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Effects of Ototoxic Drugs on Corti's Explants: Experimental Study

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Abstract
Introduction: Hearing loss represents one of the most frequent human disabilities. Hair cells, the primary sound receptors located in the inner ear are extremely sensitive, but also very fragile. The destruction of these cells in humans or in any other mammal is not followed by replacement, and therefore a permanent hearing loss results.
Material and Methods: Neonatal mousse CD1 (P0-6) were sacrificed according to the legal standards and ethics. After manual dissection of the cochleae, the entire spiral ganglion was dissected from the modiolus. The explants were treated with gentamicin, followed by incubation for 48 hours at 37°C. Normal and damaged outer hair cells (OHC) or inner hair cells (IHC) were then counted to allow for statistical comparisons between groups.
Results: A total of 20,100 outer hair cells from 64 cochleae and 4 groups were analyzed. At 3 mM of gentamicin the hair cells were almost complete damaged. The main type's alteration in the damaged outer or inner hair cells was absence of hair. The mean difference between the damaged or not damaged OHC/IHC was statistically significant ($p<0.001$).
Discussion: In our study we did not observe more damage in the basal cochlear turn when compared to the second turn. No statistically significant difference was found between the first cochlear turn of subjects on these groups, and turns 2 and 3, respectively.
Conclusion: Progressive doses of gentamicin cause increased numbers of damaged outer and inner hair cells with absence of hair (the most frequent finding).

Keywords: Hearing loss; Cochleae; Spiral ganglion; Ototoxic; Hair cells.

Introduction

Hearing loss represents one of the most frequent human disabilities [1]. It is estimated that one person in ten is directly affected, and nearly 40 percent of the population has a hearing-impaired friend or family member [1]. Hearing aids and cochlear implants have helped a lot of patients until now, but the scientific community believes that a real potential to cure deafness is available and should be pursued.

Hair cells, the primary sound receptors located in the inner ear are extremely sensitive, but also very fragile and susceptible to many types of damage including noise, aging, infection, ototoxic medications, and trauma as acquired causes of deafness and genetic conditions [2]. All the inner ear cells, including the supportive cells, are completely developed before birth. Subsequent to their destruction in humans or in any other mammal, these cells are not replaced and, therefore, permanent hearing loss results [2].

The possibility to re-grow certain human cells has recently become a clinical reality. For example, it was shown that skin cells can be cultured and grown in the laboratory and used to resurface burns. [3] At this very moment, we know that new hair cells can be developed under certain laboratory conditions. The search to identify and optimize these particular conditions and, to characterize the factors involved in the cell regrowth regulation is the heart of many research projects throughout the world. We intend to follow these research lines to bring our contribution to this scientific area

Material and Method

The animal procedures were approved by the institutional review board of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. The study was conducted between January 2008 and December 2008. Thirty-two neonatal mousse CD1 (P0-6) (Center for practical aptitudes and skills of the University of Medicine and Pharmacy, Cluj-Napoca) were sacrificed according to the legal standards and ethics, deeply anesthetized and decapitated [4]. After removal of the mandible and the skin, the skull was opened along the midline, separated into two halves and the brain was removed. Following removal of the temporal bone the bullas were opened under stereomicroscope in PBS (Sigma) sterile solution (Figure 1).

