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Ototoxic drugs: Difference in sensitivity between mice and guinea pigs

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ABSTRACT

The development of experimental animal models has played an invaluable role in understanding the mechanisms of neurosensory deafness and in devising effective treatments. The purpose of this study was to develop an adult mouse model of ototoxic drug-induced hearing loss and to compare the ototoxicity in the adult mouse to that in the well-described guinea pig model. Mice are a powerful model organism, especially due to the large availability of antibodies, probes and genetic mutants. In this study, mice ($n = 114$) and guinea pigs ($n = 35$) underwent systemic treatment with either kanamycin or cisplatin. Auditory brainstem responses showed a significant threshold shift in guinea pigs 2 weeks after the beginning of the ototoxic treatment, while there was no significant hearing impairment recorded in mice. Hair cells and neuronal loss were correlated with hearing function in both guinea pigs and mice. These results indicate that the mouse is not a good model for ototoxicity, which should be taken into consideration in all further investigations concerning ototoxicity-induced hearing loss.

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1. Introduction

In mammals, hearing largely depends on the presence of outer and inner sensory hair cells in the organ of Corti and of spiral ganglion neurons. There are multiple aetiologies that are responsible for neurosensory hearing loss (Billings and Kenna, 1999; Morzaria et al., 2004), which has led to numerous attempts to develop appropriate animal models aimed at understanding the pathophysiology and developing protective strategies. Experimentally, the loss of hair cells and/or spiral ganglion neurons can be induced either by pharmacological treatments or repetitive exposure to noise trauma. The drugs that most commonly affect the cochlea are aminoglycosides and cisplatin.

Aminoglycoside antibiotics are largely used in clinical practice, especially in multiresistant Gram-negative infections and tuberculosis. In the organ of Corti, aminoglycosides, such as kanamycin, interact with membranous phosphatidyl inositol biphosphate, which could be the first possible step in hair cell damage. Subsequent caspase-mediated apoptosis and deafness are induced by free-radical generation and iron mobilisation (Forge and Schacht, 2000; Schacht, 1999). Cisplatin is a platinum compound that is

responsible for poisoning hair cells, auditory neurons and stria vascularis (Rybak et al., 2007; van Ruijven et al., 2004). Reactive oxygen species are known to play a pivotal role in cisplatin-induced ototoxicity (Rybak, 2007; Rybak and Whitworth, 2005).

The apoptotic death of outer hair cells has emerged as a final common pathway in response to these "ototoxicants". The initial loss of outer hair cells in the cochlear basal turn is followed by a progressive loss of inner hair cells in a baso-apical direction. This hair cell loss causes secondary degeneration of auditory neurons as a result of the elimination of trophic support (Humes, 1999; Rizzi and Hirose, 2007; Rybak and Whitworth, 2005; Rybak et al., 2007). Evidence for direct cisplatin-induced neuronal toxicity has also been reported (Banfi et al., 2004; van Ruijven et al., 2004).

Animal models have played a crucial role in the development of otoprotective drugs. In vivo studies allow the observation of intricate interactions between specialized cells of the inner ear, which are required for hearing and balance functions. Typically, the cochlear effects of ototoxic drugs are studied in the guinea pig, which is the most common animal model used in hearing research (Aran et al., 1999; Rybak and Whitworth, 2005). Studying ototoxicity in mice is of the utmost interest because it allows for more flexibility and offers the potential for transgenic studies (Brown et al., 2008). In an attempt to establish a reliable mouse model of deafness, we examined the effects of kanamycin and cisplatin on auditory function in young adult (4-week-old) and adult (9-week-old) mice in comparison to the well-established guinea pig model. The primary endpoint of this study was hearing function, which was

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Table 1
Experimental groups.

Specie	Age	Treatment	Way of administration	Cumulative dose	n
Mouse	9 weeks	Saline during 14 days	Intraperitoneal		10
		Kanamycin low dose (LD) during 14 days	Subcutaneous	19.6 g/kg	5
			Intraperitoneal		12
		Kanamycin high dose (HD) during 14 days	Intraperitoneal	28 g/kg	12
	Cisplatin during 10 days	Subcutaneous	20 mg/kg	5	
		Intraperitoneal		10	
	4 weeks	Saline during 14 days	Intraperitoneal		10
		Kanamycin low dose (LD) during 14 days	Intraperitoneal	19.6 g/kg	10
		Kanamycin high dose (HD) during 14 days	Intraperitoneal	28 g/kg	20
		Cisplatin during 10 days	Intraperitoneal	20 mg/kg	20
Guinea pig	9 weeks	Saline during 7 days	Intraperitoneal		4
		Kanamycin during 7 days	Intraperitoneal	2.8 g/kg	15
		Cisplatin during 10 days	Intraperitoneal	20 mg/kg	16

A summary of all experimental groups (species, age at the beginning of the treatment, drug administration and number of animals in each group). Control groups received saline.

assessed by recording auditory brainstem responses. Histopathological phenotypes were analysed as secondary endpoints.

2. Materials and methods

2.1. Drugs

Kanamycin was obtained from Sigma–Aldrich (St. Louis, MO, USA, Kanamycin sulphate, reference # K1377). According to the manufacturer's specification, the preparation of kanamycin contains more than 95% kanamycin A. Cisplatin was obtained from Sigma–Aldrich (Cis-Platinum (II) Diamine Dichloride, reference # P4394). Control animals were injected with the same volume of vehicle.

2.2. Animals

BALB/c male mice (purchased from the animal facility of the University of Liège) and Dunkin Hartley guinea pigs (Harlan, Horst, The Netherlands, reference Hsd-Poc:DH) were maintained on a 12-h light–dark cycle and had unrestricted access to water and food. All experimental protocols were performed in accordance with the Animal Welfare Committee of the University of Liège.

