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Lipoic acid and 6-formylpterin reduce potentiation of noise-induced hearing loss by carbon monoxide: Preliminary investigation

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Abstract—Potentiation of noise-induced hearing loss (NIHL) by specific chemical contaminants and therapeutic drugs represents a distinct public health risk. Prediction of chemicals that yield such potentiation has not been successful because such agents differ markedly in structure. One mechanism for this potentiation that has garnered support is oxidative injury to the cochlea. Thus far, limited data have been published in support of this hypothesis. The current experiment was designed to further test this model using two antioxidant compounds, lipoic acid (LA) and 6-formylpterin (6-FP), and determine whether they would block potentiation of NIHL resulting from simultaneous exposures to carbon monoxide (CO) and noise in rats. Neither CO nor noise exposure at the intensity and duration selected produce persistent auditory impairment by themselves. Different groups of rats were exposed to noise alone centered at 8.0 kHz (105 dB) for 2 hours or to combined CO + noise treatment consisting of CO exposure for 1.5 hours and then exposure to CO + noise for 2 hours. Additional groups received either LA (100 mg/kg) or 6-FP (14 mg/kg) 30 minutes prior to the onset of CO + noise. Cochlear function was monitored using distortion product otoacoustic emissions, and auditory thresholds were assessed using compound action potentials recorded from the round window. Histopathological evaluation of the organ of Corti provided counts of missing hair cells in each treatment group. The CO + noise-exposure group replicated previous studies in demonstrating permanent impairment of cochlear function and associated outer hair cell loss that greatly exceeded the minimal losses observed in the group treated with noise alone. Both LA and 6-FP given 30 minutes prior to the onset of CO + noise exposure reduced cochlear impairment and loss of hair cells.

Key words: 6-formylpterin, antioxidant, carbon monoxide, lipoic acid, noise, noise-induced hearing loss, potentiation, protection, rat, rehabilitation.

INTRODUCTION

One potential risk factor for the occurrence of significant hearing loss even under conditions of relatively low noise exposure is the presence of specific chemical contaminants. Organic solvents, metals, and chemical asphyxiants are all known to have ototoxic potential [1–3]. Simultaneous and even successive exposure to certain of these agents along with noise can greatly increase

Abbreviations: 6-FP = 6-formylpterin, ANOVA = analysis of variance, CAP = compound action potential, CO = carbon monoxide, DPOAE = distortion product otoacoustic emission, f1 = first fundamental frequency, f2 = second fundamental frequency, GM = geometric mean, IM = intramuscular, IP = intraperitoneal, LA = lipoic acid, NIHL = noise-induced hearing loss, OBN = octave-band noise, OHC = outer hair cell, PBN = phenyl-N-tert-butyl-nitrone, PBS = phosphate-buffered saline, ROS = reactive oxygen species, SPL = sound pressure level, VA = Department of Veterans Affairs.

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susceptibility to noise-induced hearing loss (NIHL) both for humans and laboratory animals [3–4].

The potentiation of NIHL by carbon monoxide (CO) exposure is particularly well established, and CO serves as a useful model because it is a common toxicant with well-studied physiological effects. A series of publications from our laboratory have demonstrated that CO elevates sensitivity to permanent NIHL such that noise exposures that normally produce no permanent auditory threshold shift yield profound loss if CO is present along with the noise [5–11]. The extent of cochlear impairment is related to CO dose [9], but the loss of outer hair cells (OHCs) tends to occur preferentially in the base (high-frequency region) of the cochlea, even when noise energy is maximal within a relatively low (4.0–8.0 kHz) frequency band [7]. CO alone does not produce persistent impairment of auditory function, although it can produce transient effects during acute exposure [12].

