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Does N-acetylcysteine Improve Established Hearing Loss in Guinea Pigs?

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Abstract

Objective. To assess whether multiple injections of a powerful antioxidant can improve established sensorineural hearing loss in guinea pigs.

Study Design. Animal study.

Setting. Animal science laboratory, University of Manitoba.

Methods. A total of 16 guinea pigs were used in our study: 8 underwent unilateral intracochlear neomycin injection, and 8 underwent unilateral saline to serve as controls. After a period of 3 weeks for hearing loss to stabilize, 4 guinea pigs from each group received weekly intraperitoneal injections of N-acetylcysteine (NAC) for 4 weeks. Click auditory brainstem response (ABR) testing was conducted at baseline, weekly after the start of NAC injections, and after the last injection. Pure tone ABR tests were conducted prior to intracochlear injections and at completion of the study.

Results. Click ABR thresholds were significantly worse in ears treated with neomycin ($P < .001$), as expected, but not significantly different when treated with NAC ($P = .664$). Thresholds for pure tone ABR were also not statistically different in neomycin-treated ears with or without NAC ($P > .99$).

Conclusions. The aggressive antioxidant therapy performed in this study was not successful in improving established hearing loss via an antioxidant regimen that is known to change the oxidation-reduction potential in the cochlea.

Keywords

N-acetylcysteine, ototoxicity, neomycin, antioxidants

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Oxidation-reduction (REDOX) mechanisms underlie most chemical reactions and are thought to be responsible for a variety of physiologic and pathologic processes.[1] The mechanism of sensorineural hearing loss (SNHL) due to ototoxicity or aging is commonly accepted as an imbalance between REDOX damage and repair mechanisms.[2-4] It seems possible that restoration of REDOX imbalance with powerful antioxidants may reverse established hearing loss, which is the hypothesis underlying this study. Many articles in the literature deal with prevention of aminoglycoside-induced hearing loss, but treatment of established hearing loss is rarely attempted. In this study we created SNHL using an aminoglycoside model and then tried to treat it with antioxidants. Aminoglycosides are known to have vestibulotoxic and cochleotoxic properties, likely secondary to the apoptosis of hair cells secondary to the formation of free radicals.[5,6] The prevalence of aminoglycoside-induced ototoxicity is 3% to 25%, with high-frequency audiograms demonstrating up to 62% prevalence of hearing loss.[7,8]

N-acetylcysteine (NAC) is an antioxidant precursor to glutathione, used in the treatment of Tylenol overdose, and it neutralizes reactive oxygen and nitrogen species.[9] Previous studies have shown that a single 400-mg/kg dose of NAC is effective at preventing SNHL if delivered before or soon after an ototoxic agent, though none have shown improvement once hearing loss has stabilized.[6,10,11] Our laboratory has found that administration of a powerful antioxidant can delay presbycusis in mice.[12] Unpublished data from our laboratory show that NAC alters the REDOX potential in perilymph up to 24 hours, so it seemed plausible that the same dose of NAC might alleviate SNHL. The objective of this study is to assess the efficacy of multiple NAC injections at reducing established SNHL. Our hypothesis is that weekly intraperitoneal injections of NAC will improve neomycin-induced hearing loss in guinea pigs.

Methods

Animals and Drugs

A total of 16 male guinea pigs were studied. Guinea pigs were given 2 days to acclimate before testing began. The study was reviewed and approved by the Research Ethics and

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Table 1. Allocation of Animals' Ears by Systemic Treatment (N = 16).[a]

Group: ear	Injection
NAC	
Left	Saline
Right	Nothing
No treatment	
Left	Saline
Right	Nothing
NAC ± neomycin	
Left	Neomycin
Right	Nothing
Nothing ± neomycin	
Left	Neomycin
Right	Nothing

Abbreviation: NAC, N-acetylcysteine.
[a]n = 4 per group.

Compliance Board at the University of Manitoba. The animals were housed and cared for by the Animal Care Committee of the University of Manitoba Bannatyne Campus. Power analysis from data in our laboratory suggested that a sample size of 16 should provide 80% power to detect a 15-dB difference in thresholds at a significance level of $P = .05$.

We desired to test the effect of systematic (intraperitoneal) NAC on hearing loss introduced by intratympanic neomycin. We needed to control for the effects of NAC as well as the intratympanic injection itself. To permit paired statistical analysis, treatment of ears within individual animals was varied, as indicated in **Table 1**. Click auditory brainstem response (ABR) testing was carried out weekly to detect a possible trend in thresholds over the 4 weeks of treatment. Significant changes by frequency may be important as well, so pure tone thresholds were tested before (baseline) and after the 4 weeks of treatment.