2.3. Treatments

Animals were randomly divided into 11 groups, as summarized in Table 1. Animal body weights were recorded daily at the time of dosing. At either 4- or 9-week-old, mice were treated as follows: (1) saline for 14 days ($n = 10$ at both ages), (2) a "low dose" of kanamycin, 700 mg/kg intraperitoneal (i.p.) or subcutaneous (s.c.) injection twice daily for 14 days, (3) a "high dose" of kanamycin, 1.4 g/kg i.p. injection twice daily for 14 days, (4) a daily dose of cisplatin (2 mg/kg i.p. or s.c.) during two sessions on 5 consecutive days, separated by 2 days of rest. Mice were sacrificed 35 days after the first injection, except for a cohort of 4-week-old mice belonging to the "high-dose" kanamycin regimen ($n = 10$) and to the i.p. cisplatin group ($n = 10$), which were sacrificed after 15 days to assess apoptosis. As aluminum can inactivate cisplatin (Prestayko et al., 1979), we used aluminum-free material.

Guinea pigs were divided into three groups, as follows: (1) "control" ($n = 4$), which received saline i.p. for 7 consecutive days; (2) "kanamycin" ($n = 15$), which received 400 mg/kg/day kanamycin i.p. for 7 consecutive days; and (3) "cisplatin" ($n = 16$), which received 2 mg/kg/day cisplatin i.p. for two sessions on 5 consecutive days. They were sacrificed 35 days after the beginning of the treatment, with the exception of eight kanamycin-treated and eight cisplatin-treated guinea pigs, which were sacrificed after 15 days to assess apoptosis. In each group, the survival rates were calculated by the Kaplan–Meier method and differences evaluated with log rank test (GraphPad Prism 4.0 software, San Diego, CA, USA).

2.4. Auditory assessment

Auditory brainstem responses (ABR) were recorded prior to any treatment in order to confirm normal hearing. ABR were then recorded weekly until sacrifice. For sedation, animals spontaneously breathed isoflurane (1.5%) in 1 L/min oxygen. Body temperature was maintained with an electric heating pad. Subcutaneous needle electrodes were inserted to the vertex (active), the mastoid (reference) and the hind limb (ground). Ear probes were gently introduced into the external canal. Using Intelligent Hearing System (IHS, Miami, FL, USA) hardware and software, click stimuli and pure tones of 16 kHz were delivered monaurally with calibrated ER2 Insert Earphones and High Frequency Transducers (IHS). The frequency most sensitive to

ototoxic injury is 16 kHz (Church et al., 2004; Dickey et al., 2005; Li et al., 2001; Minami et al., 2004). Stimuli for each condition were presented 1024 times at the rate of 19.3 s⁻¹. Stimulation levels were decreased with 10 dB steps until no response peaks were detectable and were subsequently increased with 5 dB steps until the response reappeared. Hearing thresholds were determined by the minimal sound pressure level that was able to evoke a reproducible electrophysiological response. The thresholds were verified at least three times for each stimulus condition. An increase in the hearing threshold was the primary endpoint of this study. Normality of the data distribution was assessed using the Kolmogorov–Smirnov test. Data were analysed using a two-way analysis of variance (ANOVA). The between-group variable was the ototoxic treatment while the time was the within-group variable (i.e., a repeated measure). Bonferroni method was used for post hoc analyses. Results were considered statistically significant if $P < 0.05$ for both the ANOVA and the post-test ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$). GraphPad prism software (Graph Pad, San Diego, CA, USA) was used to compute the analyses.

2.5. Tissue preparation

Mice were sacrificed by neck dislocation, and guinea pigs by CO₂ overdose. After sacrifice, the temporal bones were collected and stored overnight in 4% paraformaldehyde before decalcification in EDTA (4% in phosphate-buffered saline (PBS), pH = 6.4) for a total of 72 h for mice or 2 weeks for guinea pigs. The left ear was then cryoprotected in 20% sucrose for 24 h before being frozen for cryostat sectioning into 14- μ m thin sections. The right otic capsule was removed, and the cochlea was carefully isolated from the surrounding bony tissue, taking care to preserve the organ of Corti. Basal turn of the organ of Corti was separated, delicately unrolled and immediately processed for immunofluorescent staining.

2.6. Toluidine blue staining

Sections were stained with a solution of toluidine blue (2 g/L in Walpole's buffer [27 g/L sodium acetate, 1.2% acetic acid in distilled water, pH 7.4]) for 80 s and then washed two times for 5 min in Walpole's buffer. The coloration was fixed in 50 g/L ammonium molybdate. Sections were washed in distilled water and then dried for 15 min before mounting with coverslips (Safe Mount™, reference # 006 47520, Labonord, Templemars, France).

2.7. Immunofluorescence

Tissue sections or basal turns of the organ of Corti were incubated with PBS + 0.1% Triton X-100 for 5 min, followed by incubation with PBS + 0.3% Triton X-100 + 0.25% gelatine for 30 min at room temperature. Samples were then incubated overnight with a specific neuronal antibody directed against β III-tubulin (1/1000, clone TUJ1, Covance, Princeton, NJ, USA) or with an antibody directed against cleaved caspase-3 (1/1000, Promega, Madison, WI, USA). After three rinses in PBS, a secondary antibody conjugated to fluorescein isothiocyanate (the F(ab')₂ fragment of the affinity-purified antibody, 1/500, Jackson ImmunoResearch, West Grove, PA, USA) and phalloidin conjugated to tetramethylrhodamine B isothiocyanate (1/1000, Sigma–Aldrich) were added for 1 h at 37 °C. After three rinses in PBS, the samples were incubated in the presence of the DNA dye TOPRO-3 (1/2000, Invitrogen, Carlsbad, CA, USA) and then embedded in Vectashield hardset (Vector Labs, Burlingame, CA, USA). Samples were observed with a FV1000 Olympus confocal microscope. For the whole-mount preparations (i.e. basal turns of the organ of Corti), each cochlea was evaluated qualitatively for the presence or absence of outer and inner hair cells, with a particular attention to the basal turn of the cochlea.