The acute effect of CO on hearing appears to involve an oxidative stress mechanism because the loss in function can be reduced or eliminated by administration of either a spin trap agent (phenyl-N-tert-butyl-nitrone [PBN]) or inhibition of reactive oxygen species (ROS) formation via the xanthine oxidase pathway (allopurinol) [13]. A two-step model is proposed to explain the potentiation of NIHL by CO. First, it is hypothesized that moderate levels of noise exposure initiate ROS formation, which is buffered via a homeostatic antioxidant system under normal conditions. This possibility has not been directly tested, although it is well-known that intense noise exposure can enhance production of ROS and generate oxidative stress. Second, it is hypothesized that if moderate noise exposure is combined with exposure to chemicals that either generate ROS on their own (e.g., CO) or disrupt intrinsic ROS-buffering pathways (e.g., acrylonitrile), intrinsic antioxidant pathways can become overwhelmed and the resulting oxidative stress could yield cochlear injury and dysfunction.

The ability of two antioxidant drugs, lipoic acid (LA) and 6-formylpterin (6-FP), to block the potentiation of NIHL by CO was investigated in this study. LA has a complex antioxidant action, being able to directly scavenge free radicals and also acting to regenerate other antioxidants such as glutathione and vitamins C and E [14]. LA has been shown to have significant protective effects in multiple organ systems, including the liver [15], kidneys [16], and brain [17]. In the cochlea, LA has also been shown to slow the development of topical aminoglycoside ototoxicity in guinea pigs [18], block both cisplatin- and carboplatin-induced ototoxicity in rats [19–20], and reduce age-related hearing loss in mice [21].

Very limited data are available concerning the biological action of 6-FP in vivo. However, 6-FP, like many other pterins, is known to be a potent inhibitor of xanthine oxidase [22]. From in vitro studies, 6-FP can apparently have differential effects on cells. This agent can promote the generation of ROS, increase apoptosis, and suppress cell proliferation [23]. However, 6-FP also inhibits DNA fragmentation in vitro when cells are treated with tumor necrosis factor-α/actinomycin D. At the same time, 6-FP is known to scavenge superoxide anion radical (O_2^-) with an efficiency more than 100 times higher than PBN and to have a neuroprotective effect in the gerbil global brain ischemia model that also surpasses that of PBN [24].

METHODS

Subjects

A total of 37 male Long-Evans rats (225–250 g, 7–8 weeks old) obtained from Harlan (Indianapolis, Indiana) were used in these experiments. The subjects were housed with free access to food and water in their home cages. Temperature was maintained at 21 ± 1 °C, and lights were on from 6:30 am to 6:30 pm. The Jerry L. Pettis Memorial Department of Veterans Affairs (VA) Medical Center Institutional Animal Care and Use Committee approved all the experimental protocols. All efforts were made to minimize the number of animals used in these experiments.

Chemicals

The LA was purchased from Sigma-Aldrich (St. Louis, Missouri), and the 6-FP was received as a gift from Dr. Keisuke Makino of the Institute of Advanced Energy, Kyoto University, Japan.

Procedures

This experiment was designed to determine whether LA and 6-FP pretreatments would protect the cochlea against the potentiation of NIHL by CO. For this purpose, Long-Evans rats were exposed to different combinations of CO, LA, 6-FP, and/or noise. Experimental groups and treatment schedules are detailed in the **Table**.

Table.
Experimental groups and treatments.

Treatment Group	Noise	CO	Antioxidant	Time
Control (n = 8)	—	—	—	—
Noise Alone (n = 10)	100 dB OBN/8.0 kHz	—	—	2 d
CO + Noise (n = 12)	100 dB OBN/8.0 kHz	CO 800 ppm	—	2 d
LA + CO + Noise (n = 4)	100 dB OBN/8.0 kHz	CO 800 ppm	LA 100 mg/kg IP	2 d
6-FP + CO + Noise (n = 3)	100 dB OBN/8.0 kHz	CO 800 ppm	6-FP 14 mg/kg IP	2 d

6-FP = 6-formylpterin, CO = carbon monoxide, IP = intraperitoneal, LA = lipoic acid, OBN = octave-band noise.

Since previous studies showed no permanent hearing impairment after exposure to CO alone [6–7,13,25–26], rats were not exposed to that treatment.

Chen and Fechter showed that carboxyhemoglobin levels approach steady state within 30 minutes of exposure onset and stabilize by 90 minutes of exposure [5]. Therefore, CO exposure began for the appropriate subjects 90 minutes prior to the onset of noise to assure carboxyhemoglobin equilibration. Noise exposures lasted 2 hours and were designed to yield very mild permanent NIHL.