NAC was administered at 400 mg/kg weekly for 4 weeks. This dose has been effective in our laboratory and was suggested by literature to offer adequate protection with no side effects.[13,14] Injections were initiated 3 weeks following creation of hearing loss and were performed on a weekly basis for 4 weeks.

During data collection, animals were anesthetized with an intraperitoneal injection of a 10:1 mixture of ketamine/xylazine with 65 mL/kg of ketamine. Cochlear function was evaluated by pure tone ABR testing prior to creation of hearing loss and on week 7. Click ABR was performed prior to creation of hearing loss and then weekly from weeks 3 to 7.

Creation of Hearing Loss

Under isoflurane general anesthesia, animals that were shown to have normal hearing on baseline ABR underwent intracochlear neomycin or saline injections. Intracochlear injections were performed by making relaxing incisions in the posterosuperior aspect of the external auditory canal to access the round window. Some removal of the scutum was required, and the tympanic membrane was excised. Once the scutum was visualized under microscopy, a tuberculin syringe with a 27-gauge needle was used to introduce 0.1 mL of neomycin (40 nM) or 0.1 mL of 0.9% saline through the round window into the cochlea of the left ear. Previous research by this laboratory showed healing of the tympanic membrane postincision and that stabilization of hearing loss occurs in 2 weeks.[15,16] ABR was conducted 3 weeks after the intracochlear injection to ensure stabilization of hearing loss.

Electrophysiologic Recording

ABR responses were recorded by placing transcutaneous needle electrodes on the postauricular temporal bone of the skull over the right and left mastoids and a ground electrode in the subcutaneous tissue in the abdomen. ABR testing was performed with a computer-based auditory research system (System II; Tucker Davis). Two hundred fifty pure tone pips were averaged at 3000, 6000, 12,000 and 24,000 Hz in intensities decreasing from 100 to 10 dB in 10-dB steps via high-frequency transducers. For click ABRs, 250 click presentations were delivered via the EAR-2 transducers. The threshold was defined as the highest intensity at which there is no response. Pairwise comparisons in univariate analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons were used to determine the significance of changes in click threshold over the 4 weeks of NAC injections and to assess the difference in pure tone thresholds across ear treatments. The significance level of $P = .05$ was assumed, and SPSS version 27 (IBM) was used for analysis.

Results

Click ABR

After the underlying assumptions for parametric testing were met, paired tests indicated that differences between ears within the same animal across treatment groups differed significantly when neomycin was administered (**Table 2**). This finding was necessary to demonstrate that we established hearing loss with neomycin.

The most relevant outcome for this study was whether or not the addition of NAC improved hearing in ears treated with neomycin. To address this question, pairwise comparisons in ANOVA were conducted contrasting the ears that did receive neomycin were contrasted with those that did not. Differences in ABR thresholds were significantly different in the neomycin-treated ears vs the nontreated ears; yet, within the neomycin-treated ears, the threshold difference between ears treated with and without NAC was not significant ($P = .664$), indicating that administration of NAC did not change ABR thresholds significantly. These P values are shown in **Table 3**, and the data are shown in **Figure 1**. It was possible that ≥ 1 treatments might show a trend over the 4 weeks of injections, but this was not supported statistically (univariate ANOVA, $P = .062$).

Pure Tone ABR

The Levene test of equality of variances suggested that the variances did not differ significantly and assumptions of

Table 2. Differences Between Ears Within the Same Animal Across Treatment Groups.

		Threshold, dB, mean (SD)	
Systemic treatment	Significant? (*P* value)	Injection	Nothing
NAC	No (.598)	28 (19)[a]	32 (24)
No treatment	No (.325)	23 (19)[a]	17 (20)
NAC ± neomycin	Yes (<.001)	64 (22)[b]	23 (19)
Neomycin	Yes (.002)	61 (13)[b]	28 (23)

Abbreviation: NAC, N-acetylcysteine.
[a]Saline.
[b]Neomycin.

Table 3. *P* Values for Differences in ABR Thresholds.

	NAC	Nothing	Neomycin and NAC
Nothing	.577		
Neomycin and NAC	<.001	<.001	
Neomycin only	<.001	<.001	.664

Abbreviations: ABR, auditory brainstem response; NAC, N-acetylcysteine.

