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Original Article

# N-acetylcysteine Prevents Gentamicin Ototoxicity in a Rat Model

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**OBJECTIVE:** The possible preventive effect of N-acetylcysteine (NAC) in gentamicin ototoxicity was studied with auditory brain stem responses (ABRs), otoacoustic emissions (OAEs), and histopathological investigation of the cochlea.

**MATERIALS and METHODS:** This study is conducted on 36 rats in three groups. Gentamicin, gentamicin plus NAC, and NAC alone were intraperitoneally administered for 15 days. The rats were sacrificed to study the cochleas after testing hearing levels.

**RESULTS:** ABR thresholds and OAEs were attenuated in the gentamicin group, in which apoptosis was detected with histopathological investigation. The group that received NAC in addition to gentamicin had better ABR thresholds and better OAEs. The histopathological evidence of apoptosis in was considerably less in this group.

**CONCLUSION:** Gentamicin ototoxicity can be detected by ABR and OAE testing in rats, and NAC may protect the cochlear cells from apoptosis.

**KEYWORDS:** Ototoxicity, gentamicin, NAC, n-acetylcysteine, hearing

## INTRODUCTION

Gentamicin is an aminoglycoside antibiotic with a major side effect, i.e., ototoxicity sometimes resulting in permanent hearing loss [1] used against. However, it is still used in developing countries, including Turkey, because of its well-known effect against Gram-negative aerobic bacterial infections, reasonable price, and over the counter availability. First author of this paper has a tragic memory in which an old colleague was lost during rehabilitation for vestibulotoxicity and ototoxicity. The reason of toxicity was previous gentamicin injections only for a week, as suggested by a colleague, for treating intractable external otitis.

Gentamicin characteristically causes ototoxicity with similar patterns of hearing losses in both ears. It was known to be impossible to heal after started. The exposure first attenuates the higher pitch sounds and then lower pitch sounds [2]. Up to 33% hearing loss and 15% vestibulopathy were reported [3]. However, initial hearing loss in high frequencies is difficult to be detected since high frequency audiometry is not a custom test but a test for advanced investigation. Therefore, the percentage of the actual loss may have been underestimated.

Gentamicin destroys outer hair cells particularly at the basal turn, and if the dosage is further increased, the histopathological effects are observed in inner hair cells. Nerve fibers seem to be affected, independent of the situation of the sensory cells [2].

Continuous ambulatory peritoneal dialysis (CAPD) may cause peritonitis and is treated with aminoglycosides. A study about hearing functions of patients with CAPD has been previously conducted, where peritonitis versus non-peritonitis patients were compared [4]. In that study, it was found that intraperitoneal aminoglycoside causes hearing loss, severity of which ranges from mild to profound. Hearing loss severity correlates with repetition of treatment and total dose of antibiotics.

There are a few agents, including deferoxamine, 2.3-dihydroxybenzoate, steroids, α-tocopherol, α-lipoic acid, salicylates, trimetazadine, thymoquinone, mannitol, β-carotene, vitamins C and E, and magnesium [5-14], that claim to prevent gentamicin ototoxicity. NAC also was shown to protect the sensory cells of the cochlea in a culture medium from the ototoxic effect of gentamicin [15].

This study was presented at the 2013 Schucknect Society Meeting, 10-11 June, 2013, Boston, MA, USA.

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**Table 1.** Preliminary gentamicin ototoxicity trial on 16 rats

| Group | Treatment protocol | Result |
|---|---|---|
| 1st group n=4 | Gentamicin 80 mg/kg Wistar-Albino | Died before ototoxicity |
| 2nd group n=4 | Gentamicin 80 mg/kg Sprague-Dawley | No ototoxicity |
| 3rd group n=4 | Gentamicin 100 mg/kg Sprague-Dawley | No ototoxicity |
| 4th group n=4 | Gentamicin 120 mg/kg Sprague-Dawley | Ototoxicity detected |

**Table 2.** N-acetylcysteine against Gentamicin ototoxicity on 36 rats

| Group | Treatment protocol |
|---|---|
| 1st group n=12 | Gentamicin 120 mg/kg+1 cc saline |
| 2nd group n=12 | Gentamicin 120 mg/kg+N-acetylcysteine 500 mg/kg |
| 3rd group n=12 | N-acetylcysteine 500 mg/kg+1 cc saline |

The author of this paper had conducted another study, in which patients previously treated with intraperitoneal vancomycin for CAPD peritonitis were evaluated. Intraperitoneal aminoglycoside had caused a low and high frequency dose-related hearing loss. In that study, N-acetylcysteine (NAC) had prevented ototoxicity and had a curative effect on hearing loss at high-frequencies [16].

New, but scarce, literature data supports our finding, showing the formation of new synapses after cessation of the toxic drug [17].

The present study aims to set a rat model to histopathologically investigate the protection of N-acetylcysteine against gentamicin ototoxicity; thus, in the following studies, mediators of action and dose-related changes can be evaluated even with other agents.

## MATERIALS and METHODS

This study was approved by the Erciyes University Ethics Committee on Animal Research and was conducted at the Experimental Animal Study Laboratory of the Erciyes University. This is a prospective, controlled animal study about gentamicin ototoxicity.