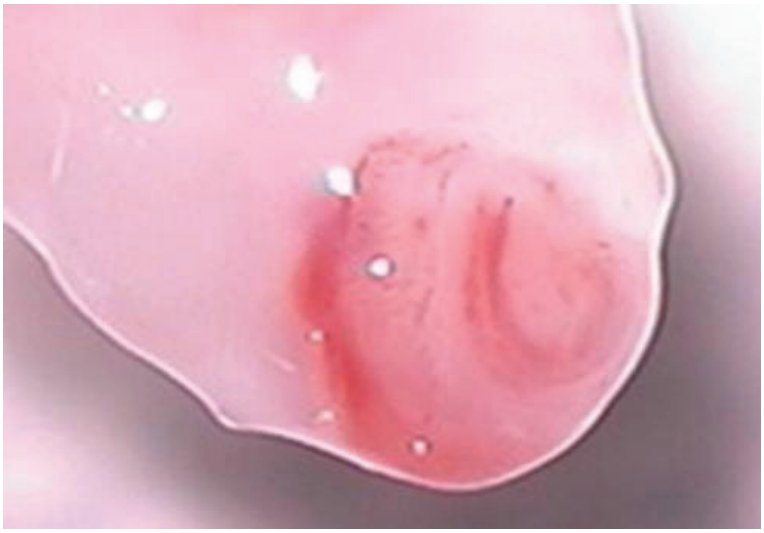

Figure 1. CD1 cochleae specimen P3

Further dissection of the sixty-four cochleaes for attaining spiral ganglion cells and modiolus was carried out by opening the bony cochlear capsule carefully and exposing the cochlear parts of the membranous labyrinth. After removal of the spiral ligament the organ of Corti with the stria vascularis was separated from the spiral ganglion and the modiolus. Finally, the entire spiral ganglion was dissected from the modiolus, followed by transferring them to transparent membrane Millicell, 12mm - Millipore. The cultures were incubated for 30 min at 37°C with 5% CO_2 on DMEM milieu (Sigma) with following contains: 6g/L glucose, 5% fetal bovine serum, 10µg/ml transferrin, 25µg/ml insulin, 60µM putresceine, 30nM selenium, and 30nM progesterone. The Corti's explants were maintained in these conditions for one month without any infections (Figure 2).

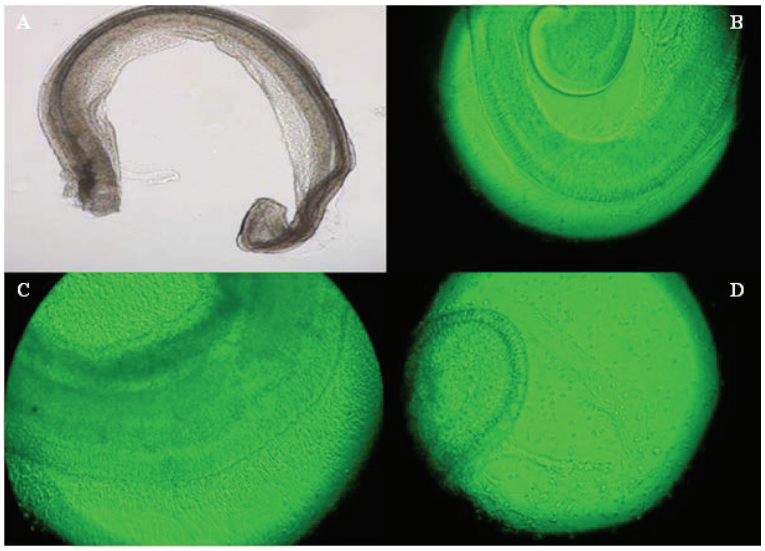

Figure 2. Corti's explants (P2): a.1st day, × 200; b. 1st day, × 400; c.4th day, × 400; 10th day; × 400.

The explants were treated with gentamicin, twelve for each concentration of aminoglycoside, followed by incubation for 48 hours at 37°C. Sixteen explants were considered as witness. We used the following concentrations of gentamicin: 0.1mM, 0.5 mM, 1 mM and 3mM, respectively.

To appreciate the ototoxic effect of gentamicin the Corti's explants were colored with tetrametylrodamine B isotiocianat (TRITC) (phaloidine extracted from *Amanita Phalloides*) (Sigma), followed by incubation for 15 min with PBS-Triton X100 1%. The fixed cultures were exposed to 50μg/ml phaloidine-TRITC for 45 min at 20ºC and washed with PBS.

