

Functional and morphological analysis of different aminoglycoside treatment regimens inducing hearing loss in mice

LUKAS HORVATH^{1,2*}, DAVID BÄCHINGER^{1*}, TIM HONEGGER¹,
DANIEL BODMER² and ARIANNE MONGE NALDI¹

¹Department of Otorhinolaryngology, Head and Neck Surgery, University of Zurich, University Hospital Zurich, 8091 Zurich;

²Department of Biomedicine and The Clinic for Otorhinolaryngology, University Hospital Basel, 4031 Basel, Switzerland

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Abstract. Aminoglycoside ototoxicity is common in clinical practice but reliable protective agents currently do not exist. Aminoglycoside regimens causing ototoxicity in different laboratory animals are under investigation. The assessment method used most commonly to determine auditory effects is the auditory brainstem response (ABR). Distortion product otoacoustic emissions (DPOAE) have been used less frequently. A precise recommendation on the specific method to assess peripheral auditory function before and after aminoglycoside toxicity in mice does not exist. In order to evaluate various mouse models for ototoxic injury caused by various aminoglycoside regimens, there is a need for performing preliminary tests in small cohorts before large experiments. The aim of our study was to investigate different aminoglycoside regimens that cause substantial ototoxic damage *in vivo*. Aminoglycosides are safe and produce a detectable hearing threshold shift in a small cohort of mice that can be used as a model for preliminary tests. Different ototoxic regimens were assessed by ABR and DPOAE measurements pre- and post-treatment. Further, the sensory cell loss was quantified by counting hair cells in the cochlea. It was revealed that an ototoxic regimen with kanamycin twice daily for 15 consecutive days is safe, well tolerated and produces an early significant hearing threshold shift detected by DPOAE in a small cohort of mice. The study compared ABR and DPOAE in mentioned regimens for the first time and illustrated that DPOAE is well suited for detecting hearing threshold shifts in high frequencies before ABR threshold shifts occur in accordance with

predominating outer hair cell damage mainly in the basal turn of the cochlea.

Introduction

Hearing impairment is one of the most relevant chronic disorders in humans worldwide with an increasing incidence in industrial countries. According to the WHO, 360 million people currently suffer from disabling hearing loss (1). Congenital deafness, aging, acoustic trauma or ototoxins (e.g., uremic, drug induced) are the most common causes of partial or total hearing loss. Hearing loss due to ototoxic drugs such as aminoglycosides or platinum-based chemotherapeutic agents can be either reversible and temporary or irreversible and permanent. The severity of the effect depends on several factors including the level of the dose, duration of treatment, genetic predisposition of the patient or animal and route of administration (2-4). The hearing loss is typically sensorineural, bilateral and progressive (5). Hearing loss is a major limiting factor in the clinical use of aminoglycosides and represents one of the main preventable causes of deafness (6-8). An otoprotective compound is yet to be found.

Early and accurate detection of cochlear damage during aminoglycoside administration is a major concern for health care professionals. The treatment of choice to prevent drug-induced ototoxicity is once-daily dosing and careful monitoring of serum drug concentrations as well as auditory testing. At present, finding otoprotective compounds is of vast interest. Cochlear hair cell damage *in vitro* by aminoglycosides such as Gentamicin and Kanamycin has been described in literature by several different research groups and is an established known phenomenon. *In vivo* experiments are performed to investigate drug-induced cochelotoxicity and tolerance while co-morbidities ought to be avoided. In order to research otoprotection in aminoglycoside-induced hearing impairment, a reliable model for preliminary tests performed in small cohorts needs to be established. Common experimental animals have included guinea pigs, rats and chinchillas. The larger rodents' pharmacokinetic profiles differ significantly from those of small rodents such as mice (9-11). In the past, large rodents have been used not only because of their body size and easier breeding or surgical intervention but also because of the greater drug-induced toxicity of their

Correspondence to: Dr Arianne Monge Naldi, Department of Otorhinolaryngology, Head and Neck Surgery, University of Zurich, University Hospital Zurich, Frauenklinikstrasse 24, 8091 Zurich, Switzerland
E-mail: arianne.monge@kispi.uzh.ch

*Contributed equally

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auditory system compared to mice (3,12,13). Nevertheless, mice are widely used and suitable as research models and can be genetically modified. Genetic standardization and relative ease of engineering make the mouse a preferred model for hearing research *in vivo*, especially when studied at a young age (up to 20 weeks) (5,12). Furthermore, the inner ear anatomy of mice is similar to that of humans (14). The mouse has served as a successful model in inner ear research, including auditory and vestibular disorders (15), inner ear development, noise-induced hearing loss and drug-induced ototoxicity (13,16–18). Yet, mouse models have shown high resistance to aminoglycoside-induced cochlear damage (13). Some researchers have combined aminoglycosides and loop diuretics to take advantage of their synergistic effect to increase the rate of cochlear injury (17,19–22).