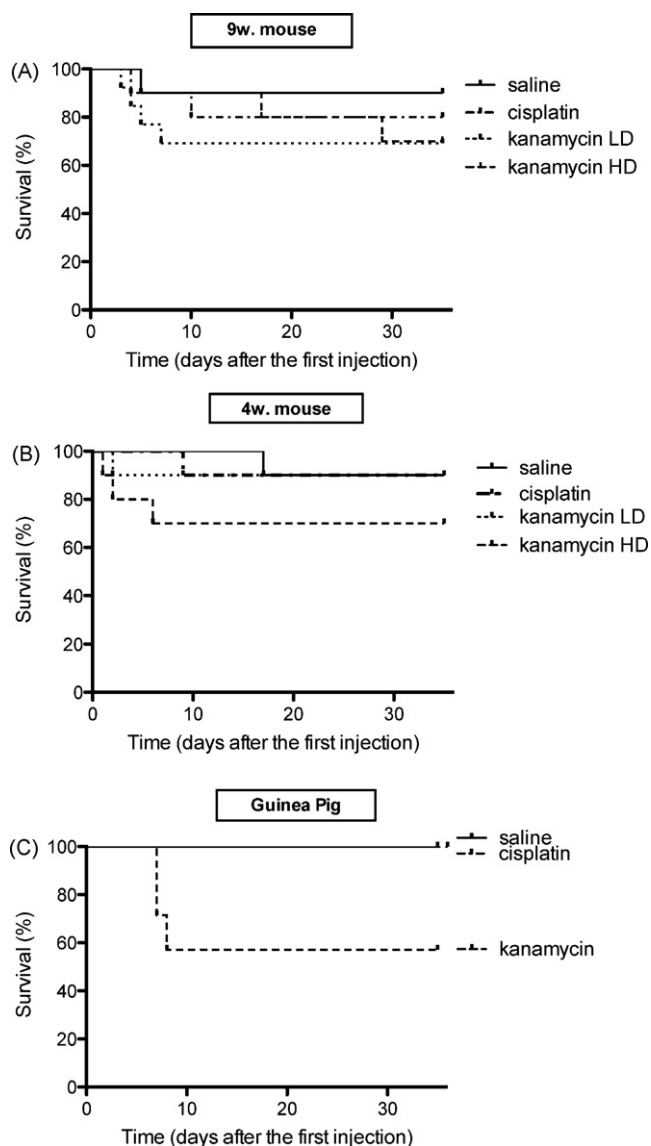


Fig. 1. Kaplan–Meier survival curves of 9-week-old mice (A), 4-week-old mice (B) and guinea pigs (C) that received i.p. injection of saline (—), cisplatin (---), low dose (LD) of kanamycin (····), or high dose (HD) of kanamycin (— · —). None of the curves are statistically different from each other (log rank $P=0.3734$ for 9-week-old mice, $P=0.6841$ for 4-week-old mice, $P=0.6271$ for guinea pigs).

2.8. Spiral ganglion neuron quantification

For each animal, four non-adjacent midmodiolar sections were examined. An observer, who was blinded to the treatment condition, counted all β III-tubulin immunopositive cells that co-stained with TOPRO-3. The results were expressed as the number of neurons per mm^2 of spiral ganglion (mean \pm standard deviation). Using GraphPad Prism program, a one-way ANOVA or t -test was used when appropriate.

3. Results

3.1. Effect of systemic ototoxic drugs on survival

Acute toxicity, measured by the lethal dose 50 (LD50) was shown to be 6.6 mg/kg for cisplatin and 17.5 g/kg for kanamycin in mice (Kahn, 2007). Fig. 1 shows Kaplan–Meier survival curves for animals receiving a systemic treatment. In both mice and guinea pigs, survival rate was higher in animals receiving saline than ototoxic drugs. However, even at high cumulative doses, the effect of

ototoxic treatment on the survival rate was not significantly different from each other (log rank $P>0.05$). Taken together, these findings suggest that adequate sublethal doses of ototoxic drugs were used in this study.

3.2. Effect of ototoxic drugs on hearing function

To quantitatively assess hearing loss in toxic drug-treated animals, the ABR were measured weekly. The ABR threshold was defined as the lowest sound level at which the response peaks were clearly present. The saline-treated animals, either mice or guinea pigs, showed ABR potentials that were consistent in sensitivity, form and latency with many strains of mice and guinea pigs with normal hearing (Huang, 1980; Zhou et al., 2006) (Fig. 2A and D). None of the groups of toxic drug-treated mice showed a significant increase in their hearing threshold. There was no difference between groups, at any time point and for all recorded frequencies both in 4- (Fig. 2A–C and G) and 9-week-old mice (data not shown). In contrast, the toxic drug-treated guinea pigs displayed elevated thresholds at all frequencies tested (Fig. 2E, F, H and I). The mean hearing threshold after 5 weeks was 64.3 dB-SPL (± 15.9 dB) in the kanamycin group, 61.9 dB-SPL (± 21.1 dB) in the cisplatin group and 31.2 dB-SPL (± 7.2 dB) in the saline group. Cisplatin-treated guinea pigs showed a dramatic shift in the hearing threshold 2 weeks after the beginning of the treatment (70.9 dB \pm 19.2 dB). Moreover, the loss of the Preyer's reflex was observed after 2 weeks in all ototoxic drug-treated guinea pigs.