After previously mentioned processes were completed, the specimens were taken to a scanning Zeiss microscope with reverse phase (AxioObserver Z1), with HBO100 fluorescent lamp and filters for TRITC (544 nm excitation and 572 nm emission). The images were taken with monochrome AxioCam MRm and processed with Axiovision 4.6 software. Hair cell integrity was defined based on the analysis of their stereocilia. Hair cells with perfect stereocilia were considered healthy. Hair cells with missing or deformed stereocilia were considered damaged. Normal and damaged outer hair cells (OHC) or inner hair cells (IHC) were then counted to allow for statistical comparisons between groups. The percentage of normal and damaged hair cells in the first three turns of each cochlea was recorded for each group.

Descriptive statistics and statistical analyses (mean values and their 95% CI, analysis of variance and Mann-Whitney U test) were performed using SPSS version 13.

Results

The mean number of OHC decreased to 38 cells/μm at 0.1 mM gentamicin, and IHC were not affected by this dose (Table 1). If we increased gentamicin dosage to 0.5 mM we observed a massive loss of OHC cells, but only a small part of IHC was damaged. At 3 mM of gentamicin the hair cells were almost completely damaged. (Figures 3 and 4) A total of 20,100 outer hair cells from 64 cochleae and 4 groups were analyzed, averaging 335 outer hair cells per cochleae.

In terms of hair cell architecture, the main types of alteration in the damaged outer hair cells were absence of hair (the most frequent finding), distorted pattern from 'v' to 'w', hair cell tumefaction and fusion. Damage severity decreased in intensity from the first and second turns to the apex and from the first row of outer hair cells to the third. The apical turn was not considered as it processes lower frequency, and ototoxic drugs affect mainly higher frequency ranges. This turn also presents naturally disarrayed hair cells, which hinders thorough anatomic assessment.

Table 1. The mean number of hair cells/µm/length of cochleae after incubation with different concentrations of gentamicin

Concentration of gentamicin (mM)	Mean n° OHC		Mean n° IHC		95% CI
	/100µm	%	/100µm	%	
witness	43.4	*100*	13.3	*100*	
0.1 mM	38.5	*88.7*	12.9	*96.99*	[14.51-16.94]
0.5 mM	25	*57.6*	11.8	*88.72*	[22.33-25.81]
1 mM	7.8	*17.97*	5.8	*43.6*	[29.54-39.14]
3 mM	1.3	*2.99*	0.7	*5.26*	[56.85-58.48]

The mean difference between the damaged and undamaged OHC was statistically significant (Anova one-way test, df=3, F= 3.465, p=0.0182). The mean difference between the damaged or not damaged IHC was statistically significant (Anova one-way test, df=2, F= 5.770, p= 0.008). Statistical analysis was also done on the findings from the first cochlear turn of subjects on these groups, and tests were repeated for turns 2 and 3. Likewise, no statistically significant difference was found between groups (Mann-Whitney U test, p=0.65 for first cochlear turns; p=0.83 for second turns; p=0.36 for third turns).