Antioxidant treatments (LA and 6-FP) were administered 30 minutes before noise onset. LA was administered by intraperitoneal (IP) injection at a concentration of 100 mg/kg. This concentration was chosen because it was found to be effective against carboplatin [20] and cisplatin [19] ototoxicity. Moreover, Elsayed et al. used a total dose of 75 mg/kg LA (25 mg/kg daily for 3 days) in studies of noise-induced oxidative stress [27].

Previous studies in gerbils showed that 6-FP provided neuroprotection from ischemia at doses of 7.1 to 8.6 mg/kg. In this study, 6-FP was administered at a concentration of 14 mg/kg. We dissolved 14 mg of 6-FP in 1 mL of 0.1M NaOH (pH 13). This solution was immediately diluted with 4 mL of 0.1M phosphate-buffered saline (PBS) to make a 3 mg/mL solution. The rats received IP injection of 5 mL/kg of this solution.

Noise Exposure

Noise exposures were conducted in a ventilated reverberant 40 L Plexiglas® cylinder. The subjects were placed within small wire-cloth enclosures (15 × 13 × 11 cm) within the chamber. They were conscious and free to move within the enclosures. Broadband noise was generated by a function generator (model DS335; Stanford Research Systems, Inc; Sunnyvale, California) and bandpass filtered (model 9002; Frequency Devices, Inc; Haverhill, Massachusetts) to provide octave-band noise (OBN) with center frequency of 8.0 kHz. The roll-off for the filter system was 48 dB/octave. This signal was amplified by an SAE 2200 power amplifier (Scientific Audio Electronics, Inc; Los Angeles, California) and fed to speakers (Vifa model D25AG-05; Tymphany; Videbaek, Denmark) located approximately 5 cm above the subjects' wire-cloth enclosure. Sound intensity was measured at the level of the rats' pinnae by a Quest Type 1 sound pressure meter with a 1/1 octave filter set (models 1700 and OB300, respectively; Oconomowoc, Wisconsin) and was 100 dB in the OBN centered at 8.0 kHz. Sound level in the exposure chamber was maximal and essentially flat between 6.3 and 10.0 kHz. The levels were approximately 7 dB lower at 5.0 and 12.5 kHz. The acoustic intensity was approximately 20 dB below maximum at 4.0 and 16.0 kHz. Noise levels varied less than 2 dB within the space available to each animal. The intensity and duration of the noise exposure (105 dB sound pressure level [SPL] for 2 hours) was chosen in order to obtain limited but discernible NIHL.

CO Exposure

CO was delivered into the inhalation chamber via polyethylene tubing connected to a compressed CO tank with a regulator equipped with a microvalve. The flow of CO was monitored by a Top Trak 82213-0V1-PV1-V1-LF flow meter (Sierra Instruments; Monterey, California). Air-exchange rate in the exposure chamber was 20 L/min as monitored by a Top Trak 821-I-PS mass flow meter. This flow rate provided approximately one air change every 2 minutes. At regular intervals during exposure, the actual CO concentration inside the chamber was monitored with a CO meter (model CO-220; Fluke Corp; Everett, Washington) and the CO flow was fine-tuned in order to maintain the CO concentration at 800 ppm. The CO concentration in the exposure chamber reached the desired level within 30 minutes of exposure onset.