To identify a mouse model for cochlear injury, different research groups (5,9,12,13,16–18,22,23) have investigated aminoglycoside ototoxicity in different strains of mice, which has yielded variable results for similar drug dose regimens. Also, similar dose regimens in identical mouse strains have produced dissimilar results. Kendall *et al.* (24), have suggested the use of C57BL/6J strains in auditory research instead of C57BL/6N, as the latter have genetically drifted within their subpopulation and show irregular auditory thresholds among different populations. A robust *in vivo* mouse model for aminoglycoside-induced ototoxicity remains to be established. The most commonly used method to assess functional auditory effects is the auditory brainstem response (ABR). Less frequently, distortion product otoacoustic emission (DPOAE) have been used. Wu *et al.* (13), presented an adult mouse model for kanamycin ototoxicity using ABR only. We, however, assessed different dose regimens for gentamicin- and kanamycin-induced ototoxicity, that is capable of causing substantial cochlear damage in young mice while maintaining a low mortality rate and being able to detect cochlear damage in a small cohort, which can be used as a model for future preliminary cochelotoxic tests.

Materials and methods

Animals. Four- to 6-week-old C57BL/6J mice supplied by Charles River (Charles River, Freiburg im Breisgau, Germany) were used to assess the optimal aminoglycoside dose regimen needed to induce ototoxic hair cell injury for purposes of research. Mice were divided and housed in cages (Type 2 IVC; Allentown Inc., Allentown, PA, USA) in groups of four (M1–M4), with wood shaving litter (Lignocel select premium; J. Rettenmaier & Söhne GmbH, Rosenberg, Germany) under standard conditions: 12:12 h photoperiod, room temperature between 20 and 22°C, relative humidity of 45–55% and 20–22 air changes per hour. Standard mouse food (cat. no. 3436, Kliba Nafag Provimi Kliba AG, Kaiseraugst, Switzerland) and water were available *ad libitum*. All mice were allowed to acclimatize to the animal facility for at least one week before testing was begun. Health monitoring was performed following FELASA recommendations (25). All animal procedures were carried out according to our approved animal research protocols (permission number 19/2011, Veterinary Department of Zurich, Kantonales Veterinäramt Zürich, Switzerland). Due to the previously described high mortality rates with systemic

gentamicin administration (5), the local veterinary department (Kantonales Veterinäramt Zürich, Switzerland) permitted us to use a limited cohort of animals (n=16) for the experiments. Three groups received different aminoglycoside treatment protocols including gentamicin, kanamycin and kanamycin plus furosemide. The fourth group served as a control and received no treatment. Every mouse was checked daily during the experiment for weight and activity behavior.

Drug administration. The details of dosing and timing are listed in Table I and Fig. 1A. Mice from each experimental group were treated with the corresponding protocol. Kanamycin (Sigma Aldrich, Buchs, Switzerland) was dissolved in physiological saline to obtain the desired concentration according to the instructions provided by the supplier. Each mouse from the gentamicin (Hexal, Holzkirchen, Germany) group received a once-daily dose by intraperitoneal (i.p.) drug administration at 8:00 a.m. for 7 days. Each mouse from the kanamycin group received twice daily subcutaneous (s.c.) drug administration at 8:00 a.m. and 8:00 p.m. for 15 days. Kanamycin plus furosemide (Sanofi-Aventis, Vernier, Switzerland) was administered as a single dose in which furosemide was infused into the tail vein within 5 min after kanamycin s.c. injection. The dosage administered was selected on the basis of previously published and weight-adapted ototoxic protocols using gentamicin at 200 mg/kg body weight (BW) (12), kanamycin at 800 mg/kg BW (5), kanamycin plus furosemide at 1,000 mg/kg BW plus 100 mg/kg BW (22). The injections were adjusted daily according to body weight and were administered using a 1-ml syringe and 25-gauge needle in a standardized manner by the same person in the same setting, at the same time each day.

Measures of auditory function. The ABR and DPOAE measurements were performed on all animals before starting the treatment protocols, at 1-week and the two groups with kanamycin were also tested at 3 weeks post-treatment (Fig. 1A). The DPOAE were performed one day after ABR measurements, therefore in two separate anesthetics. Mice were anaesthetized by i.p. injection of a combination of ketamine (65 mg/kg body weight, Graeb, Switzerland), xylazine (13 mg/kg body weight, Bayer HealthCare, Leverkusen, Germany) and acepromazine (2 mg/kg body weight, Fatro S.p.A., Ozzano dell'Emilia, Italy). After loss of the withdrawal reflex, the animals were placed on a heating pad in a soundproof chamber. Photos showing the method for hearing assessment are provided as supplementary material (Fig. S1).