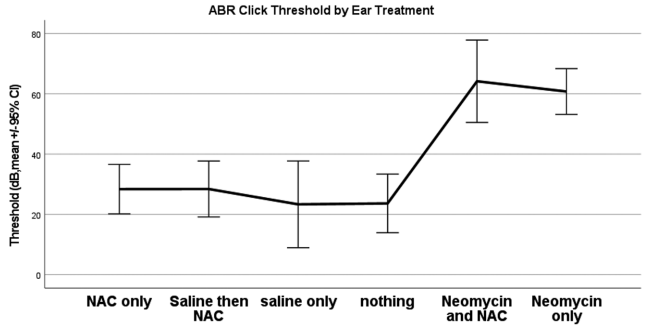

Figure 1. Final ABR click thresholds. Mean ± 95% CIs are shown for ear treatments. Thresholds for ears treated with neomycin only or neomycin and NAC were significantly worse than the other 4 categories. ABR, auditory brainstem response; NAC, N-acetylcysteine.

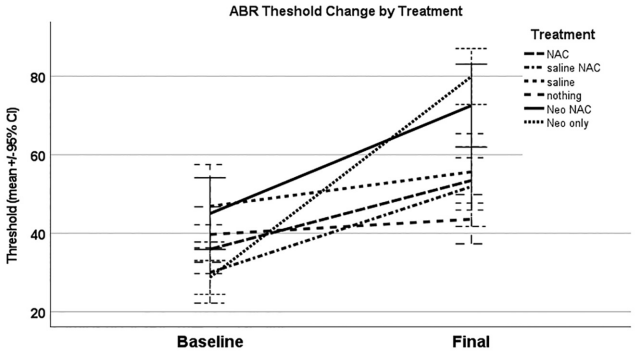

Figure 2. Baseline and final pure tone ABR threshold (dB) change by treatment (mean ± 95% CI). Final thresholds were highest for the ears treated with neomycin. These were the "neomycin/NAC" and "neomycin only" ears. Pairwise comparisons of the difference between those groups (mean ± SD, –8.07 ± 5.4 dB; $P > .99$) was not statistically significant, indicating that 4 weeks of NAC did not restore hearing. ABR, auditory brainstem response; NAC, N-acetylcysteine.

normal distribution were met, justifying the use of parametric statistics. Univariate ANOVA was carried out with the final ABR threshold as the dependent variable and with ear treatment and frequency as the independent variables, and the model was significant ($P = .001$ with 95% power). Final thresholds differed significantly from baseline by ear treatment ($P < .001$; **Figure 2**), as expected with neomycin injection; however, these were not significantly different across the 4 frequencies tested by treatment ($P = .25$; **Figure 3**), so all frequencies were considered together.

Pairwise comparisons of thresholds across the 6 treatment groups were carried out using Bonferroni adjustment for multiple comparisons. Mean differences, standard errors, and *P* values are shown in **Table 4**. The cell most relevant to our hypothesis is that comparing neomycin alone and neomycin with NAC, which is not statistically significantly different ($P > .99$).

Discussion

This study is one of the most robust animal studies completed evaluating the effect of antioxidant therapy on established hearing loss.[17-19] Although previous studies have demonstrated the efficacy of antioxidants in preventing hearing loss related to ototoxic mediation, we were unable to find any that adequately examined the use of antioxidant therapy in established hearing loss secondary to ototoxic exposure. The study

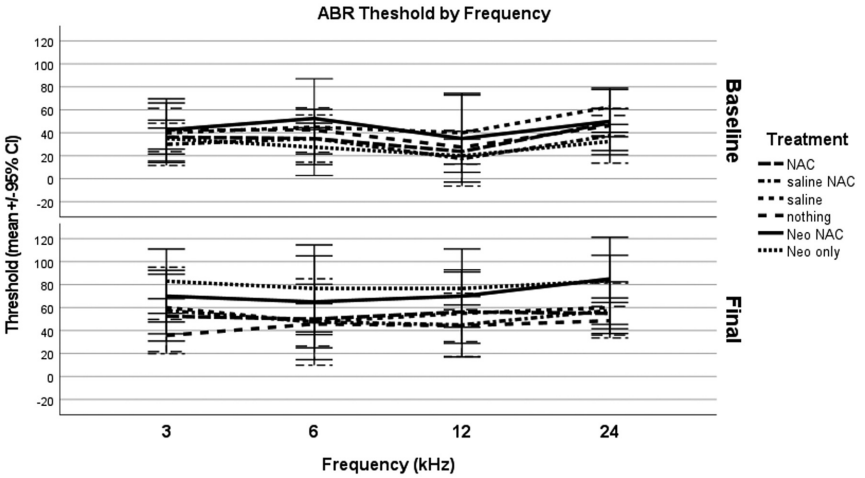

Figure 3. Baseline and final ABR threshold (dB) by test frequency (kHz). Analysis of variance indicated that final thresholds differed significantly across treatments ($P < .001$) but not by frequency ($P = .25$) and not for frequency by treatment ($P = .986$). The adjusted R^2 was 0.913, indicating that 91% of the variability of the data was accounted for by the model. ABR, auditory brainstem response; NAC, N-acetylcysteine.

