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Reduction of noise-induced hearing loss using L-NAC and salicylate in the chinchilla[1]

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Abstract

The effects of a combination of two antioxidant compounds were studied in a chinchilla model of noise-induced hearing loss. After obtaining baseline hearing thresholds using inferior colliculus evoked potentials, chinchillas were exposed for 6 h to octave band noise centered at 4 kHz (105 dB SPL). Post-noise thresholds were obtained 1 h after the noise exposure, and then animals received either saline or salicylate and *N*-L-acetylcysteine combination. Another group received antioxidant treatment 1 h prior to noise. Hearing was tested at 1, 2 and 3 weeks post-noise. Subsequently, the cochleae were harvested, and cytocochleograms were prepared. There was a 20–40 dB SPL threshold shift at 3 weeks for tested controls. Permanent threshold shifts (PTS) were significantly reduced ($P < 0.05$) to approximately 10 dB for the pre-treatment group at week 3. The PTS for the post-treatment group at week 3 was similar to the pre-treatment group at 1 and 2 kHz (0–10 dB) but was intermediate between the control and pre-treatment groups at 4 and 8 kHz (23 dB). Animals pre-treated with antioxidant had a significant reduction in hair cell loss but those post-treated with antioxidant had no protection from hair cell loss. These findings demonstrate the feasibility of reduction of noise-induced hearing loss using clinically available antioxidant compounds.

Key words: Antioxidant; Loud noise; Evoked potential; Hair cell; Chinchilla

1. Introduction

Hearing loss due to noise exposure is an increasing problem in industrialized and developing societies. Noise is a prevalent worldwide 'pollutant', with 600 million persons estimated (Alberti, 1998) working in environments with hazardous levels of noise (50–60 million in the USA and Europe). The US government spends over 250 million dollars in compensation each year for military-related noise-induced hearing loss (D. Ohlin, US Army Hearing Conservation Program, personal communication). Mechanical hearing protection is essential and effective, however, inherent limitations allow a significant percentage of permanent hearing loss to occur after relatively short military noise exposures (Attias et al., 1994). Hence, a pharmacological preventative or rescue agent for noise-induced hearing loss (NIHL) would be an important element of a comprehensive approach to maintaining inner ear functional integrity.

The damage associated with some types of noise ex-

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posure has been linked to the creation of free radical or reactive oxygen species (ROS). Acoustic over-stimulation has been shown to increase intra-cochlear ROS (Yamane et al., 1995; Ohlemiller, 1998). High levels of ROS, induced by noise, activate the up-regulation of cochlear antioxidant enzyme activity (Jacono et al., 1998) and modulate the key antioxidant compound reduced glutathione (GSH) (Yamasoba et al., 1998b; Kopke et al., 1999). A variety of agents with antioxidant properties have been shown to attenuate threshold shifts and/or hair cell loss when given prior to a damaging noise exposure (Seidman et al., 1993; Quirk et al., 1994; Hu et al., 1997; Yamasoba et al., 1998a,b; Kopke et al., 1999; Komjathy et al., 1998; Liu et al., 1999; Hight et al., 1999). When administered to the inner ear before, or even shortly after noise exposure, protein trophic factors have also been demonstrated to prevent NIHL (Keithley et al., 1998; Shoji et al., 1998).

Auditory threshold shift after acoustic overexposure begins during exposure, however, hair cell loss in the noise-damaged cochlea may not begin for 3–5 days and continues for a period of time following both continuous (Fredelius et al., 1988) and impulse (Hamernik and Henderson, 1974) noise exposure. Models of excitotoxicity used in the CNS suggest that ongoing ROS damage may be related to spreading excitotoxicity or initiation and propagation of ROS chain reactions (Mattson and Scheff, 1994). Hence, a potential therapeutic window exists for pharmacological intervention to 'rescue' hearing loss after the cessation of noise exposure.

The present study investigates the efficacy of utilizing a combination of FDA-approved antioxidant compounds (salicylate plus *N*-L-acetylcysteine (L-NAC)) to either prevent or attenuate the level of NIHL in a chinchilla model. Both agents may be administered orally, which is a desirable characteristic. Low dose salicylate is known to scavenge hydroxyl free radicals (Ohlemiller, 1998) and in so doing to form the iron chelator dihydroxybenzoate. Iron chelators can prevent ROS formation by inhibiting the Fenton reaction (Yamasoba et al., 1998a). Salicylate may also inhibit the transcription factor nuclear factor-κB, a potential activator of inflammatory or cell death pathways (Kopp and Ghosh, 1994; Yin et al., 1998) or induce heat shock proteins which are known to provide a protective effect for the cochlea (Altschuler et al., 1996). L-NAC is another potentially effective candidate because it acts as an ROS scavenger as well as a neuroprotective agent by increasing intracellular GSH. L-NAC is a well-tolerated antidote to ROS-induced liver damage due to acetaminophen overdose (Kopke et al., 1997). GSH ester applied to the round window membrane of chinchillas substantially prevents noise-induced hair cell loss (Hight et al., 1999).