Assessment of Outer Hair Cell Function: Distortion Product Otoacoustic Emissions

Subjects received distortion product otoacoustic emission (DPOAE) testing prior to experimental treatment and 1 hour, 1 week, and 4 weeks postexposure in order to trace both immediate and permanent alterations in cochlear function as reflected in the cubic distortion product amplitude (2 × first fundamental frequency [f1] – second fundamental frequency [f2]). The f1 and f2 primary tones were generated by a dual-channel synthesizer (model 3326A; Hewlett Packard; Palo Alto, California) and attenuated, under computer control, with customized software. The f1 and f2 primary tones (f2/f1 = 1.25) were then presented through two separate earphones (realistic dual radial horn tweeters; Radio Shack; Tandy Corp; Ft. Worth, Texas) and delivered through a probe to the outer-ear canal, where they were acoustically mixed to avoid artifactual distortion. Ear-canal SPLs, measured by an emissions microphone assembly (model ER-10B+; Etymotic Research, Inc; Elk Grove Village, Illinois) embedded in the probe, were sampled, synchronously averaged, and Fourier analyzed for geometric mean (GM) frequencies [$(f1 \times f2)^{0.5}$] ranging from 5.6 to 19.7 kHz (i.e., f2 = 6.3–22.5 kHz) by a computer-based digital signal processing board. Corresponding noise floors were computed by averaging the levels of the ear-canal sound pressure for five frequency bins above and below the DPOAE frequency bin (±54.0 Hz). For test frequencies above 20.1 kHz, a computer-controlled dynamic-signal analyzer (model 3561A, Hewlett Packard) was used. The related noise floors were estimated by averaging the levels of the ear-canal sound pressure for the two fast Fourier transformation frequency bins below the DPOAE frequency (i.e., for 3.75 Hz below the DPOAE).

DPOAEs were measured as DP-grams. Specifically, DP-grams describe emission levels in response to primary tones set at f1 intensity (L1) = 65 dB SPL and f2 intensity (L2) = 55 dB SPL as a function of the GM frequencies, which ranged from 2.9 to 56.3 kHz (f2 = 3.2 to 63.0 kHz) in 0.1-octave increments. For both stimulus protocols, DPOAEs were considered to be present when they were at least 3 dB above the noise floor. Between f2 = 20.0 and f2 = 25.0 kHz, the DP-grams display an artifactual notch due to the resonance of the rat's outer auditory meatus that prevents the primary frequencies from reaching the eardrum with adequate intensities and increases the noise floor obtained within this range. Candreia et al. previously observed a similar notch in the mouse [28]. This phenomenon was recently described in detail by Martin et al. [29].

The animals were lightly anesthetized by intramuscular (IM) injection of xylazine (7 mg/kg) and ketamine (44 mg/kg) and placed on a heating table in order to maintain body temperature at 38 °C. The probe was inserted in the right auditory canal; the same ear was subsequently used for compound action potential (CAP) determination.

Assessment of Auditory Threshold

Four weeks following exposure, a time interval designed to permit recovery of any temporary threshold shifts, auditory thresholds were assessed in all subjects. The subjects were anesthetized with xylazine (13 mg/kg IM) and ketamine (87 mg/kg IM), and normal body temperature was maintained with a heating unit that was built into the surgical table. The temperature of the cochlea was also maintained with a low-voltage high-intensity lamp. The auditory bulla was opened via a ventrolateral approach to allow the placement of an insulated silver wire electrode onto the round window. A silver chloride reference electrode was inserted into the neck muscle. The CAP signals evoked by pure tones were amplified 1,000 times between 0.1 and 1.0 kHz with a preamplifier (model P15; Grass Instrument Co; Quincy, Massachusetts). The sound level necessary to generate a visually detectable CAP response on a digital oscilloscope (approximate response amplitude of 1 μV) was identified based on the average of four sweeps. Pure tones for eliciting CAP were generated by an SR530 lock-in amplifier (Stanford Research Systems, Inc). A programmable attenuator controlled the tone intensity, and the output of the attenuator was amplified by a high-voltage amplifier and then delivered to the sound transducer in the rat's external auditory meatus. Auditory thresholds were determined for tones of 2, 4, 6, 8, 12, 16, 20, 24, 30, 35, and 40 kHz using tone bursts of 10-ms duration with a rise-fall time of 1.0 ms. The repetition rate of the tone bursts was 9.7 Hz. Sound levels were calibrated with a 1/2 in. probe microphone (model 4015; ACO Pacific, Inc; Belmont, California) located near the eardrum. Calibrations were conducted for each subject.