ABR testing. Hearing was evaluated by ABR as previously described (26). Needle electrodes were inserted subcutaneously at the vertex (active electrode), in the ipsilateral mastoid region (reference electrode) and in the lumbar region (ground electrode). Tone bursts of 4, 8, 12, 16, 24, 32 kHz were generated with SigGenRP software (Tucker-Davis Technologies TDT, Gainesville, FL, USA). The stimuli were delivered through a closed acoustic system and were calibrated using a sound level meter (Precision Integrating Sound Level Meter Type 2218; Brüel & Kjaer, Naerum, Denmark) and an ear simulator (Type: 4157, Brüel & Kjaer, Naerum, Denmark). Sounds were delivered from a free-field electrostatic speaker (ES1, TDT) placed into the ear canal. The ABR recordings were

Table I. Treatment regimens and mortality rate.

Drug/Group	Dose, rate, route	No. of deaths/No. of injected mice (mortality rate) (%)
Kanamycin	800 mg/kg/12 h, 15 days, s.c	0/4 (0)
Kanamycin + furosemide	1,000 mg/kg, s.c. + 100 mg/kg i.v.	0/4 (0)
Gentamicin	200 mg/kg/24 h, 7 days, i.p.	2/4 (50)

Dose per kilogram (kg) body weight. i.p., intraperitoneal; s.c., subcutaneous; i.v., intravenous.

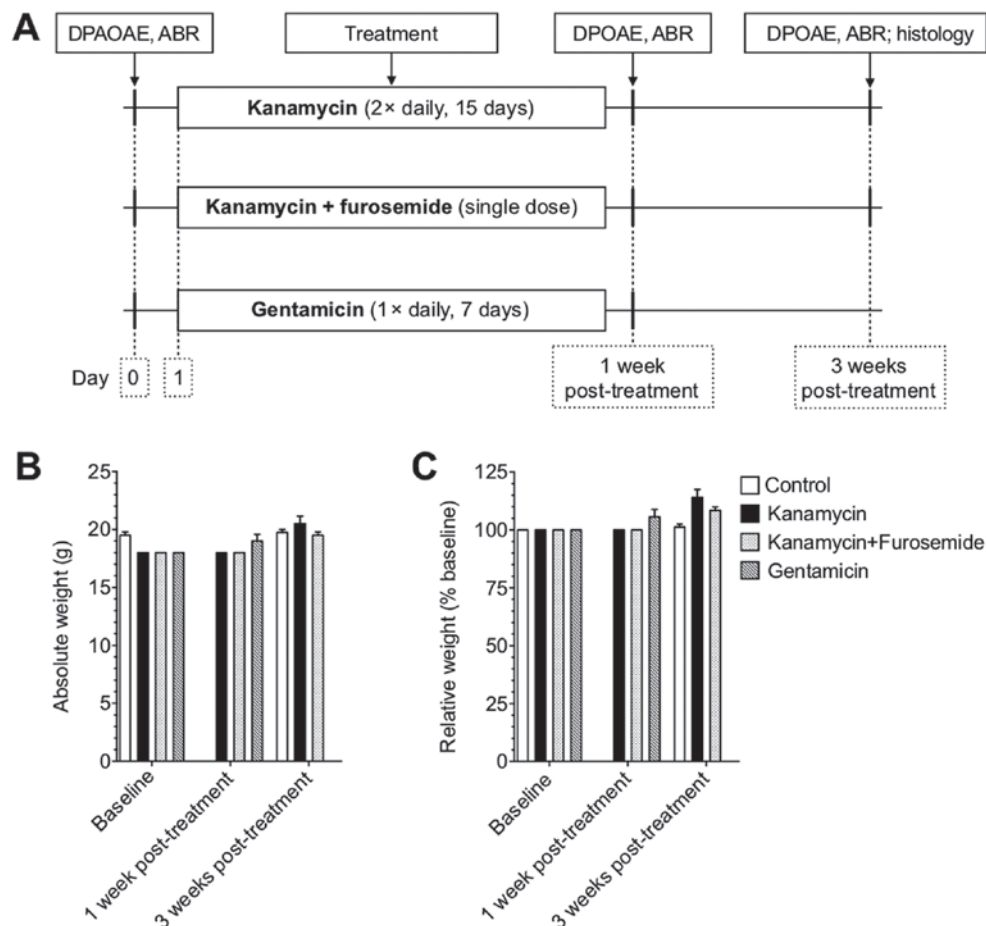


Figure 1. (A) Schematic diagram describing the experimental procedure and timeline of hearing assessment by ABR and DPOAE at 1 day pre-treatment, 1 and 3 weeks post-treatment, following sacrifice and histological studies. Treatment groups are listed by drug name. (B) Absolute and (C) relative weight in grams of mice during treatment period. ABR, auditory brainstem responses; DPOAE, distortion product otoacoustic emissions.

obtained with a Tucker-Davis Technologies (TDT) System III workstation running BioSig RP. The tone-type sounds of 5 ms duration were presented at a rate of 10 per second and reduced in level from 80 dB SPL to 5 dB SPL in 5-dB steps. The ABR waveforms were averaged in response to 300 tones. Hearing threshold was defined as the lowest level that induced the appearance of a visually detectable peak in the response waveform.