2. Materials and methods

Fifteen female adult *Chinchilla laniger* were divided equally into three experimental groups. Each animal received IC electrode implants 1 week prior to noise exposure. Baseline hearing thresholds were taken within 2 days of the initial noise exposure (Fig. 1). The three groups consisted of a saline control group, a pre-noise treatment group, and a post-noise treatment group, the latter two hereafter referred to as 'pre-treatment' and 'post-treatment' groups.

Pre-treatment animals received L-NAC (325 mg/kg) and salicylate (50 mg/kg) by intraperitoneal injection 1 h prior to and 1 h following noise exposure and then twice per day (b.i.d.) the following 2 days. The post-treatment group received the same dosage, although in this case at 1 h following noise and b.i.d. the ensuing 2 days. Saline-noise animals were injected with a similar volume of saline over the same schedule as the post-treatment group. All three groups had audiologic tests performed pre-noise, 1 h post-noise, and once a week for 3 weeks. Shortly after the last audiometric determination, animals were humanely killed, and the temporal bones were harvested and subsequently stained with a vital dye to indicate the presence of living hair cells.

The care and the use of the animals in this study were approved by the Animal Care and Use Committee of Naval Medical Center San Diego in accordance with the guidelines of the Declaration of Helsinki.

2.1. IC electrode placement

Animals were anesthetized with a ketamine/xylazine mixture and placed in a stereotactic head holder. Fur covering the cranium was removed and a midline scalp incision was made. Periosteum was stripped from the calvarium, and the underlying bone was treated with silver nitrate solution (10%) and then coated with cyanoacrylate cement. Using a diamond burr and high speed drill, a 1 mm burr hole was produced 1 mm anterior, and lateral to bregma. A 0.5 cm Teflon-coated tungsten rod electrode (reference) was then inserted and fixed using dental cement. Two more burr holes were drilled 1 mm lateral to midline and 5 mm anterior to the bullae. Using a micromanipulator, two 1 cm Teflon-coated tungsten rod electrodes were inserted individually. Electrode placement was confirmed using a real-time click-evoked auditory brainstem voltage response. The electrode was then fixed in place with dental cement. The skin wound was allowed to granulate around the skullcap. All animals received an antibiotic prophylactically for 72 h post-operatively (enrofloxacin, 2.5 mg/kg by intramuscular injection).

2.2. Evoked potential measurement

Animals were awake and lightly restrained in a plastic tube during the 30 min recording procedure. Digitally-generated stimuli consisted of tone pips (4 ms Blackman rise/fall ramp, 0 ms plateau, and constant alternating phase) at octave intervals 1, 2, 4, and 8 kHz. All acoustic stimuli were routed through a computer-controlled attenuator to an insert earphone (Etymotic Research ER-2). The sound delivery tube of the insert earphone was positioned approximately 5 mm from the tympanic membrane. Earphone sound delivery was calibrated using a coupler attached to the sound level meter approximating the distance from the earphone to the tympanic membrane. Five hundred samples were collected from the recording electrode, amplified (50 000–75 000×), filtered (100–1500 Hz), and fed to an A/D converter computerized on a signal processing board. Stimuli at a rate of 23/s were varied in 10 dB descending steps until threshold was reached, then 5 dB ascending steps were presented to confirm threshold. Earphone inserts on the tested ear were removed, and controls during which no sound was presented were determined for comparison. Threshold was defined as the mid-point between the lowest level at which a clear response was evidenced and the next lower level where no response was observed.

2.3. Noise exposure

One week was allowed to elapse after placement of the IC electrode in order to allow surgical healing prior to noise exposure. Our protocol was developed from the procedure of Hu et al. (1997). Specifically, an octave band noise centered at 4 kHz was generated by a standard audiometer (GSI 16), selected to white noise, routed through an attenuator (HP 350 D), a bandpass filter (Krohn-Hite 3550R), and a power amplifier (Crown D150A model 716) to an audiometric loudspeaker suspended directly above the animal's cage. The sound spectrum output of the system was confirmed using a Larson and Davis model 800B sound level meter, centering the octave bandwidth at 4 kHz. In order to ensure consistent noise exposure conditions, the noise output of the system was periodically monitored using a sound level meter (Larson and Davis 800B). Also, a pre-amplifier (Larson and Davis model 825) and a condenser microphone (Larson and Davis, LDL 2559) were positioned within the cage at the level of the animal's head. Each animal was exposed continuously to the noise at a level of 105 dB SPL for 6 h. During the noise exposure, the animal was unrestrained in a small wire cage with ad lib food and water access. When the animals were not being exposed to noise, they were housed in a quiet animal colony.