Hair Cell Counts

Immediately after CAP measurements, rats were decapitated and the cochleae harvested. Within 2 minutes, the cochleae were fixed by perilymphatic perfusion with 1 mL of a trialdehyde fixative (3.0% glutaraldehyde, 2.0% formaldehyde, 1.0% acrolein, and 2.5% dimethyl

sulfoxide in PBS at pH 7.4). Following the primary 24-hour fixation, the tissue was first washed with 0.1M PBS, postfixed with 2 percent osmium tetroxide in water for 2 hours, and finally washed again with 0.1M PBS. The organ of Corti was dissected in 70 percent ethanol and mounted in glycerin to allow counting of the hair cells. Cells were counted as present when either the stereocilia, the cuticular plate, or the cell nucleus could be visualized. No attempt was made to assess the degree of possible cellular damage to existing cells. The frequency-place map established by Müller was used to superimpose the frequency coordinates on the length coordinates of the organ of Corti [30]. This map reflects the fact that the cochlea is organized in a tonotopic fashion with high-frequency sound producing maximum stimulation of cells in the base and low-frequency sound in the apex. A cochleogram showing the percentage of hair cell loss as a function of distance from the base of the cochlea was plotted for each animal. The results were averaged across each group of subjects for comparison between groups. The custom programs used for counting cochlear hair cells, plotting, and averaging cochleograms, were developed by R. Lataye and Dr. P. Campo from L'Institut National de Recherche et de Sécurité, Nancy, France.

Statistical Analysis

We analyzed the DPOAE and CAP data using two-way analysis of variance (ANOVA) and performed Bonferroni post hoc tests using GraphPad Prism (version 4.0, GraphPad Software; San Diego, California). Baseline, 1-hour, 1-week, and 4-week postexposure DPOAE amplitudes were analyzed with repeated measures ANOVAs with experimental treatment as a between-subjects factor and f2 and time relative to treatment as within-subject factors. Planned post hoc comparisons were performed between treatment groups with the Bonferroni test. The significance threshold was set at $p = 0.05$. We analyzed CAP thresholds with two-way ANOVAs to evaluate the effects of experimental treatment (between-subjects factor) at different frequencies (within-subject factors). We performed Bonferroni post hoc tests between experimental groups. The significance threshold was set at $p = 0.05$.

RESULTS

Distortion Product Otoacoustic Emissions

Figure 1 shows DP-grams obtained from the untreated control group at baseline and at time points equivalent to those for experimental subjects. This figure shows clearly that the DPOAE response is highly reproducible over the 4- to 5-week experimental period. As stated in the "Methods" section (p. 1054), the DP-grams of all subjects, including control subjects, displayed a notch between 20.8 and 25.6 kHz. Therefore, this range of frequencies was excluded from statistical analysis and was not taken into account in interpretation of the results.

The DP-grams for each experimental group at baseline and 1 hour, 1 week, and 4 weeks after the different treatments are shown in **Figure 2(a)–(d)**. At 1-hour postexposure, DPOAE amplitudes were profoundly reduced between 5.5 and 58.8 kHz in all treated groups. Only at frequencies below the OBN did exposed subjects show DPOAE amplitudes significantly different from the noise floor. At 1 week postexposure, the animals exposed to noise alone showed substantial recovery of DPOAE amplitudes at all frequencies that were impaired at the 1-hour postexposure time point; their DPOAE amplitudes were reduced less than 15 dB from control levels at all frequencies. By contrast, rats that received combined exposure to CO + noise (**Figure 2(b)**) showed persistent reductions in DPOAE amplitudes relative to baseline at