Normal hearing in all rat ears was determined in the baseline examination, hearing measurements with auditory brain stem response (ABR), and distortion product otoacoustic emission (DPOAE) tests.

### Preliminary Trial

First, a preliminary trial for ototoxicity on 16 rats was conducted. Gentamicin (80 mg/kg; Genta, I.E Ulagay, Turkey) was administered to 4 Wistar Albino rats, which died before ototoxicity was determined with hearing tests. Then, 3 set of 4 Sprague-Dawley rats were administered gentamicin, first 80 mg/kg, followed by 100 mg/kg and 120 mg/kg until resultant ototoxicity (Table 1).

### Study Design

Once the gentamicin model for ototoxicity was established, 36 healthy female rats were assigned into three groups, to be daily treated intraperitoneally, for 15 days. To observe the protective effect of N-acetylcystein (NAC) (Asist, Hüsnü Arsan, Turkey) on gentamicin ototoxicity, the groups received gentamicin, gentamicin plus NAC, or NAC alone. To equalize the injections, the 1st and 3rd groups received 1 cc extra saline (Table 2).

At the 21st day, OAE and ABR were repeated. Two random animals from each group were sacrificed for histopathological evaluation.

### OAE Measurement

The Otodynamics ILO-288 Echoport equipment (Otodynamics Ltd., London, UK) was used for a measuring distortion product otoacoustic emissions (DPOAE).

The sound stimulus that composed DPOAE comprised two simultaneous permanent pure tones at different frequencies (ratio of f1 and f2=1.22) at 80 Db SPL (L1=L2).

DPOAE were tested at seven frequencies: 1001, 1501, 2002; 3003, 4004, 6006, and 7996.

### ABR Measurement

ABR responses were recorded by electrode needles subdermally placed with the active electrode at vertex, ground electrode on the glabella, and reference electrodes on the right and left mastoid regions.

Clicks were used as auditory stimuli with following settings: bandpass filters of 100-3000 Hz and a repeat rate of 21/s.

The ABR threshold was defined on the fifth wave. The threshold was determined by starting at 70 dB, and when an appropriate wave form was obtained, by decreasing the volume by 20 dB each time. If an appropriate wave form does not occur, 90 dB was tried. When the wave was about to disappear, 10 dB decrements were instituted. The volume of the last wave, before it disappeared is the threshold if repeatability was confirmed, and the threshold determination was developed over two tests.

### Histopathological Evaluation

The removed tissues were fixed for a day in neutral formalin. They were then placed in an EDTA solution for the decalcification of osseous tissues. This was followed by an overnight washing under a water flow. The tissues were dehydrated in an alcohol series, and transparency was achieved using xylene. Serial sections (5-µm thickness) were mounted to polylysine-coated slides. The figures were obtained from the basal turn of the cochlea.

### TUNEL Method

A specific kit was used for detecting DNA fragmentation and apoptotic cell death *in situ*. Sections were stored at 60°C overnight in an oven in order to facilitate deparaffinization. To complete deparaffinization, the sections were reacted with xylol for 2 rounds of 15 min each. The sections were then placed into an alcohol series of 100%, 96%, and 80% by 10-min intervals. After 2 washings in distilled water for 5 min, the sections were incubated with 20 µg/mL proteinase K. Next, the endogen peroxidase activity was blocked in the sections, and they were allowed to react with 3% hydrogen peroxide (TA-015-HP; Lab Vision, Fremont, CA) after washing with PBS. Subsequently, the sections were incubated in a balanced buffer for 15 min and then incubated with a TdT enzyme (77 µL reaction buffer+33 µL TdT en-

**Table 3.** Pretreatment and post treatment auditory brainstem response (ABR) thresholds between the groups (median value)

| Group | Pretreatment | Post treatment | p |
|---|---|---|---|
| Gentamicin | 13.33±7.02 | 44.58±13.51 | <0.001* |
| Gentamicin+N-acetylcysteine | 14.58±5.88 | 16.67±7.02 | 0.059 |
| N-acetylcysteine | 14.58±5.88 | 17.08±6.90 | 0.096 |

* - statistically significant

zyme, 1-µL TdT enzyme) at 37°C for 60 min. Further, the sections were placed in pre-warmed stopping/washing buffer at room temperature for 10 min before incubating them with anti-digoxigenin for 45 min. PBS washing was performed after every step. After the washing, DAB staining was used for detecting TUNEL-positive cells. For background staining, methyl green was applied for 5 min. The stained slides were dehydrated using an alcohol series and placed in xylol for 20 min. The slides were then covered by entellan and thin glass. Finally, the slides were observed using a photolight microscope equipped with a computer.

Two pathologists, who were blinded to the experiment, independently evaluated the TUNEL scores. The average number was determined by counting the TUNEL-positive apoptotic cells in randomly selected fields of each case. A total of 100 TUNEL-positive or -negative cells were counted in each case, and the TUNEL-positive cells were provided as a percentage. The cells in necrotic regions and those having a poor morphology and borders between sections were excluded.