2.4. Histologic examination

Following auditory tests (i.e. at 3 weeks post-noise exposure), the animals were heavily anesthetized with ketamine (30 mg/kg) and xylazine (1 mg/kg). Each temporal bone was quickly removed from the skull. The cochlea was exposed and slowly perfused through the oval window and round window with a solution of 0.2 M sodium succinate and 0.1% nitrotetrazolium blue in 0.2 M phosphate buffer (pH 7.4 at 37°C). Samples were then immersed in the same solution for 1 h at 37°C. Lastly, the cochlea was rinsed with buffer and fixed with 4% paraformaldehyde for 24 h. Cochleae were dissected and sections of the organ of Corti were mounted on glass slides and examined for hair cell loss under a light microscope at 400× magnification. Missing or non-viable hair cells were noted by the absence of blue vital stain in the area of inner and outer hair cells. An experienced but experiment-blinded observer counted missing hair cells over the length of the basilar membrane per cochlear turn utilizing specialized software. A cytocochleogram was developed for inner and outer hair cells for each cochlea, and cytocochleogram means were computed and graphed.

2.5. Statistical analyses

A three-way ANOVA with interaction model was used for analyzing the effect on hearing thresholds over time for each ear being treated as repeated measures. Where a significant effect included more than two levels, pair-wise comparisons of the levels were made by Scheffé methods (post hoc test). A one-way ANOVA was used to analyze the hearing threshold data at the 3-week time point at the termination of the experiment, and the Newman–Keuls multiple comparison tests (post hoc) were used to analyze the hearing threshold treatment effects when significant differences were identified. To analyze cytocochleogram statistics, the area under the curve representing missing inner or outer hair cells was determined for each cochlea using AutoSketch R2 for Windows (Autodesk, Inc.). Mean areas under the curve were compared across treatment groups using one-way ANOVA. When differences were found, Tukey's post hoc analysis was applied to identify significant differences. A P-value smaller than 0.05 was considered significant.

3. Results

3.1. Audiometry

Baseline audibility threshold averages for both right and left ear evoked potentials at 1, 2, 4 and 8 kHz are

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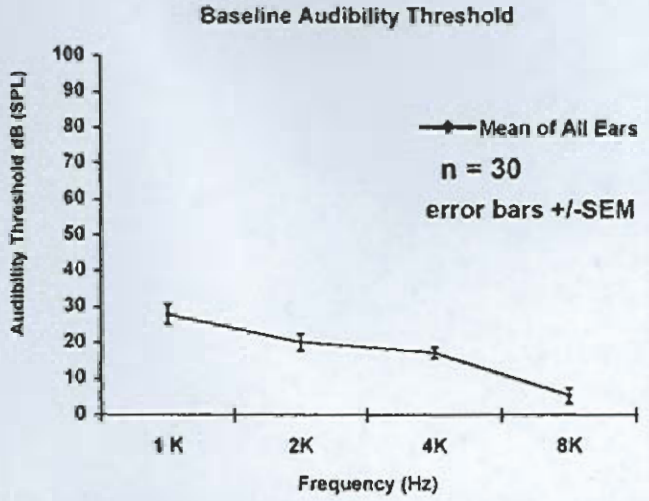

Fig. 1. Baseline hearing threshold. Pre-noise audibility thresholds derived from IC evoked potentials for the subjects of this study are shown for 1, 2, 4, and 8 kHz frequencies. The audibility thresholds range from a high of 28 dB sound pressure level (SPL) at 1 kHz to a low of 5.3 dB SPL at 8 kHz. These are mean values of sums of left and right ears prior to noise exposure. There were a total of 15 subjects and 30 ears. Error bars represent S.E.M.

represented in Fig. 1. Thresholds ranged from a maximum of 28 to a minimum of 8 dB SPL at 1 to 8 kHz, respectively. These thresholds are consistent with independently published normative data (Henderson et al., 1973; Hu et al., 1997).

Auditory threshold shifts (group means for left and right ears) are displayed (Fig. 2A–D) as post-noise thresholds (dB SPL) minus baseline threshold (dB SPL). Means were plotted as a function of treatment group (saline-noise, antioxidant pre-treatment and antioxidant post-treatment), over time (0, 1, 2, 3 weeks) and by threshold test frequency (1, 2, 4, and 8 kHz).