DPOAE testing. DPOAE at 2f1-f2 were obtained 24 h following ABR recordings from mice that were anesthetized as described above. We used the Real-time Signal Processing System III from Tucker-Davis Technologies and procedures described previously (27). The primary tones produced by

two separate speakers (ES1, TDT) were placed as a combination microphone/speaker system in the animal's sealed ear canal near the tympanic membrane. The DPOAE recordings were made with a low-noise microphone ER 10B (Etymotic Research, Elk Grove Village, IL, USA). All stimuli were digitally synthesized at 200 kHz using TDT SigGen software. Primary tone frequencies (f1 and f2) differed by a factor of 1.25 and were presented initially at 65- and 50-dB SPL respectively. The test frequencies were at 4, 8, 16, 32 kHz and levels were reduced in 10-dB steps from 80 to 30 dB. A fast Fourier Transform (FFT) was performed to obtain the magnitude of the 2f1-f2 distortion product. A peak at 2f1-f2 in the spectrum was accepted as a DPOAE if it was 6 dB above the noise floor in the same frequency.

Hair cell count. Mice were transcardially perfused with freshly prepared 4% paraformaldehyde dissolved in PBS (pH 7.2) during anesthesia. The cochleae were removed and the round and oval windows were opened. The cochleae were postfixed for 48 h in the same fixative, decalcified for 96 h in RDF Mild Decalcifier (CellPath Ltd., Newtown Powys, Wales, UK) and embedded in paraffin. Serial sections (2 μ m) were cut using a HM 355S Automatic Microtome (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Sections were collected on SuperFrost Plus slides (Thermo Fisher Scientific, Inc.), dried on a heating plate at 37°C overnight and stained with H&E. Observer-blinded inner hair cell (IHC) and outer hair cell (OHC) counts at each of the basal, middle and apical cochlear turn were performed on para-midmodiolar sections obtained from five non-overlapping 20- μ m segments in each cochlea. For each segment, three consecutive sections were mounted and the best-preserved section was used for counting. The presence of a hair cell was assumed if a nucleus was clearly visible in the typical anatomical location next to the tunnel of Corti. Additionally, hair cells were distinguished from other cells, e.g., supporting cells, by their denser and therefore darker stained nuclei. Images were acquired using an AxioCam ICc5 (Carl Zeiss Microscopy GmbH, Jena, Germany) on a Leica DM RB light microscope (Leica Camera AG, Wetzlar, Germany) and processed with Adobe Photoshop CS5 software (v.12.0, Adobe Systems, San Jose, CA, USA).

Statistical analysis. ABR, DPOAE and hair cell counts were analysed by two-way ANOVA with Dunnett's multiple comparison testing using Prism (v.6.0 for Apple Macintosh, GraphPad Software, San Diego, CA, USA). Data are presented as mean \pm SEM. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Systemic administration of kanamycin is a safe method to cause hearing damage in young mice. The administration of kanamycin in two different regimens (Table I) yielded a mortality rate of 0%. None of the animals appeared ill and no weight change $>15\%$ was observed during and after treatment (Fig. 1B and C). In contrast, systemic administration of previously reported ototoxic doses of aminoglycosides (5,12–22), has been associated with adverse effects. The gentamicin-group had the highest systemic toxicity with a mortality rate of 50%. One mouse was found dead within 24 h after the first injection and another mouse died within 24 h after the last injection on day 7.

Hearing assessment. Hearing impairment due to ototoxic effects was assessed by measuring frequency-specific ABR and DPOAE threshold before and after antibiotic treatment. After conferring with the veterinary department, the gentamicin group experiment was dismissed on Day 7 after occurrence of a 50% mortality rate. The control group was measured on day 0 and day 21. At baseline, average hearing threshold of all mice was 13 dB SPL (range \pm maximum 15 dB) for the 16 kHz tone ABR, where mice are reported to hear most sensitively (28).

DPOAE recordings showed significant threshold shifts in the higher frequencies at early stage. The kanamycin regimen caused significant cochlear damage, which was detectable by DPOAE, showing threshold shifts in the higher frequencies (8, 16 and 32 kHz). Combined with furosemide as a single dose, a significant threshold shift was detected at 32 kHz. In the gentamicin group, DPOAE were not significantly altered post treatment (Fig. 2A–C). The control group had no significant changes in DPOAE threshold.

ABR recordings showed a tendency toward an increase of hearing thresholds. The overall threshold shift between pre- and post-treatment for the kanamycin only group (Fig. 2D) ranged from 12–18 dB (mean 14.3 dB). The kanamycin-furosemide group (Fig. 2E) had overall threshold shifts of 5–13 dB (mean 9.6 dB) between pre- and post-treatment. The gentamicin group (Fig. 2F) as well as the control group showed no substantial threshold shifts. When focused on individual animal threshold measurements, the shifts showed a heterogenic variance between the mice and between the two ears of the same mouse (data not shown). No statistical significant threshold shifts were observed in any group by ABR, however ABR show a trend for hearing threshold elevation after kanamycin-induced ototoxicity.