**Immunohistochemical Method**

For immunohistochemical staining, the remaining serial sections were assigned, incubated at 60°C overnight, and the slides were deparaffinized by xylene and dehydrated through an alcohol series. The sections were boiled for 15 min in citrate buffer (10 mM, pH 6.0) using a microwave oven to retrieve antigen. Furthermore, for preventing endogenous peroxidase activity, they were placed in hydrogen peroxidase for 15 min The sections were incubated in a blocking serum (Ultra V Block, TP-060-HL; NeoMarker, Fremont, CA) for 10 min and then with primary antibodies, including caspase-3 (Lab Vision) and -9 (Lab Vision) in a moist environment at room temperature for 60 min. The antigen-antibody complex was fixed with biotinylated secondary antibodies and streptavidin-peroxidase for 20 min. Labeling was performed using DAB, and background staining was achieved using Mayer's hematoxylin and covered by mounting medium. The images were captured using a camera attached to an Olympus microscope (CX31, Germany).

Except for omission of the incubation period with the primary antibody, control samples were similarly processed. Two pathologists, blinded to the study, independently evaluated the immunolabeling scores. Scores of the staining intensity of the slides was semi-quantitatively assigned. The staining intensity was decided weak, moderate, or strong and valued 1, 2, or 3, respectively. The score was obtained from the following: SCORE=ΣPi (i+1), where i is the intensity of staining, and Pi is the percentage of stained cells for each intensity, varying from 100% to 0%.

**Statistical Analysis**

SPSS for Windows 16.0 (SPSS Inc.; Chicago, IL, USA) was used for analyzing the findings of this study. The data were expressed as means±SD. One-way Kruskal-Wallis test were used for comparing ABR and DPOAE results between groups before and after drug administration. To compare the positivity of the TUNEL method, the caspase (-3 and -9) and p53 expressions among groups, Mann-Whitney U test was used. P values of less than 0.05 were considered statistically significant.

**Table 4.** Pretreatment and post treatment Distortion Product Otoacoustic Emission (DPOAE) responses in all frequencies (mean value)

| | Gentamicin+Saline | | Gentamicin+N- acetylcysteine | | Saline+N- acetylcysteine | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | Sd | Mean | Sd | Mean | Sd | KW | p |
| Pre 1000 | 0.467 | 5.472 | −0.500 | 10.911 | −0.296 | 5.576 | 0.227 | 0.893 |
| Post 1000 | −7.129 | 11.800 | −1.979 | 11.579 | 1.521 | 7.812 | 7.726 | 0.021* |
| Pre 1500 | 6.913 | 9.594 | 5.158 | 9.172 | 1.846 | 6.291 | 4.294 | 0.117 |
| Post 1500 | −2.483 | 9.816 | 5.842 | 9.182 | 1.700 | 7.650 | 8.179 | 0.017* |
| Pre 2000 | 9.93 | 9.44 | 7.03 | 6.81 | 8.76 | 6.41 | 1.728 | 0.422 |
| Post 2000 | −6.37 | 9.95 | 7.37 | 6.40 | 9.25 | 5.43 | 34.718 | 0.000** |
| Pre 3000 | 11.00 | 9.12 | 12.18 | 7.17 | 11.34 | 5.81 | 0.049 | 0.976 |
| Post 3000 | −3.24 | 19.56 | 11.18 | 6.35 | 10.85 | 5.84 | 21.322 | 0.000** |
| Pre 4000 | 43.38 | 9.52 | 47.28 | 8.79 | 43.40 | 10.82 | 2.488 | 0.288 |
| Post 4000 | 6.40 | 26.17 | 46.50 | 9.33 | 44.60 | 10.18 | 34.878 | 0.000** |
| Pre 6000 | 26.82 | 6.10 | 26.09 | 6.91 | 28.76 | 8.41 | 1.693 | 0.429 |
| Post 6000 | −0.09 | 13.26 | 25.57 | 6.88 | 27.97 | 8.68 | 41.775 | 0.000** |
| Pre 8000 | 28.15 | 3.07 | 26.02 | 6.31 | 29.63 | 4.36 | 5.700 | 0.058 |
| Post 8000 | 3.12 | 13.72 | 25.67 | 6.79 | 27.89 | 7.84 | 3.420 | 0.000** |

Gentamicin treatment markedly decreased distortion product otoacoustic emission (DPOAE) responses in all frequencies. DPOAE changes in other two groups were not marked. KW: Kruskal-Wallis value