As shown (Fig. 2A–D), 6 h sound exposure produced an initial threshold shift ranging from approximately 55 dB at 1 kHz (Fig. 2A) to approximately 80 dB at the higher frequencies (Fig. 2B–D). The post-treatment group receiving the antioxidants 1 h after exposure demonstrated threshold shifts similar to those of the saline-noise group except at 1 kHz, where the shift was only 33 dB. The initial threshold shifts were reduced for the pre-treatment group compared to the saline-noise group at all frequencies. This suggests that the antioxidants were able to reduce temporary threshold shifts induced in this model given that noise exposure was standardized and calibrated for all groups. There was evidence of recovery of threshold shift among all three groups. However, the threshold shift recovery for the saline-noise group stabilized over weeks 2 and 3, resulting in no significant threshold improvement for any of the frequencies tested. In contrast, both the pre- and post-treatment groups showed statistically significant improvement over the entire time period for all frequencies except 1 kHz (see Table 1), where the interaction effect with time was not significant ($P=0.739$). There was an overall treatment effect for both pre- and post-treatment at 1 kHz compared with the saline-noise condition ($F_{2,28}=7.4678$; $P=0.013$ and $P<0.01$, respectively on post hoc analysis; see Table 1), but the former two conditions' effects were not statistically different from each other. Hearing thresholds at 1 kHz improved (compared to saline-noise) from week 0 to week 1 and from week 1 to week 2, but not from week 2 to week 3. This finding diminished the comparison of the treatment conditions with time.

As seen in Fig. 2, the permanent threshold shift observed for the pre- and post-treatment groups was reduced compared to that of the saline-noise group. The threshold shift at 3 weeks for controls varied between 20 and 40 dB SPL from 1 to 8 kHz. The permanent threshold shift for the pre-treatment animals was significantly reduced to approximately 0–10 dB. The permanent threshold shift for the post-treatment group at 3 weeks was similar to the pre-treatment group at 1 and

Table 1
Summary of threshold shift statistical results

	F	P^b	df
1 kHz			
Treatment (overall effect)	7.4678	<0.01	2, 28
Pre-treatment[a]		0.013	2, 28
Pre-treatment by time	0.5869	ns	6, 24
Pre-treatment at 3 weeks[a]	10.680	<0.001	2, 28
Post-treatment[a]		0.008	2, 28
Post-treatment by time	0.5869	ns	6, 24
Post-treatment at 3 weeks[a]	10.680	<0.001	2, 28
2 kHz			
Treatment (overall effect)	7.6273	<0.01	2, 28
Pre-treatment[a]		0.007	2, 28
Pre-treatment by time	5.0637	<0.001	6, 24
Pre-treatment at 3 weeks[a]	9.285	<0.01	2, 28
Post-treatment[a]		0.013	2, 28
Post-treatment by time	5.0637	<0.001	6, 24
Post-treatment at 3 weeks[a]	9.285	<0.01	2, 28
4 kHz			
Treatment (overall effect)	6.6610	<0.01	2, 28
Pre-treatment[a]		0.005	2, 28
Pre-treatment by time	3.8650	<0.001	6, 24
Pre-treatment at 3 weeks[a]	8.151	<0.01	2, 28
Post-treatment[a]		ns	2, 28
Post-treatment by time	3.8650	<0.001	6, 24
Post-treatment at 3 weeks[a]	8.151	<0.05	2, 28
8 kHz			
Treatment (overall effect)	9.1020	<0.01	2, 28
Pre-treatment[a]		0.002	2, 28
Pre-treatment by time	2.9589	<0.001	6, 24
Pre-treatment at 3 weeks[a]	6.8050	<0.01	2, 28
Post-treatment[a]		ns	2, 28
Post-treatment by time	2.9589	<0.05	6, 24
Post-treatment at 3 weeks[a]	6.8050	ns	2, 28

ns = not significant.
[a]Compared to saline control.
[b]Post hoc analysis.