Table 4. Pairwise Comparisons of Threshold by Treatment.[a]

	Treatment, mean difference ± SE (*P* value)				
	NAC	Saline and NAC	Saline	Nothing	Neomycin and NAC
Saline and NAC	3.7 ± 4.5 (>.99)				
Saline	−6.6 ± 4.5 (>.99)	−10.3 ± 5.2 (.704)			
Nothing	3.1 ± 3.7 (>.99)	−0.56 ± 4.5 (>.99)	−9.7 ± 4.5 (.481)		
Neomycin and NAC	−14.1 ± 4.5 (.028)	−17.8 ± 5.2 (.010)	−7.5 ± 5.2 (>.99)	−17.2 ± 4.5 (.003)	
Neomycin only	−6.0 ± 4.7 (>.99)	−9.7 ± 5.3 (>.99)	0.571 ± 5.3 (>.99)	−9.2 ± 4.7 (.80)	−8.07 ± 5.3 (>.99)

Abbreviation: NAC, N-acetylcysteine.
[a]Pairs that are statistically significant are shaded.

objective was to assess the efficacy of multiple NAC injections at reducing established SNHL, which may have given rise to a treatment for many people. Our hypothesis was that weekly intraperitoneal injections of NAC administered over 1 month would improve neomycin-induced hearing loss in guinea pigs. The results of this study did not show that NAC improves hearing loss related to neomycin exposure.

Our findings do not rule out the possibility that other REDOX interventions may be helpful. The ototoxic side effects of aminoglycoside antibiotics seem to be related to iron chelation and free radical formation.[20] This concept is supported by reduction of ototoxicity attained by the use of antioxidants or iron chelators, including NAC and glutathione.[14,21] Animal models demonstrate generation of free radicals within the inner ear, with apoptotic cell death of the outer air cells.[22] NAC has been documented to act as a substrate for glutathione production, free radical scavenger, mitochondrial protectant, lipid peroxidation inhibitor, and necrosis inhibitor in the inner ear.[23-25] Sinswat et al found significant threshold shift in hearing following intraperitoneal gentamicin treatment, which was mitigated with simultaneous injection of the iron chelator 2,3-dihydroxybenzoate.[26] Sha and Schacht reported progressive hearing threshold shift with administration of 2,3-dihydroxybenzoate at the time of gentamicin injection.[27]

These findings have been suggested in humans.[1] Patients undergoing hemodialysis are at high risk of hearing loss, as they often require aminoglycoside antibiotics due to vascular access infections and sepsis. Feldman et al evaluated the utility of NAC in prevention of hearing loss associated with gentamicin use in hemodialysis.[28] When NAC was delivered with gentamycin exposure, there was a significant reduction of ototoxicity, with improved hearing preservation in the high audiometric frequencies. The effect of antioxidant medication has also been shown in sudden SNHL. Joachims et al reported a recovery rate of 78% in patients treated with vitamin E and prednisone, as compared with 45% for patients treated with prednisone alone.[29] However, these patients received treatment within 7 days of hearing loss onset and therefore did not have stabilized hearing loss. These studies show a likely role for antioxidant treatment at the time of exposure, whereas our results suggest that administration must occur before the hearing loss is established.

As the etiology of hearing loss is likely due to free radical damage and subsequent apoptosis of hair cells, attempts at

treatment with antioxidant medications after hearing loss is established may be futile. Our findings may not be generalizable to the fluctuating hearing change seen in Ménière's disease and autoimmune hearing loss and potentially that of sudden SNHL.

Conclusion

Aggressive antioxidant therapy regime as performed in this study was not successful in improving established hearing loss.

Author Contributions

Gia Gill, performed updated literature review and was the primary manuscript author; **Brian W. Blakley**, performed the initial literature review, obtained ethics approval, performed data analysis, and provided manuscript edits. Both authors performed intervention and testing on guinea pigs

Disclosures

Competing interests: None.

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