3.3. Effect of ototoxic drugs on the organ of Corti

In the saline-treated mice, rhodamine-phalloidin staining showed the well-defined outline of outer hair cells (Fig. 3A and B). After treatment with cisplatin (Fig. 3D and E) or kanamycin (Fig. 3G and H), there was no apparent loss of outer hair cells in either the 4- or 9-week-old mice. The organs of Corti remained intact, regardless of the dose of ototoxic drug injected. None of the mice that had received an ototoxic treatment showed any significant hair cell injury. In the kanamycin-treated groups, there was no difference in hair cell survival, even when the route of administration (s.c., i.p.) differed. In contrast, kanamycin or cisplatin administration in guinea pigs demonstrated a dramatic reduction of the number of outer hair cells, especially in the basal part of the cochlea (Fig. 3F for cisplatin, and Fig. 3I for kanamycin).

To determine if the ototoxic drugs could induce apoptosis in the organ of Corti, immunostaining with cleaved caspase-3, which is an early marker for cell apoptosis, was performed. After 2 weeks of kanamycin or cisplatin treatment, no cleaved caspase-3 was detected in mice (Fig. 3J and data not shown), while intense staining was observed in the organ of Corti, the spiral ligament and the stria vascularis of adult guinea pig sections (Fig. 3K, green).

3.4. Effect of ototoxic drugs on spiral ganglion neurons

To evaluate whether kanamycin and cisplatin affect spiral ganglion neuronal morphology, toluidine blue staining and β III-tubulin immunostaining were performed. Mice showed normal spiral ganglion neurons at all ages studied, regardless of the type of drug administered, the method of administration or the dose (Fig. 4A–F). Quantification of neuronal densities showed no differences between groups (Fig. 4M). In contrast, there was a reduction in neuronal density and a relative swelling of the cytoplasm of neuronal cells in kanamycin- or cisplatin-treated guinea pigs when compared to the saline-treated group (Fig. 4G–L). Five weeks after the beginning of the treatment, cisplatin or kanamycin treatment resulted in a statistically significant decrease in the density of spiral ganglion neurons ($P<0.01$) compared to saline-treated guinea pigs