* - statistically significant

** - statistically significant (higher significance)

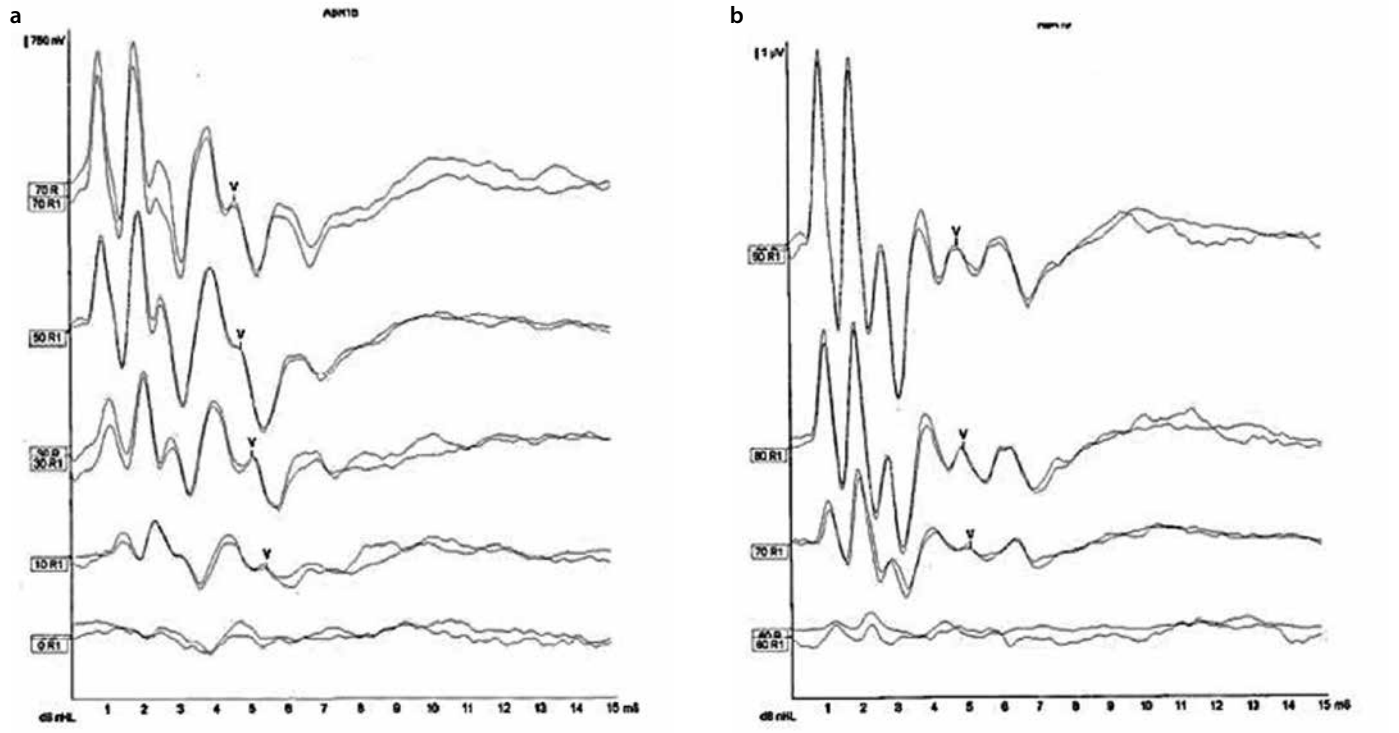


**Figure 1. a, b.** Auditory brainstem response (ABR) graph before treatment with gentamicin. ABR traces demonstrate that the hearing threshold is 10 dB (a) Auditory brainstem response (ABR) graph after treatment with gentamicin. ABR traces demonstrate that the hearing threshold is 70 dB (b)

**Table 5.** Number of TUNEL-positive cells in the ears of two randomly selected rats in each group

| TUNEL | Gentamicin | Gentamicin+N-acetylcysteine | N-acetylcysteine |
|---|---|---|---|
| 1st ear | 14 | 4 | 2 |
| 2nd ear | 11 | 5 | 1 |
| 3rd ear | 10 | 6 | 3 |
| 4th ear | 12 | 4 | 1 |

Number of TUNEL-positive cells in the gentamicin+N-acetylcysteine and N-acetylcysteine groups revealed a statistically significant decrease in the inner and outer hair cells relative to the gentamicin group ($p<0.05$).

**Table 6.** Caspase-3, -9, and p53 expressions

| | Caspase-3 | Caspase-9 | p53 |
|---|---|---|---|
| Group 1 | 232 | 236 | 248 |
| | 248 | 232 | 228 |
| | 244 | 240 | 252 |
| | 240 | 252 | 244 |
| Group 2 | 170 | 192 | 182 |
| | 177 | 189 | 185 |
| | 173 | 195 | 190 |
| | 176 | 198 | 192 |
| Group 3 | 110 | 104 | 108 |
| | 114 | 102 | 107 |
| | 116 | 100 | 105 |
| | 112 | 106 | 111 |

Caspase-3, -9, and p53 expressions were markedly higher in the gentamicin group

## RESULTS

Among all groups, baseline DPOAE values and ABR thresholds were not markedly different.

After gentamicin application, ABR thresholds were attenuated (Figure 1). There are statistically significant differences on ABR thresholds before and after gentamicin application ($p<0.05$) (Table 3). Both other groups receiving gentamicin plus NAC or NAC alone, exhibited no significant change in hearing thresholds after treatment (Table 3).

The gentamicin treatment markedly decreased DPOAE responses in all frequencies (Table 4). DPOAE responses did not change in the other two groups. As an example, Figure 2 shows DPOAE changes in 1000 H.

### Histopathological Results

### TUNEL

The gentamicin group has the highest number of TUNEL-positive (apoptotic) inner and outer hair cells (Table 5). Compared with gentamicin plus NAC and NAC groups, the number of TUNEL-positive cells was markedly high in the gentamicin group. In histopathological sections, the organ of Corti was preserved in the two groups that received NAC. Further, fewer TUNEL-positive cells were observed in the NAC group than the gentamicin group (Figure 3).