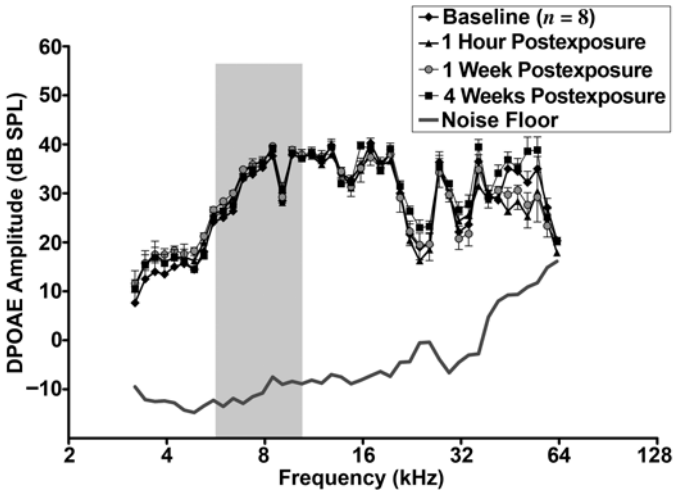

Figure 1.
Distortion product otoacoustic emission (DPOAE) amplitudes obtained in control rats before exposure, 1 hour, 1 week, and 4 weeks later. DP-grams were obtained with levels of primaries f1 and f2 set at 65 and 55 dB sound pressure level (SPL), respectively, and with f2/f1 = 1.25. Tested f2's ranged from 3.2 to 63.0 kHz (geometric mean frequencies: 2.9 to 56.3 kHz) in 0.1-octave increments. Gray area represents noise frequency range that was employed in experimental subjects. Error bars are ± standard error of the mean. f1 = first fundamental frequency, f2 = second fundamental frequency.