Kanamycin treatment causes a significant hair cell loss in the organ of Corti. Histological evaluation of the cochleae revealed a considerably reduced amount of OHC per organ of Corti (OC) in the kanamycin-only group, especially in the basal turn, compared to the control group, where no OHC loss was observed (Fig. 3). No IHC loss was found in any group and no OHC loss was found in the gentamicin group. To quantify these changes, we performed IHC and OHC counts on sections of 3 animals per group (Fig. 4). OHC loss in the kanamycin group was predominant in the basal turn of the cochlea (1.4 ± 0.2 OHC/OC vs. 3.0 ± 0.1 OHC/OC, $P < 0.0001$), less marked in the middle turn (2.3 ± 0.2 OHC/OC vs. 2.9 ± 0.1 OHC/OC, $P < 0.05$) and only a slight hair cell loss was observed in the apical turn (2.6 ± 0.16 OHC/OC vs. 3.1 ± 0.1 OHC/OC, $P < 0.05$). No significant OHC loss was found in the kanamycin-furosemide and the gentamicin group, except in the apical turn, where the kanamycin-furosemide produced a minor but significant OHC loss compared to the control group (2.6 ± 0.16 OHC/OC vs. 3.1 ± 0.1 OHC/OC, $P < 0.05$). No significant IHC loss was found in any treatment group compared to the control group.

Discussion

Hearing impairment caused by cochleotoxic agents is a prevalent clinical issue. Ongoing studies are trying to find protective agents against ototoxicity. The mouse has served as a consistent model to study the inner ear *in vivo*. So far, there have been no precise recommendations regarding ototoxic regimen in a mouse model, fulfilling requirements such as being simple to perform, safe and causing substantial inner ear damage. In order to evaluate various mouse models for ototoxic injury caused by various aminoglycoside regimens, preliminary tests in small cohorts need to be performed preceding large scaled experiments. The aim of our study was to investigate different aminoglycoside regimens that cause a