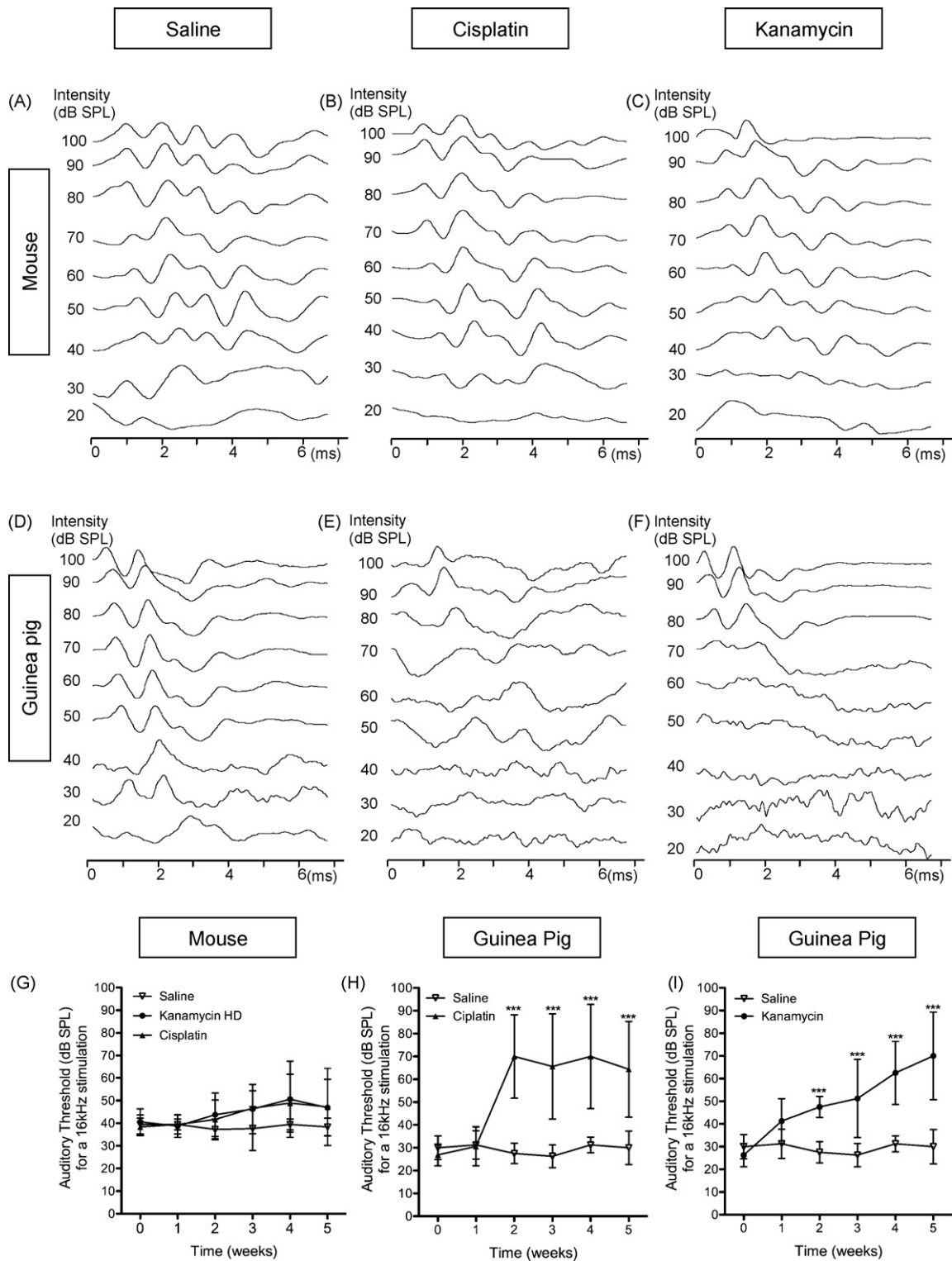


Fig. 2. A–F. Examples of 16 kHz tone burst-evoked ABR obtained from 4-week-old mice (A–C) or from guinea pigs (D–E) 5 weeks after the beginning of the treatment. Representative data from six different animals for each group are shown. There was no difference in the ABR pattern in mice receiving saline (A), cisplatin (B) or high-dose kanamycin (C). In guinea pigs, the lowest sound level to evoke a response increased after cisplatin (E) or kanamycin (F) when compared to saline (D) treatment. (G–I) The evolution of the ABR threshold over time in 4-week-old mice (G) and in guinea pigs treated by cisplatin (H) or kanamycin (I). Values are expressed as the mean \pm S.D. (G) ABR thresholds in mice treated with high-dose kanamycin (circle), cisplatin (black triangle) and saline (white reversed triangle) remained stable over a period of weeks. ABR thresholds in guinea pigs treated with cisplatin (black triangle, H) or kanamycin (circle, I) showed a significant difference compared to the saline group beginning in the second week after the start of treatment.