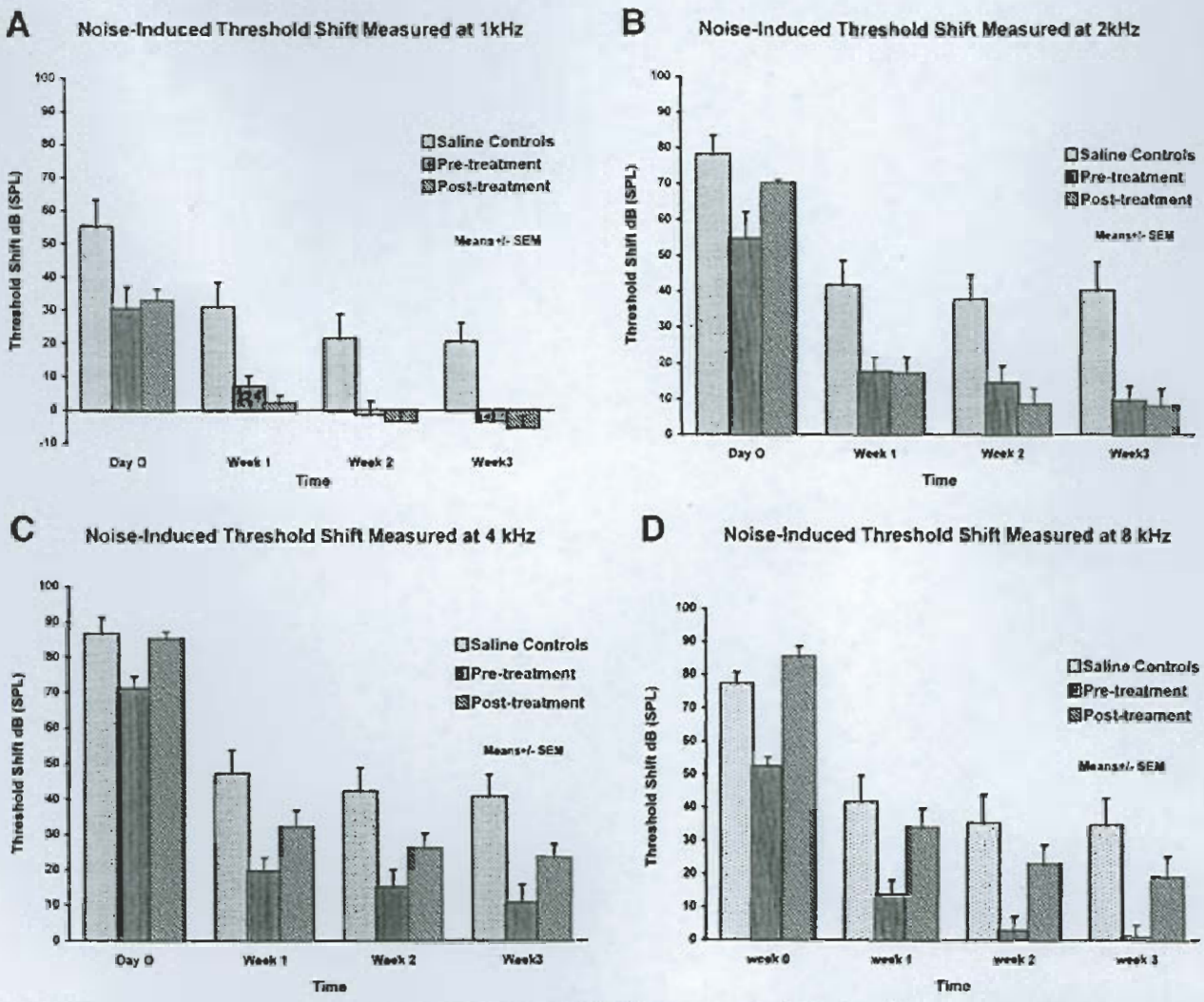

Fig. 2. A–D: Threshold shift after noise exposure. Audibility threshold shifts measured at 1, 2, 4, and 8 kHz after a 6 h 4 kHz octave band noise exposure at 105 dB SPL, as a function of time, test frequency, and treatment group. Threshold shift was calculated as the threshold for a particular frequency at a time point after noise exposure minus the baseline threshold for that frequency in dB SPL. Thresholds at time point day 0 were obtained 1 h after exposure. The data depict means of left and right ears of five animals (10 ears) in each group for a total of $n = 15$ (30 ears). Initial threshold shifts at day 0 were equivalent for saline-noise and post-treatment groups at all frequencies except at 1 kHz where the initial threshold shift was reduced for the post-treatment group. The initial threshold shifts were reduced for the pre-treatment group compared to the saline-noise group at all frequencies. There was recovery of threshold shift among all three groups. However, the threshold shift recovery for the saline-noise group tended to plateau over weeks 2 and 3. In contrast, the permanent threshold shifts that were observed for the pre- and post-treatment groups were reduced significantly ($P < 0.05$) compared to the saline-noise group. The threshold shift at 3 weeks for saline-noise animals varied between 20 and 40 dB SPL from 1 to 8 kHz. The permanent threshold shift for the pre-treatment animals was significantly reduced to about 0–10 dB. The permanent threshold shift for the post-treatment group at 3 weeks was similar to the pre-treatment group at 1 and 2 kHz (0 to 10 dB) (A and B) but was intermediate between the saline-noise and pre-treatment groups at 4 and 8 kHz (23 dB) (C and D). The post-treatment effect at 1 kHz may be explained by the reduced initial threshold shift seen at 1 kHz. Error bars represent S.E.M.

2 kHz (0–10 dB), but was intermediate between the saline-noise and pre-treatment groups at 4 and 8 kHz (23 dB). Threshold shift for the pre-treatment group was significantly lower at 1, 2, 4 and 8 kHz, ($P < 0.05$) for treatment effects averaged over time as evidenced by application of two-factor repeated measure ANOVA. The post-treatment group effect reached significance at 1, 2, 4, and 8 kHz, when week 3 was compared to week 1 ($P < 0.05$). The effect at 1 kHz may be explained by the reduced initial threshold shift observed in the post-treatment group at 1 kHz.