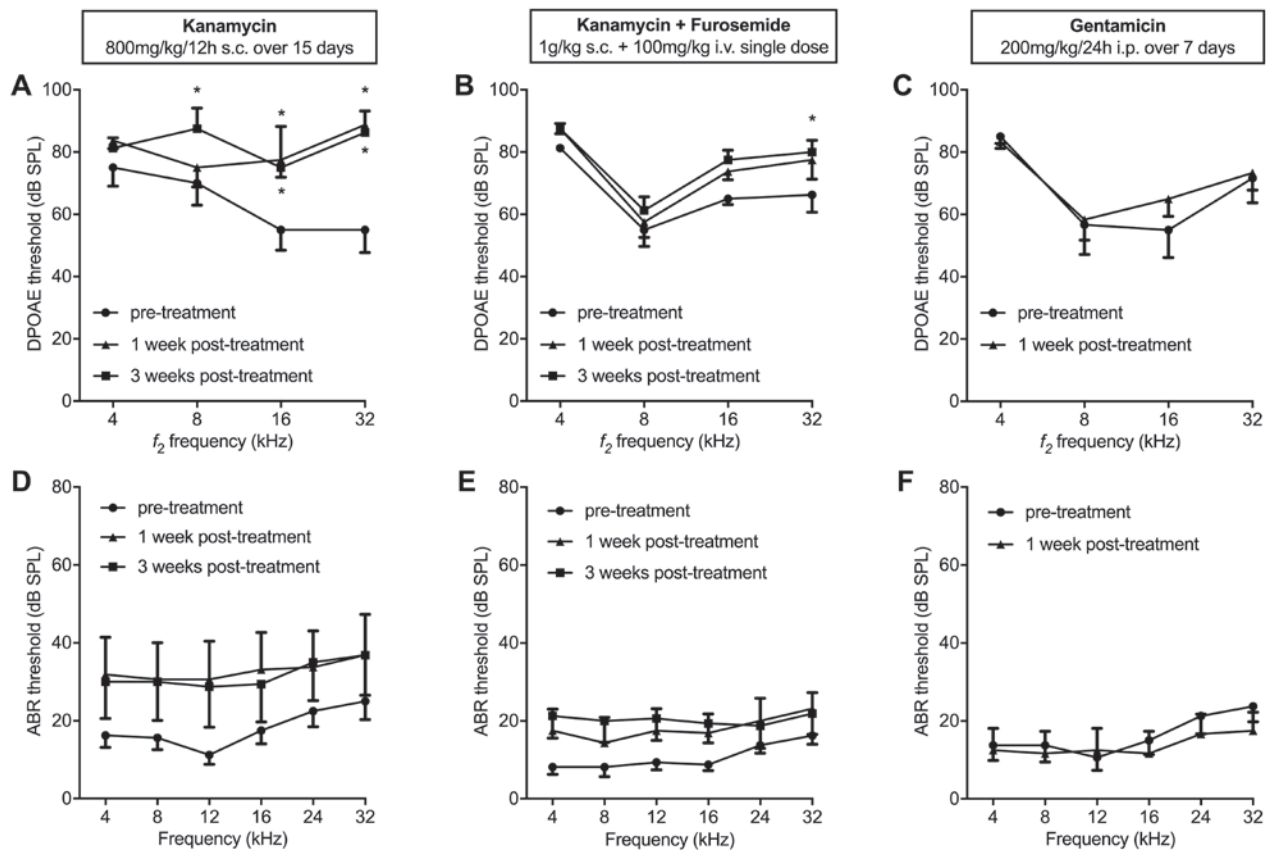


Figure 2. DPOAE overall threshold (dB SPL) in mice at pre-treatment and post-treatment. (A) Kanamycin group, (B) Kanamycin + furosemide group and (C) Gentamicin group. Frequency-specific ABR overall threshold (dB SPL) in mice at pre-treatment and post-treatment. (D) Kanamycin group, (E) Kanamycin + furosemide group and (F) Gentamicin group. Significant threshold shifts are marked with an asterisk. Data are presented as the mean \pm SEM. * $P < 0.05$ vs. controls. DPOAE, distortion product otoacoustic emissions; ABR, auditory brainstem responses; dB SPL, decibel sound pressure level; s.c., subcutaneous; i.v., intravenous; i.p., intraperitoneal; kHz, kilohertz.

substantial ototoxic damage *in vivo*, which are simple, safe and produce a detectable hearing threshold shift already in a small cohort of mice. We show that an ototoxic regimen with kanamycin twice daily for 15 consecutive days is simple to employ, well tolerated and produces a significant early hearing threshold shift in a small cohort of mice. At the same time, the small cohort itself and the lack of saline injection of the control group is a limitation to our study. We demonstrate a higher sensitivity of DPOAE, as thereby a hearing threshold shift is detectable earlier in high frequencies and correlates with the outer hair cell damage mainly in the basal turn of the cochlea. The known target of aminoglycoside ototoxicity are the hair cells of the organ of Corti, which are responsible for the mechano-sensory transduction of sound in the auditory system (29). These vulnerable hair cells are not capable of regeneration, and their damage leads to lifelong hearing impairment. ABR have been used to characterize the auditory system of various mouse strains and mutants. The DPOAE serve as a noninvasive tool to assess cochlear function, specifically that of the vulnerable OHCs. While ABR have been used as the main non-invasive method to assess auditory function in mouse models due to the simplicity and reliability of the method, DPOAE can provide a more direct means of assessing peripheral function by assessing OHC functional changes. Aminoglycosides have a narrow therapeutic range causing large spreads in hearing thresholds

leading to high standard deviations. Mice have proven to have a high resistance to aminoglycoside-induced hair cell loss and to cochlear damage in general (18). Before appreciable alterations in auditory function after aminoglycoside treatment was detected, our mouse cohorts required close to lethal doses for treatment, with the gentamicin group showing obvious systemic toxic effects. We chose to study the C57BL/6 strain because it is widely used as a transgenic modified mouse model and is therefore interesting as an *in vivo* animal model, especially to study aminoglycoside-induced ototoxicity. Age is also a factor, as young mice are more ideal as aminoglycoside ototoxicity models because they have a higher sensitivity to aminoglycosides than in adult life (12,13). Poirrier *et al* (9), hypothesized that genetic and environmental differences between mouse strains influence responses to various toxins including ototoxins. Researchers have been combining aminoglycosides and loop diuretics to study the loss of hair cells in rodents and describe a significant increase in hearing loss (16-18,23-30). The synergistic effects of a loop diuretic and an aminoglycoside occur because the aminoglycosides pass the blood-brain barrier allowing to spread effectively through the cochlea (31,32) combined with a decreased renal clearance (33). Because our kanamycin plus furosemide group received only a single dose and showed no significant hearing loss nor hair cell damage, we conclude that this dosage is insufficient in producing enough cochlear damage. In the