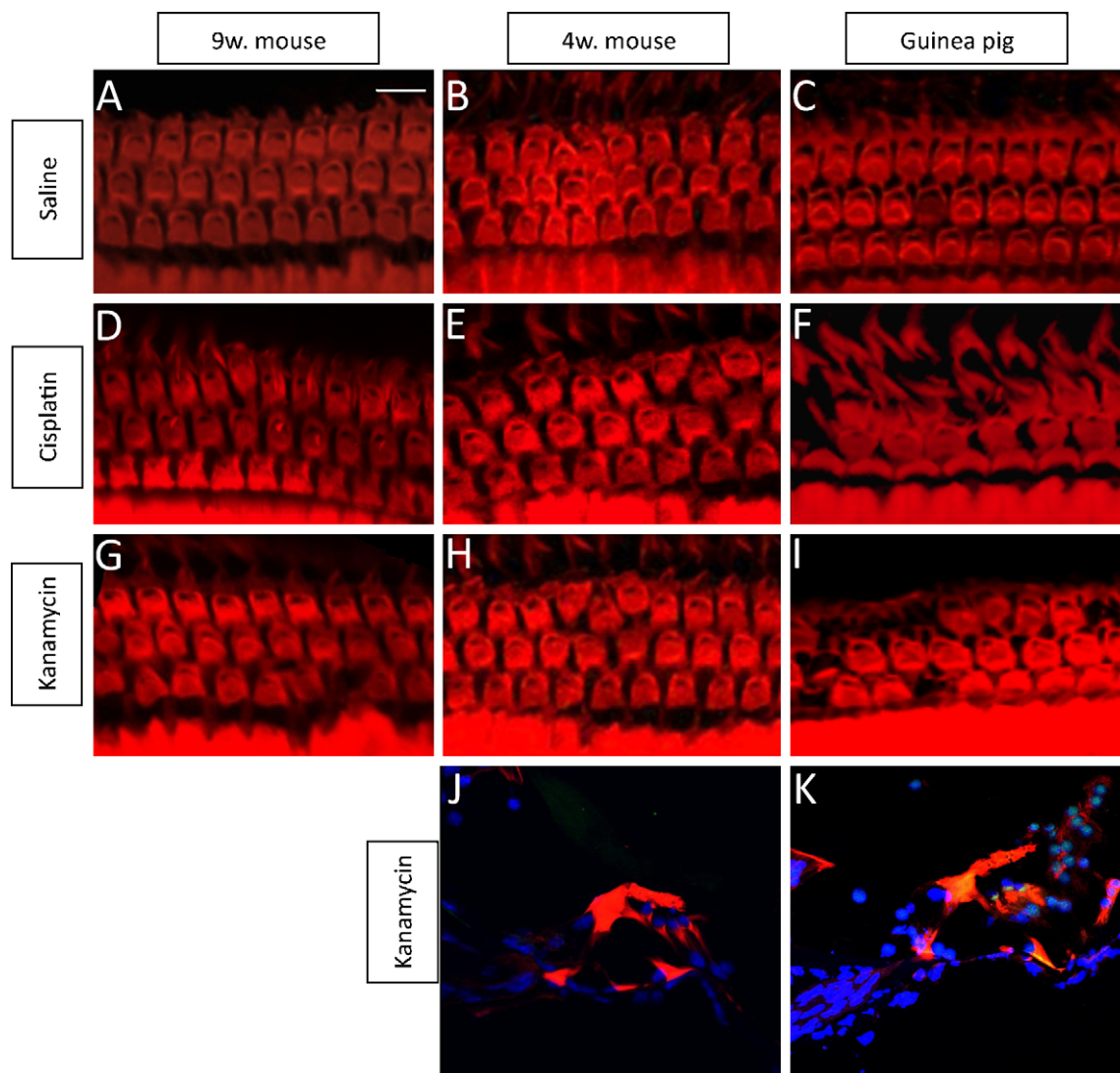


Fig. 3. (A–I) Outer hair cells in the basal turn of the organ of Corti in a 9-week-old mouse (A), a 4-week-old mouse (B), or a guinea pig (C) 35 days after saline treatment. Outer hair cells in the organ of Corti were preserved after cisplatin or kanamycin treatment both in 9-week-old mice (D and G, respectively) and in 4-week-old mice (E and H, respectively). In contrast, outer hair cell loss was observed after both cisplatin (F) and kanamycin (I) treatment in guinea pigs, mainly in the basal turn of the cochlea. (J–K) Basal turns of organ of Corti stained with antibodies against active caspase-3 (green), β III-tubulin (blue) and phalloidin (red), along with Dapi (gray), at the end of the kanamycin treatment in mice (J) and guinea pigs (K). Cleaved caspase-3 was present in the organ of Corti, the spiral ligament and the stria vascularis of guinea pigs. Scale bar shown in A = 10 μ m for A–I and 50 μ m for J–K. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

(Fig. 4N–O). Afferent fibres extended normally to the organ of Corti under all conditions (data not shown).

4. Discussion

This investigation demonstrated significant ototoxic differences between mice and guinea pigs after treatment with an aminoglycoside or cisplatin. While kanamycin or cisplatin is clearly ototoxic in the guinea pig, the BALB/c mouse is resistant to much higher doses of kanamycin or cisplatin.

Our results in mice are in line with some previously published data but discordant with others (Table 2). This discrepancy could be explained by genetic or environmental differences between American and European mouse strains. Genetic differences between mouse strains influence responses to various insults or toxins, including “ototoxicants” (Hamre et al., 1999; Peng et al., 1997; Perera et al., 2008; Schauwecker and Steward, 1997). In fact, the BALB/c strain was recently shown to be more sensitive to aminoglycoside toxicity than other strains, such as C57bl or CBA (Wu et al., 2001). The BALB/c mice in our study, however, did not display

any ototoxicity. Severinsen et al. (2006) did not show any permanent loss of hair cells in the maculae of European BALB/c mice treated by kanamycin. A potential explanation could be the genetic differences between American and European BALB/c strains. The parental BALB/c mice were derived from their original stock around the year 1913. This strain is now widely distributed and has several substrains with potential genetic diversity.

Dietary habits could also play a role in drug-induced ototoxicity. Food deprivation or treatment with glutathione-depleting drugs has been shown to exacerbate the ototoxicity of aminoglycosides (Lautermann et al., 1995a) or cisplatin (Lautermann et al., 1995b). Conversely, there could be a protective effect against drug-induced ototoxicity provided by antioxidants in the animal diet, such as alpha-tocopherol (vitamin E) (Fetoni et al., 2003, 2004a; Kalkanis et al., 2004; Sergi et al., 2004), D-methionine (Sha and Schacht, 2000), and L-acetylcysteine (Bock et al., 1983; Dickey et al., 2005; Feldman et al., 2007; Tepel, 2007). On the other hand, iron supplementation increases sensitivity to aminoglycosides (Conlon and Smith, 1998). In this study, we used a standard diet provided by SAFE (Scientific Animal Food & Engineering, France). According to the