### Immunohistology

In caspase-3-stained tissues, the strongest apoptotic expression was in the gentamicin group (Figure 4a) compared with the two other NAC-receiving groups (Figures 4b, c).

Similarly in caspase-9-stained tissues, the strongest apoptotic expression was in the gentamicin group (Figure 5a) compared with the other two NAC-receiving groups (Figures 5b, c).

In p53-stained tissues, the strongest apoptotic expression was observed in the gentamicin group (Figure 6a) than the other two NAC-receiving groups (Figures. 6b, c).

Table 6 compares the three groups. The group that received gentamicin alone reveals the highest reaction to immunohistological stain, and this was statistically significant ($p<0.05$).

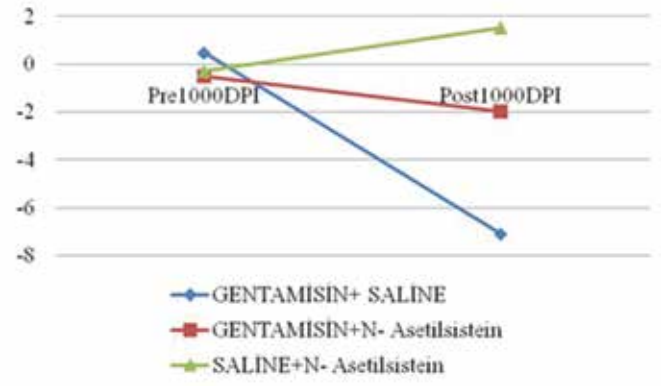


**Figure 2.** Changes in distortion product otoacoustic emissions (DPOAE) of all groups. Gentamicin decreased DPOAE responses. N-acetylcycteine (NAC) did not change DPOAE. Further, NAC prevented potential decrease in DPOAE when administered with gentamicin

## DISCUSSION

Different aspects of ototoxicity and preventive agents have been previously studied [1-9].

Regarding gentamicin dosage, there are controversial observations. A literature review supported our ototoxicity study to 80 mg/kg gentamicin for Wistar-Albino rats. However, the rats could not survive this dose, probably because of renal failure. Therefore, Sprague-Dawley rats had to be used for the study. This is also a proof for different effects of agents in different genetic background, which shows way of conducting gene therapies. Gentamicin doses of less than 120 mg/kg were not enough to cause ototoxicity in our study. We can speculate that according to Mendelian Law, some offsprings of Wistar and Sprague rats will survive the fatal dose.

In a different model of gentamicin ototoxicity, Unal et al. [14] revealed in guinea pigs that trimetazidine protects hearing, which is demonstrated both by audiological and histological findings. Cochlear histopathology in the gentamicin-receiving group showed vacuolsin stria vascularis and spiral limbus.

For histopathological investigation, we preferred to look for apoptosis at the cells of the cochlea to determine ototoxicity. The cells harmed to death by gentamicin were identified with a histochemical staining technique known as TUNEL, which exposes DNA breaks. The TUNEL method was described by Gavrieli et al. [18] Moreover, immunohistological stains for the detection of caspase (-3 and -9) and p53 expressions were used, which are also the indicators of apoptosis [19]. In both methods, apoptosis was clearly evident in the gentamicin

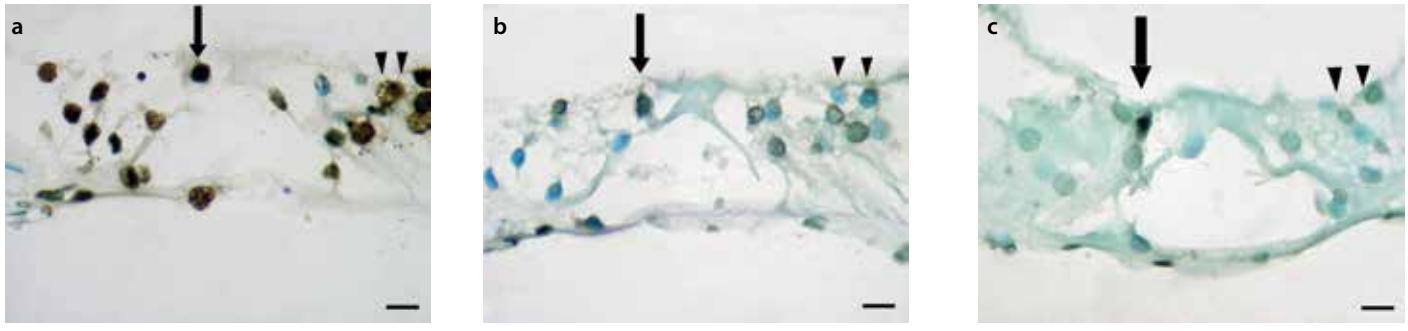


**Figure 3. a-c.** TUNEL staining of the organ of Corti in the Gentamicin group. Inner (arrow) and outer (arrow heads) hair cell nucleus are stained darker (TUNEL-positive) (compared with Figures 3b and 3c). BM: basilar membrane; TUNEL-staining method (×1000) (a), The gentamicin+N-acetylcycteine group (b) The N-acetylcysteine group (c)