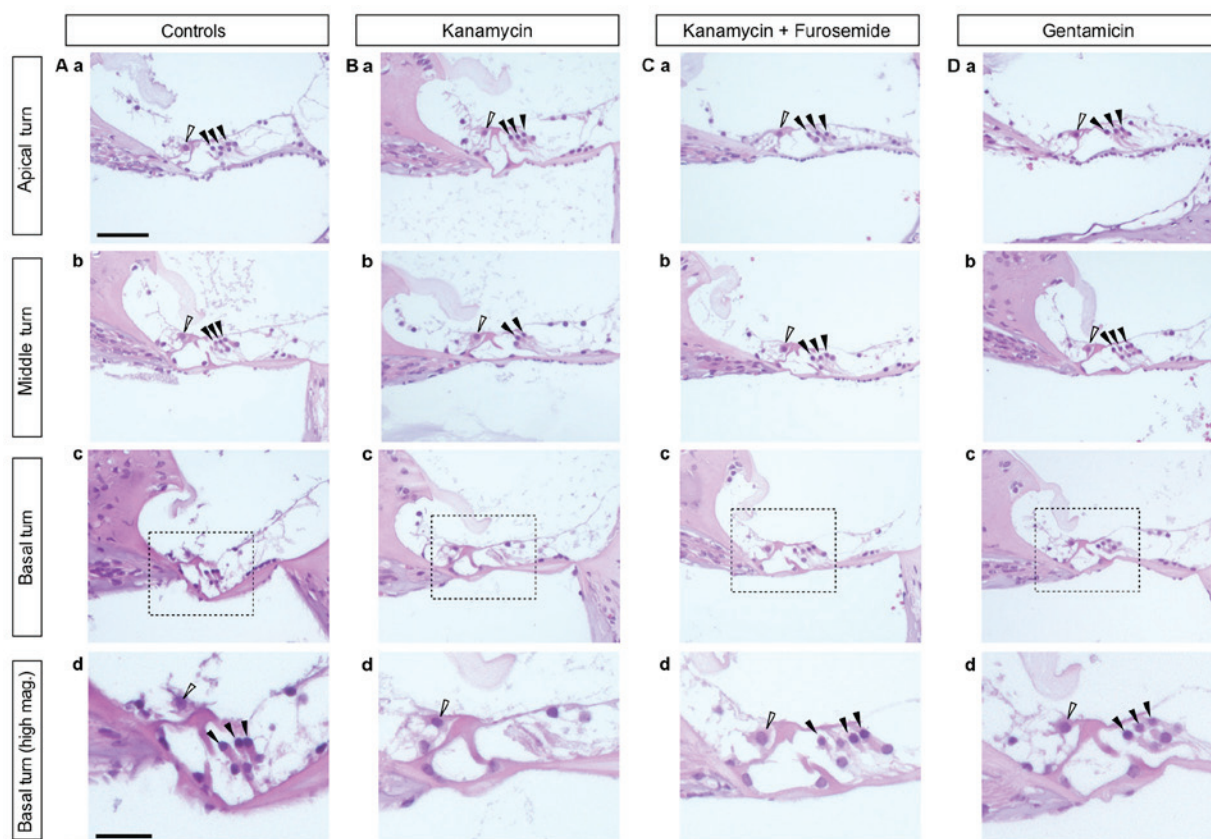


Figure 3. Representative images of the organ of Corti from bright-field microscopy of midmodiolar sections. (Aa–Ac) Control group with full hair cell preservation. (Ba–Bc) Kanamycin group demonstrating OHC loss predominantly in the basal turn of the cochlea. (Ca–Cc) Kanamycin + furosemide group with full hair cell preservation. (Da–Dc) Gentamicin group with full hair cell preservation. Ad–Dd show basal turns in high magnification. White arrow head, IHC; black arrow head, OHC; scale bar, 50 μ m. IHC, inner hair cells; OHC, outer hair cells; high mag., high magnification.

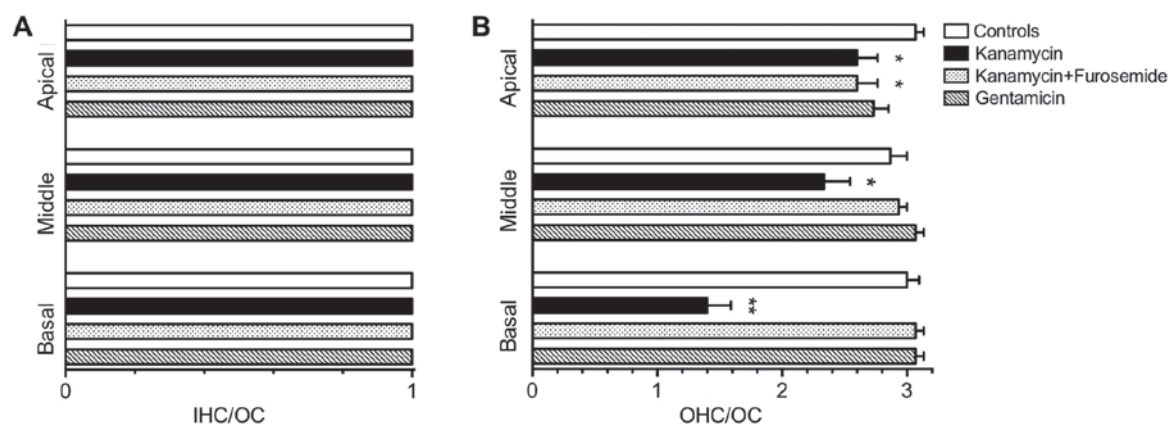


Figure 4. Counts of hair cells per OC examined for the apical, middle and basal turn of the cochlea. (A) IHC and (B) OHC counts for all groups. Results of the treatment groups were compared to those of the control group. Data are expressed as the mean \pm SEM. Error bars, absolute value of SEM. * $P < 0.05$ vs. controls; ** $P < 0.0001$ vs. controls. Dunnett's test to adjust for multiple comparisons. IHC, inner hair cells; OHC, outer hair cells; OC, organ of corti.