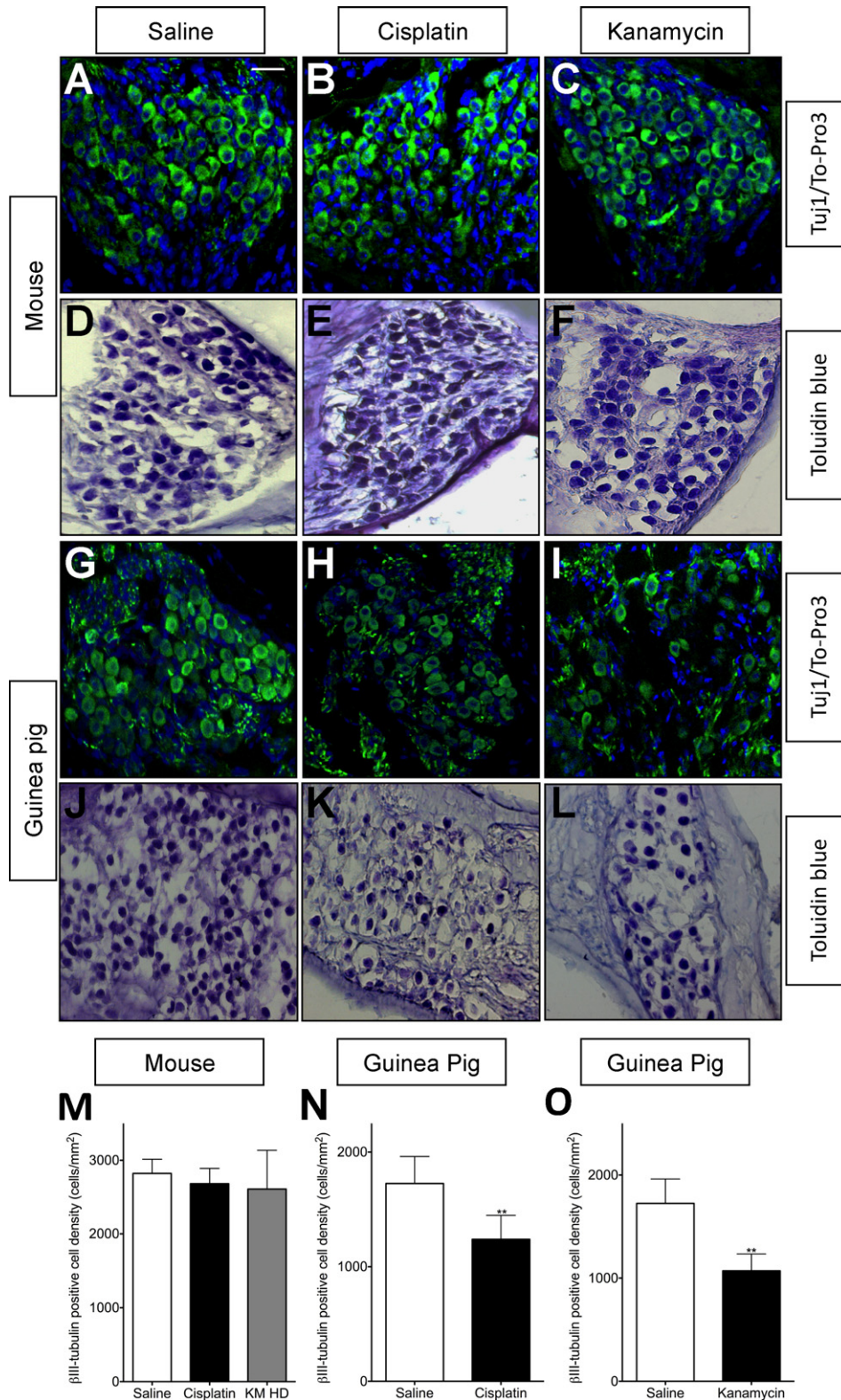


Fig. 4. Toluidine blue staining and β III-tubulin immunofluorescence (green) with TOPRO-3 nuclear counterstaining (blue) in the first coil of the cochlea on a midmodiolar section, 5 weeks after the beginning of the treatment, in a representative 4-week-old mouse (A–F) or a representative guinea pig (G–L) of each group. No difference was observed in mice receiving saline (A and D), cisplatin (B and E) or a high dose of kanamycin (C and F). In guinea pigs, toluidine blue nuclei and β III-tubulin immunoreactive profiles were more numerous in saline-treated animals (G and J) than in those treated with cisplatin (H and K) or kanamycin (I and L). A reduction in both the neuronal density and cytoplasmic swelling was observed in neurons from ototoxic drug-treated animals. (M–O) Quantification of β III-tubulin immunoreactive profiles in 4-week-old mice and in guinea pigs treated with cisplatin or kanamycin. In each animal, four non-contiguous midmodiolar sections were selected, and each coil of the cochlea was considered separately. Each cell containing β III-tubulin-positive cytoplasm and a TOPRO-3-positive nucleus was considered as a spiral ganglion neuron (SGN). Results are expressed as the number of β III-tubulin immunoreactive profiles per mm² and are presented as the mean \pm S.D. No significant differences were observed between groups of mice (M). In guinea pigs, a significant decrease in the number of neurons per mm² was observed 5 weeks after the beginning of cisplatin (black column, N) or kanamycin (black column, O) administration. Scale bar shown in A = 50 μ m for A–L. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 2
Previously published results on *in vivo* ototoxic drug-treated mice.

Animal		Toxic molecule		Dose, rate, route	Type of toxicity 0: no ototoxicity reported; A: hearing function impairment; B: change in gene/protein expression; C: hair cell loss; D: spiral ganglion neurons loss	Reference
Strain	Age	Drug	Strain			
C57		Gentamicin		200–300 mg/kg/day, 28 days, s.c. 300 mg/kg, 2/day, 14 days, s.c. 700 mg/kg/day, 6 days, s.c. 1 g/kg/day, 10 days, s.c.	0 0 0 A (inconstant)	Wu et al. (2001)
C57, CBA, BALB		Kanamycin		400–900 mg/kg, 2/day, 15 days, s.c.	A, C	Wang et al. (2003)
CBA	4 w	Kanamycin		700 mg/kg, 2/day, 15 days, s.c.	A, C	Kitahara et al. (2005b)
C57, CBA, BALB	4 w	Kanamycin		700 mg/kg, 2/day, 14 days, s.c.	B	Kitahara et al. (2005a)
CBA	4 w	Kanamycin		700 mg/kg, 2/day, 14 days, s.c.	B	Jiang et al. (2005)
CBA	4 w	Kanamycin		700 mg/kg, 2/day, 7 days, s.c.	B	Unal et al. (2005)
Albino Swiss	Adult	Gentamicin		5 mg/kg/day, 30 days, im	A, B	Jiang et al. (2006a)
CBA	4 w	Kanamycin		700 mg/kg, 2/day, 14 days, s.c.	A, B, C	Jiang et al. (2006c)
CBA	4 w	Kanamycin		700 mg/kg, 2/day, 3–11 days, s.c.	B	Jiang et al. (2006b)
CBA	4 w	Kanamycin		700 mg/kg, 2/day, 3–7 days, s.c.	B	Jiang et al. (2006b)
C57	4 w	Kanamycin		800 mg/kg, 2/day, 15 days, s.c.	A, C	Liu et al. (2007)
CBA	2 w 5 w	Kanamycin + bumetanide		1 g/kg, s.c. + 0.05 mg/kg, i.p.	B, C 0	Taylor et al. (2007)
CBA	4 w	Kanamycin + furosemide		1 g/kg, s.c. + 400 mg/kg, i.p.	B, C	Oesterle et al. (2007)
C57	12 w	Kanamycin		100 mg/kg, 2/day, 10 days, i.p.	A	Staecker et al. (2007)
C57		Gentamicin		120 mg/kg, single dose, i.p. 120 mg/kg/day, 19 days, i.p.	A (transient)	Gooi et al. (2008)
C57		Gentamicin		120 mg/kg, single dose, i.p. 120 mg/kg/day, 19 days, i.p.	0	Blakley et al. (2008)
BALBx C57	4 w	Kanamycin		700 mg/kg, 2/day, 14 days, s.c.	A, C	Taleb et al. (2009)
C57/BL6	2–5 m	Kanamycin + furosemide		1 g/kg, s.c. + 400 mg/kg, i.p.	C	Hartman et al. (2009)
CBA	22–26 m	Cisplatin		2 mg/kg/day, 12 days, i.p.	D	Bowers et al. (2002)
BALB	7 w	Cisplatin		4 mg/kg/day, 4 days, i.p.	B	So et al. (2008)
CBA	1–2 m	Cisplatin		12 mg/kg single dose, i.p. 14–16 mg/kg single dose, i.p.	0 A	Hill et al. (2008)