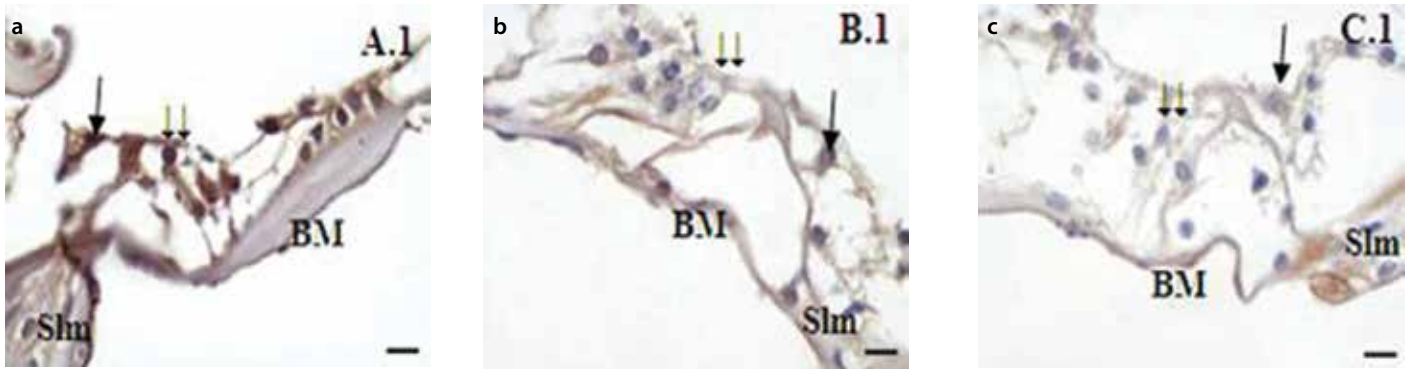


**Figure 4. a-c.** Caspase-3 stain of the gentamicin group. Inner (big arrow) and outer (small arrow) hair cells, the organ of Corti. Slm: Spiral limbus; BM: basilar membrane; Immunohistochemical staining (×1000) (a) Caspase-3 stain of the gentamicin+N-acetylcysteine group (b) Caspase-3 stain of the N-acetylcysteine group (c)

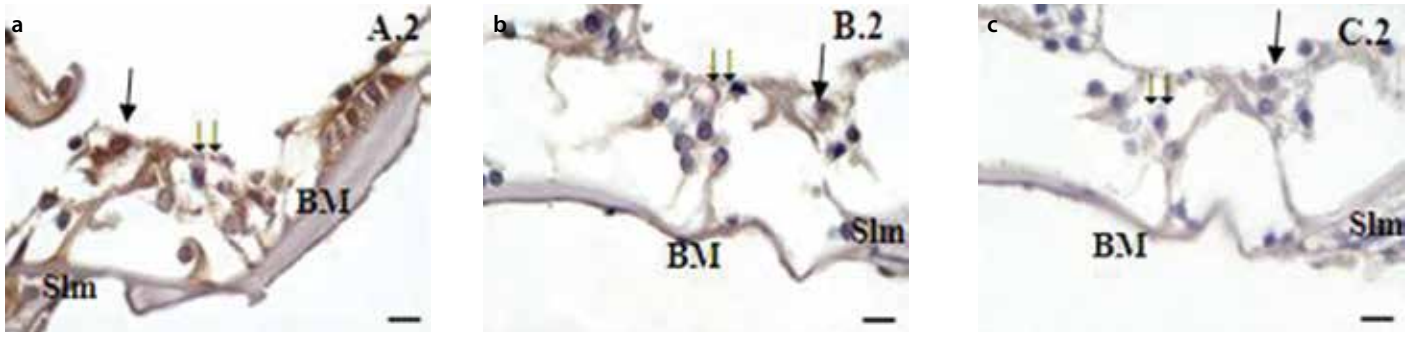


**Figure 5. a-c.** Caspase-9 stain of the gentamicin group. Inner (big arrow) and outer (small arrow) hair cells, Organ of Corti. Slm: Spiral limbus; BM: basilar membrane; Immunohistochemical staining (×1000) (a) Caspase-9 stain of the gentamicin+N-acetylcysteine group (b) Caspase-9 stain of the N-acetylcysteine group (c)