inner ear, damage is first evident as a loss of OHCs at the base of the cochlea, spreading further towards the apex with continued drug treatment (7). Clinically, the ototoxic effect of aminoglycosides is characterized by a hearing loss initially in the high frequencies corresponding to hair cell damage in the lower basal turn (7,34). We also found early detection of hearing threshold shifts in the high frequencies with kanamycin only and with kanamycin plus furosemide in a small cohort. DPOAE were able to detect these threshold changes early. Tan *et al* (35) examined the effect of sub-damaging

aminoglycoside doses on noise-induced hearing loss in guinea-pigs using DPOAE and ABR before and after treatment. Aksoy *et al* (36,37) assessed hearing in rats before and after amikacin and trimetazidine or betahistin application. Moreover, Shi *et al* (38) were one of the first to demonstrate that DPOAE are preferable to ABR testing in the early detection of gentamicin toxicity of the cochlea in guinea pigs. They suggest that DPOAE provide earlier detection of cochlear damage caused by gentamicin than do ABRs. Kakigi *et al* (39) compared ABR, DPOAE and transiently evoked otoacoustic

emissions (TEOAE) in chinchillas treated with aminoglycosides. Their results suggest that DPOAE can be used to monitor hair cell function more accurately at specific anatomical locations than can the other methods. Peguero *et al* (40) assessed hearing with both methods in different mouse strains (CBA/CaJ, 129S6/SvEvTac and 101/H). They determined that DPOAE detect early-onset OHC dysfunction despite normal ABR thresholds. It was evident in our study that DPOAE revealed hearing impairment early, while ABR still did not show any significant elevation of thresholds. Furthermore, we showed that Kanamycin alone for a prolonged application is able to cause damage in this small cohort of mice. A number of research groups have attempted to find an aminoglycoside inner ear damage regimen for rodents using different animals and strains, various drugs, doses and application methods. They have used primarily ABR for this purpose.

Three mouse groups living under identical conditions received different drug regimens to induce cochlear damage by systemic administration of high aminoglycoside doses. Significant threshold changes after kanamycin treatment were detected by DPOAE in the high frequencies (8, 32 and 16 kHz). ABR was not able to detect significant threshold changes after 3 weeks. The kanamycin group had an overall shift of 12-19 dB SPL. The combination of kanamycin and furosemide lead to threshold shifts at 32 kHz. None of the kanamycin-treated groups had any deaths during or after the experiments. Histological examination and hair cell counts particularly showed a marked hair cell loss in the basal turn of the cochlea in mice treated with kanamycin, which in accordance with the functional measurements. The DPOAE measures detected hearing impairment primarily in the high frequencies, corresponding to the basal cochlear hair cells, defining OHC damage. While ABRs reflect the summed activity of the peripheral neuronal auditory system, DPOAEs are generated presynaptically and depend only on the integrity of the OHC. Gentamicin at 200 mg/kg/24 h caused no significant threshold shift in DPOAE and ABR and was unsafe causing a high mortality rate. ABR showed a tendency towards hearing threshold shifts. While DPOAE are able to detect cochlear damage in a small cohort already, we postulate that a larger cohort is needed to show significant changes with ABR. Therefore, DPOAE can be used in preliminary experiments finding cochlear damage in early stage and Kanamycin is a preferred method for cochlear damage in mice.

In summary, the present study compares for the first time DPOAE and ABR with aforementioned aminoglycoside regimens and demonstrates that Kanamycin treatment is a simple, reliable and safe regimen to induce damage to auditory function. Despite a small cohort and high-dosed administration period, the mortality rate was 0%. Significant hearing threshold changes were produced by the kanamycin regimen in a small cohort of mice and detected by DPOAE in early stage. DPOAE can therefore be used for early detection of outer hair cell damage induced by Kanamycin 800 mg s.c. 2x/day for 15 days.

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Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

LH, DBa and AMN designed the study. LH performed drug administration, ABR and DPOAE testing. DBa and TH performed the hair cell count. DBa performed the statistical analysis. DBo contributed to the designing the study and revising the manuscript critically for important intellectual content. LH and DBa wrote the manuscript. The final version of the manuscript has been read and approved by all authors.

Ethics approval and consent to participate

The study has been granted ethics approved with consent to participate by the Veterinary Department of Zurich (permission number 19/2011, Kantonales Veterinäramt Zürich, Switzerland).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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