Abbreviations: C57: C57BL/6; BALB: BALB/c; CBA: CBA/J; W: week; M: month; D: day. In this table, we summarize previously published studies using systemic treatments by aminoglycosides or cisplatin in adult mice. We focused on the auditory effects. The effects reported may be 0/no toxicity, or involve A/the functional level (hearing impairment), B/the molecular level (change in protein or gene expression), or the cellular level (C/hair cells or D/spiral ganglion neurons loss). As the age and the strain of the mice may influence the effect of the toxins, these parameters are precised in the first two columns.

manufacturer's specification, these mouse chows contain 30 UI/kg alpha-tocopherol which may partly explain the absence of ototoxicity in this study. However, 30 UI/kg is 3–100 times lower than the dose used to reach otoprotection (Fetoni et al., 2003, 2004a,b; Kalkanis et al., 2004; Teranishi et al., 2001).

There was a dramatic difference in ototoxicity between guinea pigs and mice. A potential explanation for the lack of cochlear injury in mice could be the mouse pharmacokinetic profile at both the level of the organ of Corti and the whole organism. First, mice have a high metabolic rate and a high renal clearance, which keep serum levels of drugs very low (Walton et al., 2004; Yang and Bankir, 2005). All interspecies studies point out the importance of pharmacokinetic in the differential effect observed between species after aminoglycoside (Meza and Aguilar-Maldonado, 2007), cisplatin (Blakley et al., 2008) or other ototoxic agents (Lataye et al., 2003). Urinary excretion is the major route of platinum and aminoglycoside elimination in all species (Farris et al., 1988; King et al., 1986; Tran Ba Huy et al., 1986). In mice, the high plasma clearance allows an early urinary excretion (Walton et al., 2004; Yang and Bankir, 2005), and thus a shorter serum half-life of ototoxic drugs (Wu et al., 2001).

Second, the organ of Corti is hidden in the deep compartment of the inner ear and is protected by a blood–labyrinth barrier in a manner analogous to the way that the central nervous system is protected by the blood–brain barrier. This barrier can be roughly divided into the blood–perilymph barrier and the perilymph–endolymph barrier, although the functional division between these compartments is not fully understood. A small portion of the inner ear, the stria vascularis, is readily accessible from the systemic circulation. It is surrounded by cells that are sealed by tight junctions, which contribute to the blood–perilymph barrier (Swan et al., 2008). This barrier is most likely the major obstacle that restricts the entry of drugs into the inner ear and would thereby serve to dampen the effects of these drugs after systemic administration. Ototoxic drugs primarily affect the organisation of cell junctions (Anniko, 1985; Leonova and Raphael, 1997). A tempting speculation is that the sensitivity of guinea pigs to ototoxic drugs may be due to an essential tight junction protein with a quantitative or functional defect that is not present in mice.

Cisplatin- and aminoglycoside-induced ototoxicity is known to be mediated via injury to several terminally differentiated cellular targets in the cochlea (Rybak and Whitworth, 2005; van Ruijven et al., 2005), including the marginal and intermediate cells of the stria vascularis (Dai and Steyger, 2008; Laurell et al., 2007; Wang and Steyger, 2009). These cells are a major component of the perilymph–endolymph barrier. Whether these cells have the same properties in mice and guinea pigs remains to be determined and could provide another explanation for the difference in ototoxic drug sensitivity. Penetration of aminoglycosides into the cochlea could be less important in mice compared to guinea pigs (Dai et al., 2006). Differences in cochlear uptake are sufficient to explain different ototoxic profiles (Hellberg et al., 2009).

Several reports have concluded that the generation of reactive oxygen species (ROS) is linked both to aminoglycoside and cisplatin ototoxicity (Rizzi and Hirose, 2007; Rybak and Whitworth, 2005; Rybak et al., 2007). BALB/c mice have high levels of superoxide dismutase (SOD), which functions to block the oxidative stress generated by ROSs (Misra et al., 1991). Thus, the presence of high SOD levels in BALB/c mouse cochlear cells could reduce or abolish the ototoxicity of these drugs. This hypothesis could also be applied to other antioxidant defense and repair systems. In fact, ototoxic mechanisms are not sufficiently understood to identify reasons for species-specific differentiations.

In conclusion, these data support a marked difference between the inner ear responses of guinea pig and mouse to pharmacologically induced ototoxicity. Clearly, the intact hearing function of

the mouse is not attributable to technical errors, since the same molecules administered to the guinea pig induced cochlear injury, as has been previously described in the literature. The presented data suggest that the adult guinea pig model of cochlear toxicity remains the most reliable model for neurosensory deafness. Mouse model for neurosensory deafness must be considered with caution.

Conflict of interest

The authors report no conflict of interest.

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