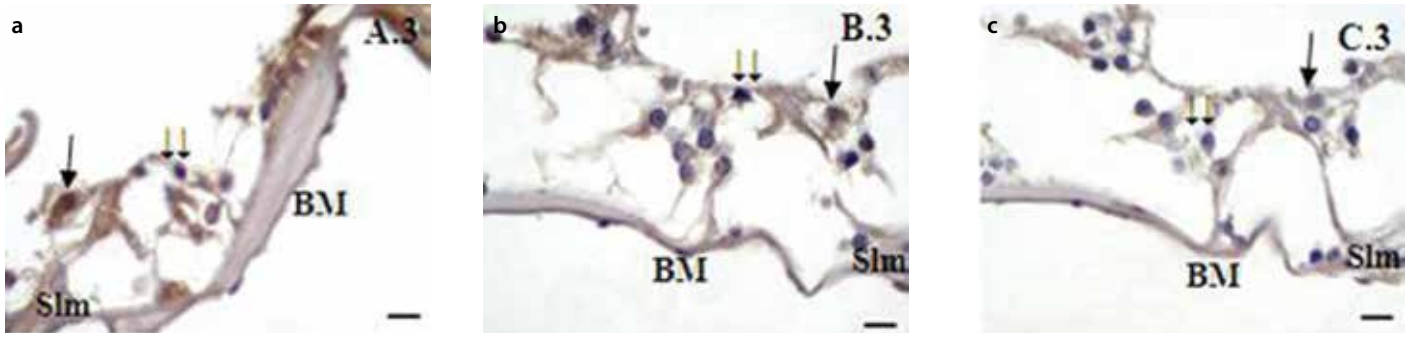


**Figure 6. a-c.** p53 stain of Gentamicin group. Inner (big arrow) and outer (small arrow) hair cells, Organ of Corti. Slm: Spiral limbus; BM: basilar membrane; Immunohistochemical staining (×1000) (a) p53 stain of the gentamicin+N-acetylcysteine group (b) p53 stain of the N-acetylcysteine group (c)

group. This was the indicator of ototoxicity. NAC, itself did not cause apoptosis, additionally protected the cells from the toxic effect of gentamicin, as there was not much apoptosis in the gentamicin plus NAC group compared with the gentamicin group.

OAE and ABR are reliable objective tests that measure hearing level [20]. We were able to clearly detect ototoxicity with these measurements in vivo. The gentamicin group revealed the worst results in both tests. NAC prevented the hearing loss caused by gentamicin; however, it did not affect hearing by itself.

As demonstrated by caspase and p53 expressions, compared with the other groups, gentamicin caused the strongest apoptotic effect in the organ of Corti. NAC blocked gentamicin, histopathologically and audiologically antagonizing its ototoxic effect. This was concordant with what we have found in our previous clinical study [16], in which NAC antagonized the ototoxic effect (of a different aminoglicoside) without interfering with the antibiotic effect.

Ototoxicity is mediated through reactive oxygen species, which results in cell death [21].

In contrast, N-acetylcysteine is an antioxidant, which originally is a mucolytic for pulmonary treatment. However, it is also used for the diseases of the lungs, liver, heart, and kidney in order to treat their toxic and ischemic injuries [22-25].

Probably NAC, in addition to its antioxidant effect, blocks a cascade where reactive oxygen species result in apoptosis in the cochlea.

Similarly, Low et al. [26] evaluated patients with head-neck cancer and claimed that NAC administered 72 h after radiation considerably reduced cochlear cell apoptosis. Free oxygen radicals in the inner ear were also decreased.

As Okur et al. [27] revealed that carboplatin ototoxicity increased nitric oxide levels and N-acetylcysteine prevented NO production, there may be different mechanisms about ototoxicity and prevention.

To our knowledge, combined audiological and histopathological findings regarding gentamycin ototoxicity and protection by N-acetylcysteine have not been previously reported. We have shown that the ABR and OAE values and numbers of apoptotic cells did not markedly change in the group receiving gentamicin and NAC, in which those receiving gentamicin alone markedly changed. Histopathological findings in the cochlea were also similar to the audiological findings. We conclude that gentamicin ototoxicity may be prevented using NAC in rats.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Erciyes University Ethics Committee on Animal Research.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - M.A.S.; Design - M.A.S., FK.; Supervision - M.A.S., M.S.; Funding - F.K., S.G.G.; Materials - M.S., F.K.; Data Collection and/or Processing - F.K., A.A.; Analysis and/or Interpretation - S.G.G., F.K.; Literature Review - M.A.S., F.K.; Writing - M.A.S., F.K.; Critical Review - M.A.S., M.S.