3.2. Hair cell counts

Fig. 4A–D illustrates mean inner and outer hair cell counts in a cytocochleogram which portrays missing hair cell percentages on the y-axis as a function of the measured percent distance from the cochlear apex. The

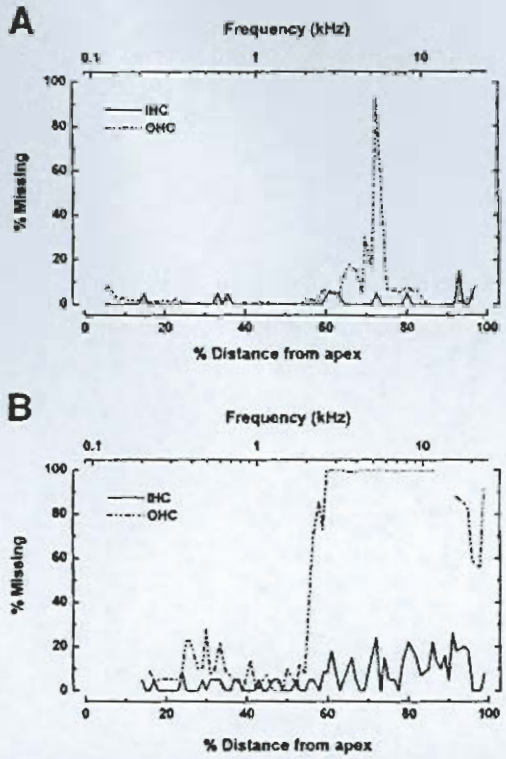

Fig. 3. A and B: Representative individual ear cytocochleogram data. These cytocochleograms depict percent missing inner (solid line) and outer (dashed line) hair cells as a function of % distance along the basilar membrane from the apex, also correlated with frequency response range. Panel A is from the right ear of a pre-treated, noise-exposed animal and demonstrates a narrow band of outer hair cell loss to over 90% as well as scattered inner hair cell loss. In contrast to this, panel B from the left ear of a saline-noise animal demonstrates a much broader band of greater than 90% outer hair cell loss and more extensive inner hair cell loss than seen in the pre-treated ear.

associated frequency region of the cochlea is plotted on the *x*-axis. Fig. 3A,B depicts individual cytocochleograms from a pre-treated noise-exposed cochlea, and a saline-treated noise-exposed cochlea, respectively. Fig. 4A demonstrates that there is very little inner or outer hair cell loss in control animals not exposed to noise. Fig. 4B shows that the 6 h 4 kHz octave band noise exposure caused substantial hair cell loss, with maximal loss occurring between 5 and 6 kHz. Sixty to 80% of the outer hair cells were lost in the region between 3 and 8 kHz on the cochlea. Fewer inner hair cells were lost (maximum of 30%) in approximately the same region of the cochlea. In contrast, as shown in Fig. 4C, there was a substantial reduction in both outer and inner hair cell loss in the pre-treatment group compared to saline-noise (20–30% outer hair cell loss vs. 60–80%, 0–10% inner hair cell loss vs. 20–30%, respectively). The outer hair cell loss reached statistical significance for pre-treatment vs. saline saline-noise groups ($P < 0.05$), but not for inner hair cell differences. As noted in Fig. 4D, there was no statistically significant difference in hair cell loss in the post-treatment group compared to the saline-noise group.

3.3. Cytocochleogram – area under the curve analysis

A multi-way ANOVA was applied to demonstrate that there were no ear-to-ear differences among all animals. Therefore, each cochlea was used in the area under the curve analysis as an independent observation giving 10 observations across three treatment groups (total of 30 observations).

Using a Kruskal–Wallis analysis, it was demonstrated that there was a significant treatment effect for both the inner hair cell (IHC) and outer hair cell (OHC) area under the curve measures. For IHC and OHC areas, the χ^2 values were 6.057 (df = 2) and 11.760 (df = 2), respectively. Dunn's multiple comparison tests determined that pre-treatment with antioxidants provided the greatest protection for auditory hair cells showing the least area under the curve for the OHC measures. Pre-treatment OHC values were significantly different from both the saline and post-treatment groups. However, for IHC measures, the pre-treatment of antioxidants was not significantly different from the saline group, but was significantly different from the post-treatment group.

4. Discussion

While transducing acoustical energy into neural signals, the cochlea produces ROS as a normal cellular byproduct. Under normal circumstances, various antioxidant defense mechanisms present in the inner ear prevent these damaging radicals from causing any permanent harm (Kopke et al., 1999). With extreme acoustic over-stimulation, normal homeostasis is no longer maintained, however, and cells may undergo a non-recoverable injury leading to a permanent NIHL. By augmenting the native antioxidant defense system during periods surrounding a noise stress, our results suggest that permanent threshold shift of hearing may be prevented or reduced.