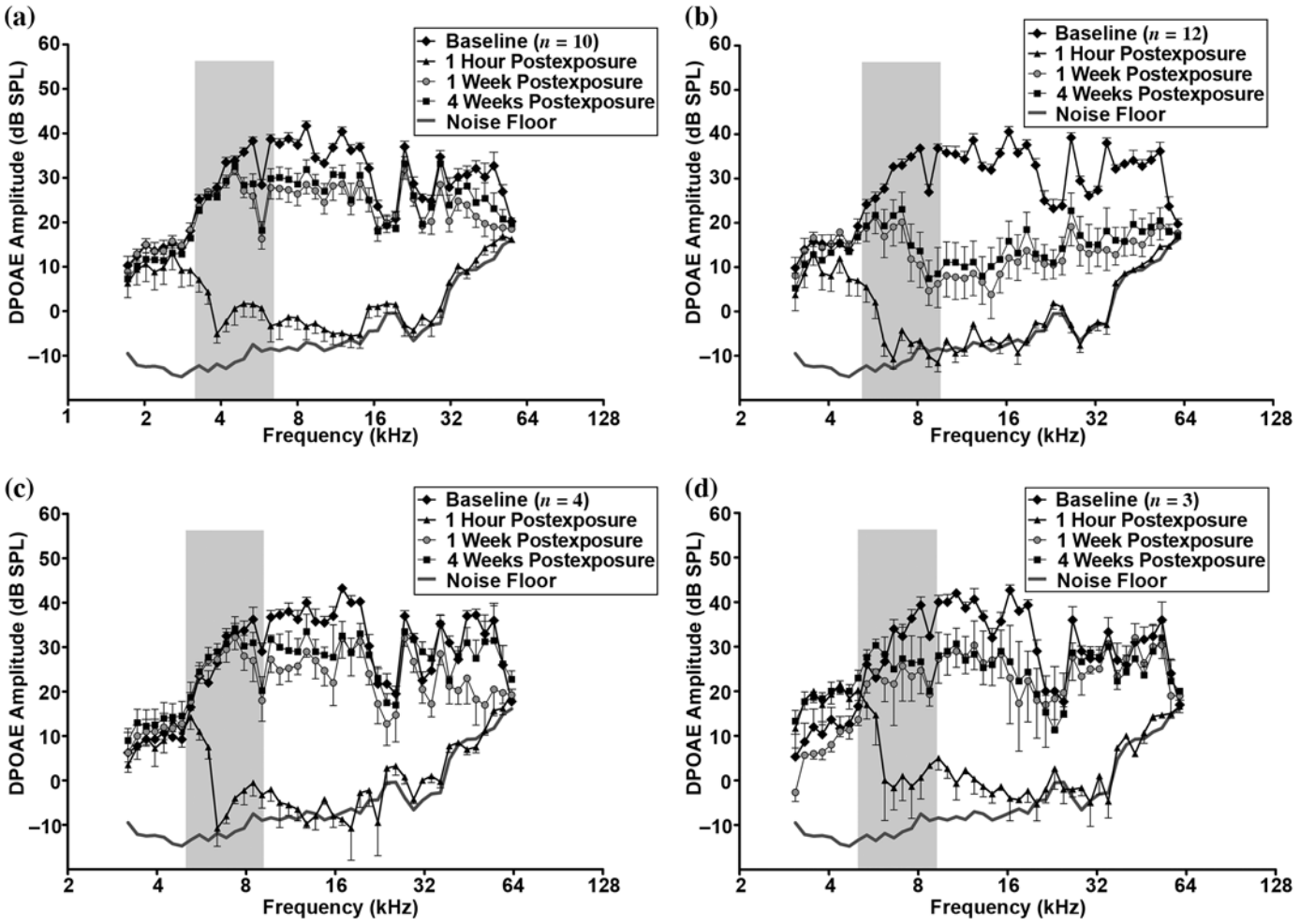

Figure 2.

Distortion product otoacoustic emission (DPOAE) amplitudes obtained in experimental treatment groups. DP-grams are shown for subjects receiving **(a)** noise alone, **(b)** carbon monoxide (CO) + noise, **(c)** lipoic acid + CO + noise, or **(d)** 6-formylpterin + CO + noise. DP-grams were obtained with levels of primaries f1 and f2 set at 65 and 55 dB sound pressure level (SPL), respectively, and with f2/f1 = 1.25. Tested f2's ranged from 3.2 to 63.0 kHz (geometric mean frequencies: 2.9 to 56.3 kHz) in 0.1-octave increments. Gray area represents noise frequency range. Error bars are ± standard error of the mean. f1 = first fundamental frequency, f2 = second fundamental frequency.

both 1 and 4 weeks following treatment. At 1 week postexposure, this reduction in DPOAE amplitudes averaged 15 dB between 5.5 and 58.8 kHz and reached 30 dB from the center of the OBN (8.0 kHz) to frequencies of 20.0 kHz. At 4 weeks postexposure, the rats that received CO + noise without benefit of antioxidant therapy still showed reductions in DPOAE amplitudes as great as 30 dB (**Figure 2(b)**).

In contrast to the ongoing impairment of DPOAE amplitudes in rats that received CO + noise, those that received pretreatments with LA and 6-FP prior to the CO + noise exposure showed DPOAE amplitudes indistinguishable from rats treated with noise only at both 1 and 4 weeks postexposure (**Figure 2(c)–(d)**). In other words, LA and 6-FP effectively blocked the potentiation of NIHL by CO. At 4 weeks postexposure, DP-grams obtained from the noise-only group and the two antioxidant + noise + CO groups (LA + noise + CO or 6-FP + noise + CO) showed modest reductions in DPOAE amplitudes relative to untreated control subjects.

The DPOAE amplitudes were analyzed by repeated measure ANOVAs performed at the different time points. The ANOVAs showed a significant effect of treatment at 1 hour postexposure ($F_{(4,32)} = 75.50$, $p < 0.001$), 1 week postexposure ($F_{(4,32)} = 6.86$; $p < 0.001$), and 4 weeks

postexposure ($F_{(4,32)} = 10.20$; $p < 0.001$). No significant effect of treatment existed at baseline. The ANOVAs also revealed significant treatment × frequency interactions (1 hour: $F_{(172,1376)} = 8.49$, $p < 0.001$; 1 week: $F_{(172,1376)} = 3.64$, $p < 0.001$; and 4 weeks: $F_{(172,1376)} = 2.47$, $p < 0.001$), pointing out that the effect of treatment is dependent on the frequency considered. Post hoc comparisons with Bonferroni multiple comparisons tests showed that at 1 hour postexposure, DPOAE amplitudes measured in animals that received noise, CO + noise, LA + CO + noise, or 6-FP + CO + noise were not significantly different from each other ($p < 0.05$) but were significantly lower than DPOAE amplitudes measured in control subjects ($p < 0.05$). At 1 week postexposure, DPOAE amplitudes measured in the CO + noise animals were significantly lower than those measured in control subjects between 6.8 and 41.6 kHz ($p < 0.05$) (except for the notch region). Noise, LA + CO + noise, and 6-FP + CO + noise DPOAE amplitudes were not significantly different from control subjects at any frequency ($p < 0.05$). Bonferroni comparisons yielded the same general results at 4 weeks postexposure as at 1 week postexposure, except that DPOAE amplitudes in the group that received CO + noise differed from those measured in control subjects over a broader range of high frequencies (7.9 to 54.8 kHz, with the exception of the notch region).