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## REFERENCES


1. Palomar Garcia V, Abdulghani Martinez F, Bodet Agusti E, Andreu Mencia L, Palomar Asenio V. Drug induced ototoxicity: current status. Acta Otolaryngol 2001; 121: 569-72. [CrossRef]
2. Ruan Q, Ao H, He J, Chen Z, Yu Z, Zhang R, et al. Topographic and quantitative evaluation of gentamicin-induced damage to peripheral innervation of mouse cochleae. Neurotoxicology 2014; 40: 86-96. [CrossRef]
3. Chen Y, Huang WG, Zha DJ, Qiu JH, Wang JL, Sha SH, et al. Aspirin attenuates gentamicin ototoxicity from the laboratory to the clinic. Hear Res 2007; 226: 178-82. [CrossRef]
4. Tokgoz B, Somdas MA, Ucar C, Kocyigit I, Unal A, Sipahioglu MH, et al. Correlation between hearing loss and peritonitis frequency and administration of ototoxic intraperitoneal antibiotics in patients with Continuous Ambulatory Peritoneal Dialysis CAPD. Ren Fail 2010; 32: 179-84. [CrossRef]
5. Mostafa BE, Tawfik S, Hefnawi NG, Hassan MA, Ismail FA. The role of deferoxamine in the prevention of gentamicin ototoxicity: a histological and audiological study in guinea pigs. Acta Otolaryngol 2007; 127: 234-9. [CrossRef]
6. Sinswat P, Wu WJ, Sha SH, Schacht J. Protection from ototoxicity of intraperitoneal gentamicin in guinea pig. Kidney Int 2000; 58: 2525-32. [CrossRef]
7. Park SK, Choi D, Russell P, John EO, Jung TT. Protective effect of corticosteroid against the cytotoxicity of aminoglycoside otic drops on isolated cochlear outer hair cells. Laryngoscope 2004; 114: 768-71. [CrossRef]
8. Fetoni AR, Sergi B, Scarano E, Paludetti G, Ferraresi A, Troiani D. Protective effects of alpha- tocopherol against gentamicin-induced Oto- vestibulo toxicity: an experimental study. Acta Otolaryngol 2003; 123: 192-7. [CrossRef]
9. Conlon BJ, Aran JM, Erre JP, Smith DW. Attenuation of aminoglycoside-induced cochlear damage with the metabolic antioxidant alpha-lipoic acid. Hear Res 1999; 128: 40-4. [CrossRef]
10. Sha S, Schacht J. Salicylate attenutes gentamicin-induced ototoxicity. Lab Invest 1999; 79: 807-13.
11. Sagit M, Korkmaz F, Gürgen SG, Kaya M, Akcadag A, Ozcan I. The protective role of thymoquinone in the prevention of gentamicin ototoxicity. Am J Otolaryngol 2014; 35: 603-9. [CrossRef]
12. Wood JW, Bas E, Gupta C, Selman Y, Eshraghi A, Telischi FF, et al. Otoprotective properties of mannitol against gentamicin induced hair cell loss. Otol Neurotol 2014; 35: 187-94. [CrossRef]
13. Le Prell CG, Ojano-Dirain C, Rudnick EW, Nelson MA, DeRemer SJ, Prieskorn DM, et al. Assessment of nutrient supplement to reduce gentamicin-induced ototoxicity. J Assoc Res Otolaryngol 2014; 15: 375-93. [CrossRef]
14. Unal OF, Ghoreishi SM, Ataş A, Akyürek N, Akyol G, Gürsel B. Prevention of gentamicin induced ototoxicity by trimetazidine in animal model. Int J Pediatr Otorhinolaryngol 2005; 69: 193-9. [CrossRef]
15. Maniu A, Perde-Schrepler M, Cosgarea M. Protective effect of L-N-acetylcysysteine against gentamycin ototoxicity in the organ cultures of the rat cochlea. Rom J Morphol Embryol 2011; 52: 159-64.
16. Tokgoz B, Ucar C, Kocyigit I, Somdas M, Unal A, Vural A, et al. Protective effect of N-acetylcysteine from drug-induced ototoxicity in uraemic patients with Continuous Ambulatory Peritoneal Dialysis peritonitis. Nephrol Dial Transplant 2011; 26: 4073-8. [CrossRef]
17. Chen L, Xiong S, Liu Y, Shang X. Effect of different gentamicin dose on the plasticity of the ribbon synapses in cochlear inner hair cells of C57BL/6J mice. Mol Neurobiol 2012; 46: 487-94. [CrossRef]
18. Gavrieli Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992; 119: 493-501. [CrossRef]
19. Cho SI, Lee JH, Park JH, Do NY. Protective effect of (-)-epigallocatechin-3-gallate against cisplatin-induced ototoxicity. J Laryngol Otol 2014; 15: 1-6. [CrossRef]
20. Olgun Y, Kırkım G, Kolatan E, Kıray M, Bagrıyanık A, Olgun A, et al. Friend or foe? Effect of oral resveratrol on cisplatin ototoxicity. Laryngoscope 2014; 124: 760-6. [CrossRef]
21. Forge A., Schacht J. Aminoglycoside antibiotics Audiol Neurootol 2000, 5: 3-22. [CrossRef]
22. Mazzon E, Britti D, De Sarro A, Caputi AP, Cuzzocrea S. Effect of on gentamycin-mediated nephropathy in rats. Eur J Pharmacol 2001; 424: 75-83. [CrossRef]
23. Arstall M, Yang J, Stafford, Betts WH, Horowitz JD. N-acetylcysteine in combination with nitroglycerin and streptokinase for the treatment of evolving acute myocardial infarction. Circulation 1995; 92: 2855-62. [CrossRef]
24. Weinbroum A, Rudick V, Ben-Abraham R, Karchevski E. N-acetyl-L-cysteine for preventing lung reperfusion injury after liver ischemia reperfusion- a possible dual protective mechanism in a dose-response study. Transplantation 2000; 69: 853-9. [CrossRef]
25. Tepel M, van der Giet M, Schwarzfeld C, Laufer U, Liermann D, Zidek W. Prevention of radiographic- contrast-agent-induced reductions in renal function by acetylcysteine. N Engl J Med 2000; 343: 180-4. [CrossRef]
26. Low WK, Sun L, Tan MG, Chua AW, Wang DY. L-N-Acetylcysteine protects against radiation-induced apoptosis in a cochlear cell line. Acta Otolaryngol. 2008; 128: 440-5. [CrossRef]
27. Okur E, Kilinc M, Yildirim I, Kilic MA, Tolun FI. Effect of N-acetylcysteine on carboplatin-induced ototoxicity and nitric oxide levels in a rat model. Laryngoscope 2007; 117: 2183-6. [CrossRef]