The present study demonstrates the potential efficacy of a combination of two FDA-approved agents in preventing, and to a lesser extent reversing, permanent noise-induced threshold shifts in a chinchilla model of NIHL. The thresholds reported in this study were similar to data published from other labs, which utilized the same animal model for NIHL (Hu et al., 1997; Henderson et al., 1973). After an intense 6 h noise exposure, we found very substantial hair cell loss as well as significant permanent shifts in evoked IC auditory thresholds. Threshold shifts and hair cell losses reported in this study were more substantial than in previous

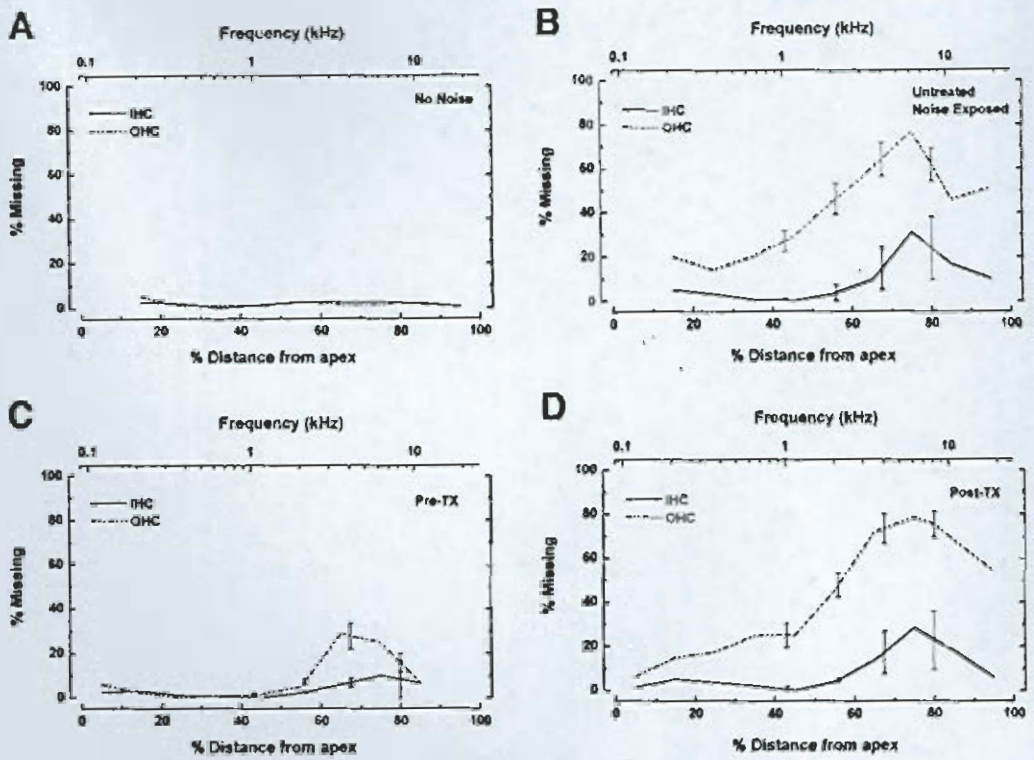

Fig. 4. A–D: Mean cytocochleogram data. Panels A–D graphically depict mean inner and outer hair cell losses in a cytocochleogram which graphs mean percent missing hair cells on the *y*-axis as a function of percent distance from the cochlear apex and associated frequency region of the cochlea on the *x*-axis. Panel A (no noise) demonstrates that there is very little inner or outer hair cell loss in non-noise-exposed animals. Panel B (untreated noise-exposed) shows that the 6 h 4 kHz octave band noise exposure at 105 dB SPL in untreated saline-noise animals caused substantial hair cell loss, with a maximal loss occurring at 5–6 kHz. Sixty to 80% of the outer hair cells were lost in the region between 3 and 8 kHz. Fewer inner hair cells were lost (maximum of 30%) in approximately the same region of the cochlea. Panel C (Pre-TX) shows there was a substantial reduction in both outer and inner hair cell loss in the pre-treatment group compared to the saline-noise group (20–30% outer hair cell loss vs. 60–80%, 0–10% inner hair cell loss vs. 20–30%; 3 weeks post-noise exposure). Panel D (Post-TX) shows there was no difference in hair cell loss in the post-treatment group compared to the saline-noise group. Error bars represent S.E.M.

experiments (Hu et al., 1997) which utilized a 4 h exposure of the same frequency spectrum and intensity of sound. By increasing the noise trauma, we increased the difference in threshold shift between untreated (saline-noise) and treated subjects.

