the potential to distinguish small changes in cochlear function, and hence show promise for monitoring cochlear function in ears exposed to noise or other hazards (Hall and Lutman, 1999). The objectivity of the measurements and the short test time required further enhance the usefulness of OAEs as a cochlear measure (Attias and Bresloff, 1996). Continuous noise exposure has been shown to cause a reduction in DPOAE amplitudes (Eddins et al., 1999). However, in a number of cases, emission shifts were unaccompanied by notable TTSs of ABR. Therefore, it appears that emissions are more sensitive to OHC changes and more appropriate for screening and monitoring ears at risk of NIHL.

DPOAE is also a sensitive measure of drug-induced hearing loss (Brown et al., 1985). Ototoxic amikacin treatment in a chinchilla model (400 mg/kg/day, i.m.) revealed that high-frequency components of DPOAEs change first, followed by lower-frequency components (Kakigi et al., 1998). In this study, although wide frequency ranges of DPOAEs were checked under 65/55 dB SPL stimuli intensities, no significant changes in amplitude of DPOAEs could be demonstrated in amikacin-treated animals, suggesting that the dose of amikacin used was a sub-damaging dose. Likewise, since the changes found in both the MN1A10 and FN1A10 groups were at the higher frequency ranges, these results lend additional evidence to suggest that measurements of DPOAE amplitudes are more sensitive in detecting ototoxic effects of OHCs than are traditional audiometric examinations.

In the present study, noise exposure alone resulted in only temporary changes of DPOAE amplitudes. However, a combination of noise exposure and a sub-damaging dose of amikacin caused marked decreases in DPOAE amplitudes from about the 4–17.7-kHz range, which persisted for as long as 28 days after the acoustic insult. These results suggest that application of amikacin may impair the recovery of hearing loss from noise exposure. This aggravated situation is not unique because carbon monoxide (Fechter et al., 1987; Young et al., 1987; Fechter et al., 1988) and toluene (Johnson et al., 1988, 1990; Morata et al., 1993; Lataye and Campo, 1997) have also been shown to aggravate NIHL. The mechanisms underlying this aggravation of noise-induced OHC damage by aminoglycosides are not yet known. Several possibilities can be suggested. First of all, the involvement of medial olivocochlear efferents

should be considered. It has been shown that protection of the ipsilateral ear from acoustic trauma occurs when the contralateral ear is simultaneously stimulated at the same frequency, but at a lower intensity (Cody and Johnstone, 1982). This protective effect can be blocked by strychnine, a known blocker of the medial efferent system of the inner ear (Puel et al., 1988). Since even a single injection of aminoglycosides can result in rapid elimination of function of this pathway (Smith et al., 1994), whether the impaired functioning of medial olivocochlear efferents during the application of aminoglycoside interferes with the recovery of OHC function after acoustic trauma needs to be clarified.

Another possibility for the impairment of recovery from noise exposure by aminoglycoside is that both of these two factors can increase free radical levels (Yamane et al., 1995; Clerici et al., 1996; Hirose et al., 1997; Lopez-Gonzalez et al., 1999; Sha and Schacht, 1999), which may result in auditory damage (Liu, 1992). Recently, protection against NIHL or aminoglycoside ototoxicity has been shown by inhibition of free oxygen radical-induced lipid peroxidation (Quirk et al., 1994) or by free radical scavengers (Hester et al., 1998; Yamasoba et al., 1999; Ohinataab et al., 2000; Sha and Schacht, 2000). Since the potential cytotoxic effects of free radicals are mitigated by several antioxidant enzymes and scavengers in living tissues, the overall levels of free radicals in living tissues are determined by the balance between the production of free radicals and the cellular antioxidant defense capacity. Amikacin administered immediately after noise trauma might further disturb this balance and impair the recovery from NIHL.

The third possibility is that both noise exposure and aminoglycosides might disturb calcium homeostasis. In guinea pigs, acute acoustic trauma caused morphological changes of cells in the organ of Corti and are correlated with changes in cellular calcium distribution (Maurer et al., 1993). The authors suggested the possible role of calcium in cell damage and necrosis after acute noise trauma. In addition, previous studies (Corrado et al., 1989; Schacht, 1993; Tan et al., 2001) revealed that calcium is involved in a possible mechanism of the acute ototoxic effects of aminoglycosides on OHCs. The role of disturbed intracellular calcium homeostasis by the combination of noise and amikacin is worthy of clarification.

The fourth possibility is that both noise and aminoglycoside-induced ototoxicity might be, in part, an excitotoxic process involving activation of *N*-methyl-D-aspartate (NMDA) receptors (Segal and Skolnick, 1998; Harvey and Skolnick, 1999; Masuko et al., 1999; Segal et al., 1999; Jager et al., 2000). NMDA antagonists have been shown to be involved in protection against noise- or aminoglycoside-induced ototoxicity (Basile et

al., 1996; Duan et al., 2000). It is thus plausible that impairment of NIHL by aminoglycosides might involve the augmentation of noise-induced excitotoxicity.

A final possibility is that neurotrophins are not only essential to the survival of neuron cells (Ernfors et al., 1996; Staecker et al., 1996; Miller et al., 1997), but also important in regulating OHC function. Recently, neurotrophic factors, such as neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) showed differential protective effects in the attenuation of noise-induced (Keithley et al., 1998; Agerman et al., 1999; Yamasoba et al., 1999; Shoji et al., 2000b) or aminoglycoside-induced (Ruan et al., 1999; Yagi et al., 1999) hair cell damage. Since it has been shown that different concentrations of NT-3 or GDNF result in different OHC survival (Shoji et al., 2000a,b), it is possible that in animals exposed to noise and aminoglycosides, changes in the amount of these neurotrophic factors may affect OHC functions. However, the exact mechanisms of noise–drug interactions remain to be determined.

Although gender susceptibility of kanamycin ototoxicity has been claimed by other authors (Mills et al., 1999), no gender differences were demonstrated in the present study. Amikacin and kanamycin are known to possess similar potency of cochleotoxicity (Basile et al., 1996). Therefore, the discrepancy could be due to the fact that we used different animals (guinea pig vs. rat), lower doses (100 vs. 400 mg/kg/day) and shorter duration (10 days vs. 13–21 days) of aminoglycoside antibiotic. These experimental findings certainly infer that, for patients, whether male or female, who are likely to be exposed to loud noises in their environments, subsequent aminoglycoside administration could produce ototoxicity.

In summary, amikacin (100 mg/kg/day, i.m., for 10 days) caused no significant changes in DPOAE amplitudes or ABR thresholds, while broadband noise (105 dB SPL, 12 h) alone only resulted in TTS of ABR and a temporary decrease of DPOAE amplitudes for 4 days. However, amikacin significantly interfered with the recovery from noise-induced changes in DPOAE amplitudes in guinea pigs treated with a combination of noise exposure and amikacin. The present results indicate that the application of aminoglycosides should be carefully monitored by wider frequency ranges of DPOAEs for those patients who have suffered from moderate noise trauma.

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