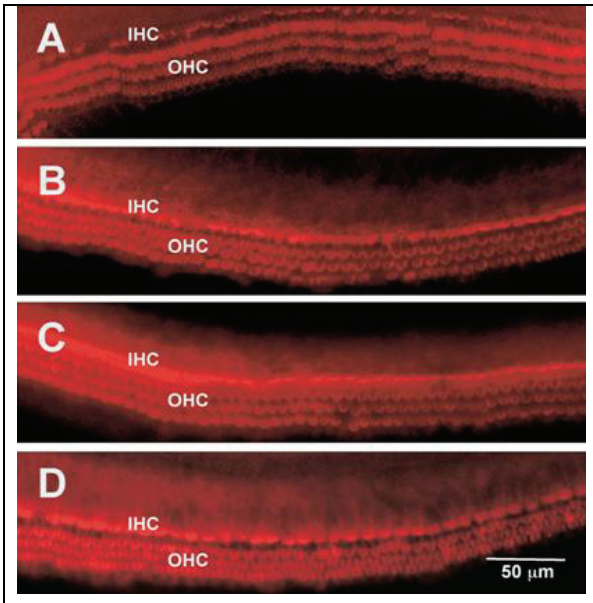

Figure 3. IHC and OHC before incubation with gentamicin

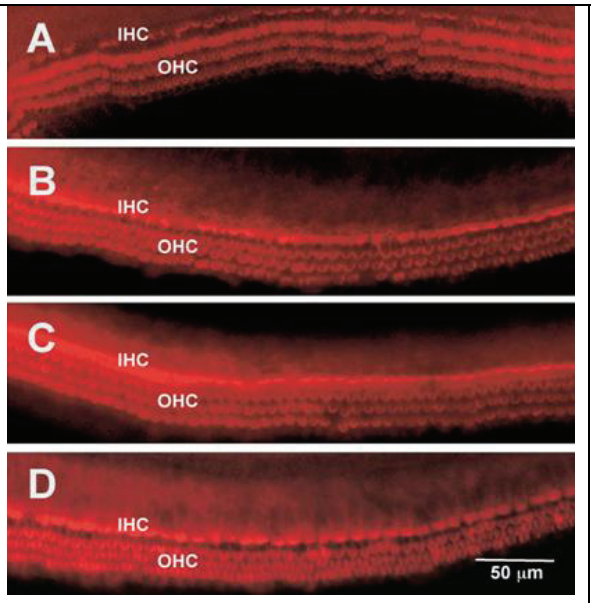

Figure 4. IHC and OHC after incubation with gentamicin: A. 0.1mM; B. 0.5mM; C. 3mM; D. 1mM.

Discussion

Although reliable data on drug-induced hearing loss in the world's population are not available, we know that deafness amounts to a significant occurrence, mainly in cases where aminoglycosides and chemotherapy drugs are used in a continuous fashion. Additionally, drug-induced hypoacusis is irreversible and introduces severe social and psychological burdens in the lives of patients and that if their families. Therefore, more research on ototoxicity and interventions to protect the inner ear are required.

Aminoglycosides are the most studied ototoxic drug, whether it is for historical reasons or clinical relevance. [1, 2] Many reports were published on aminoglycoside ototoxicity and they provide significant knowledge on inner ear anatomy, physiology, and biochemistry, thus opening

the path for the development of less toxic drugs and more effective means of protecting and preventing damage against the organ of Corti.

According to Oliveira and Bernal [2], the damage introduced by aminoglycosides in the organ of Corti affects mostly the outer hair cells and progresses towards the base and then the apex of the cochlea. In the basal turns, the first row of OHC is the first to be damaged, followed by the second and third rows. The sequence of damage coincides with the height of outer hair cells, being the basal turn the first to be damaged, followed by the second, third, and then the apical cells. The stria vascularis may also be involved, and marginal cells may be structurally affected.

The identification of sensorial cell and organ of Corti endogenous defense mechanisms in the form of antioxidant and detoxification enzymes catalase, superoxide-dismutase, glutathione-peroxidase, reductase, S-transferase, and glutathione, gave a fresh breath to the research done on ototoxicity and inner ear protection, notably on protection against oxygen-reactive substances [5, 6].

We looked at a total of 64 cochleae and 20,100 OHC (mean 335 OHC per cochlea). In our study, differently from the cited publications, we did not observe more damage in the basal cochlear turn when compared to the second turn. This is possibly due to the high degrees of damage found in these groups, as this pattern is lost with damage progression and cochlear turns with about 100% damaged OHC in all three rows are found.

Morphological analysis showed that the most frequent OHC damage type was the absence of stereocilia, followed by stereocilia deformity (fusion and tumefaction). This finding is consistent with other publications in the literature [7-9].

We believe that studies on the endogenous defense mechanisms adopted by the hair cells of the organ of Corti done concurrently with genetic research [10, 11] and functional assessments form the rational path towards achieving concrete results that will enable us to better manage damage and prevent inner ear toxicity.

Conclusion

Progressive doses of gentamicin cause increased numbers of damaged outer and inner hair cells with absence of hair (the most frequent finding). Damage severity decreased from the first and second turns to the apex and from the first row of outer hair cells to the third. No statistically significant difference was found between cochlear turns.

Financial Disclosure

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Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

All authors equally contributed to draft the article.

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