Despite the intensity of this noise exposure, the antioxidant combination, when applied shortly before the noise exposure, reduced permanent threshold shifts by 75%, and inner and outer hair cell loss by over 50%. These data are consistent with previous studies using other antioxidant compounds or compounds with antioxidant actions, administered either systemically (Seidman et al., 1993; Attias et al., 1994; Quirk et al., 1994; Yamasoba et al., 1998b; Komjathy et al., 1998), or applied to the round window membrane (Hu et al., 1997; Shoji et al., 1998; Keithley et al., 1998; Liu et al., 1999; Hight et al., 1999) prior to noise exposure. Administration of the antioxidant agents utilized in this study resulted in a reduction in threshold shift and/or hair cell loss, further supporting the hypothesis that ROS play a significant role in the causative mechanisms of NIHL. The pharmacological agents used in this study, when compared to other published agents (described above), may prove suitable in future clinical trials to prevent NIHL. The ability to use an oral agent would by inference be useful for groups exposed to periodic noise trauma at known intervals. Whether or not these agents are protective for impulse noise is not known.

Administration of the antioxidant compounds after noise exposure significantly reduced permanent threshold shift but evidenced no effect in preventing hair cell loss in our model. Improvement of hearing thresholds in the post-treatment group despite hair cell losses, which were equivalent to the untreated saline-noise group, is unclear. There could have been an enhancement of cellular repair processes or prevention of ongoing sub-lethal damage in the surviving hair cells, which could not be detected with our histological techniques, our procedure identified only viable hair cells. Thus, the enhanced cochlear function in the post-treatment group may have been a result of enhanced function in the remaining hair cells (i.e. compared to saline-noise controls). The relative lack of effectiveness when

the antioxidants were given after the noise is not clear, but the duration and intensity of the noise exposure may have exceeded the agent's antioxidant capacity when given after the noise exposure. Consequently, the noise-induced burst of ROS may have initiated a precipitation of excitotoxicity similarly observed after CNS trauma or ischemia (Mattson and Scheff, 1994), or it may have activated self-propagating chain reactions of lipid peroxidation or other oxidation cascades which were not effectively countered by our antioxidant combination (Mattson and Scheff, 1994).

It has been previously reported that loss of hair cells of the organ of Corti is not the only characteristic of hearing loss. The degree of hair cell loss following noise trauma is sometimes difficult to correlate with the auditory threshold shift (Lataye and Campo, 1996; Liberman and Beil, 1979; Ades et al., 1974) and with the total dose of noise (Erlandsson et al., 1987). It has been reported that the orderliness of the stereocilia, along with cellular changes such as swelling of the synapses at the base of outer and inner hair cells (Spoendlin, 1979; Robertson, 1983; Canlon et al., 1992), is as important as the absence of hair cells (Ulfendahl et al., 1993). In the present study, we too report this imperfect correlation of hair cell loss with hearing loss. As reported previously, we agree that two distinct mechanisms of acoustic trauma may take place within the organ of Corti, i.e. metabolic and mechanical damage (Lataye and Campo, 1996; Ades et al., 1974). While simple hair cell loss is easily observed, at what point do degenerative changes render a hair cell non-functional or partially or intermittently functional? And of equal importance, how does this correlate with auditory threshold measurements?

Alternatively, the burst of ROS may initiate necrosis and/or apoptosis (programmed cell death) pathways (Raffray and Cohen, 1997; Liu et al., 1998; Nakagawa et al., 1997) that could not be prevented or terminated by these compounds given under these conditions. A recent study by Pirvola et al. (2000) demonstrated DNA fragments of hair cell nuclei after ototoxic drug and noise exposure in vitro and in vivo. Pirvola and colleagues report that the c-Jun N-terminal kinase pathway, which is associated with apoptosis, is activated in hair cells after noise exposure.

On-going hair cell loss leading to transient gaps in the reticular lamina, with exposure of hair cells to toxic endolymph, might play a role in propagation of the injury after noise cessation (Bohne, 1976; Bohne and Rabbit, 1983; Fredelius, 1988). This catabolic time course may not be amenable to antioxidant reversal.

As with other biological systems, a concentration effect of toxin (in this case toxic noise) may produce zones of necrosis in areas of maximal concentration surrounded by zones of injury where the concentration is reduced. The injured cells may be repaired or undergo programmed cell death depending on the severity of the injury and availability of agents which might induce repair (Raffray and Cohen, 1997). Further study is needed to optimize the conditions and agents that may reverse hearing loss when the agents are administered after noise exposure. Although further pre-clinical study is warranted, these data suggest that it may be feasible to augment mechanical hearing protection with pharmacological antioxidant agents to more completely prevent permanent hearing loss due to excessive noise.

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