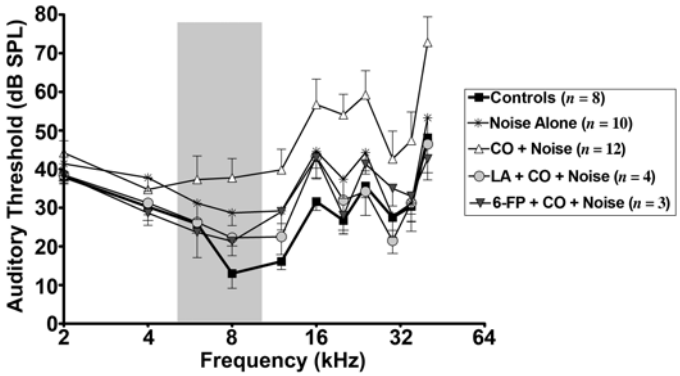

Figure 3.
Effects of different experimental treatments on compound action potential (CAP) thresholds measured 4 weeks postexposure for frequencies ranging from 2.0 to 40.0 kHz. For reference, CAP thresholds obtained in controls are included. Gray area represents noise frequency range. Error bars are ± standard error of the mean. 6-FP = 6-formylpterin, CO = carbon monoxide, LA = lipoic acid, SPL = sound pressure level.

Compound Action Potential

Figure 3 presents the disruption of CAP thresholds measured 4 weeks postexposure in the different experimental groups. Thresholds from the animals that received CO + noise exposure were significantly impaired between 8.0 and 40.0 kHz, with loss of acuity averaging 27 dB relative to untreated control subjects. Rats that received noise alone, 6-FP + CO + noise, or LA + CO + noise had slightly elevated thresholds compared with control subjects (<15 dB between 8.0 and 16.0 kHz). Little, if any, disruption in CAP thresholds was noted among these three treatment groups at higher test frequencies. A trend toward less threshold impairment was found in rats that received either of the two antioxidants + CO + noise than among rats that received only noise. However, this difference was quite small, approaching only 5 dB. We consider this step-size to be the limit of sensitivity for our test method, especially in light of the small group sizes for the antioxidant + CO + noise groups.

The overall effect of treatment on CAP thresholds was significant ($F_{(4,32)} = 3.38$; $p = 0.02$). Bonferroni post hoc tests revealed that the thresholds from the CO + noise group were significantly different from control subjects between 8.0 and 40.0 kHz ($p < 0.05$). Noise alone, 6-FP + CO + noise, and LA + CO + noise thresholds were significantly higher than control thresholds at 8.0 and 16.0 kHz only ($p < 0.05$).

Histopathological Data

To assess the magnitude of cochlear damage, we counted hair cells from cochleae harvested from the same animals used for physiological studies. The hair cell data are presented as cochleograms that display the percentage of hair cell loss as a function of distance from the apex of the cochlea. The cochleae from control subjects showed limited and sporadic hair cell loss averaging less than 1 percent (**Figure 4**).

The average cochleograms obtained in animals exposed to noise alone, CO + noise, LA + CO + noise, or 6-FP + CO + noise are presented in **Figure 5**. Noise-alone animals (**Figure 5(a)**) displayed some damage limited to the extreme base of the cochlea, which corresponds to a region represented by frequencies above 40.0 kHz. The extent of OHC loss was approximately 20 percent in this region.

The cochleae from rats exposed to CO + noise (**Figure 5(b)**) exhibited more substantial damage, extending over the basal third of the organ of Corti, than the noise-only subjects. The loss observed in the combined

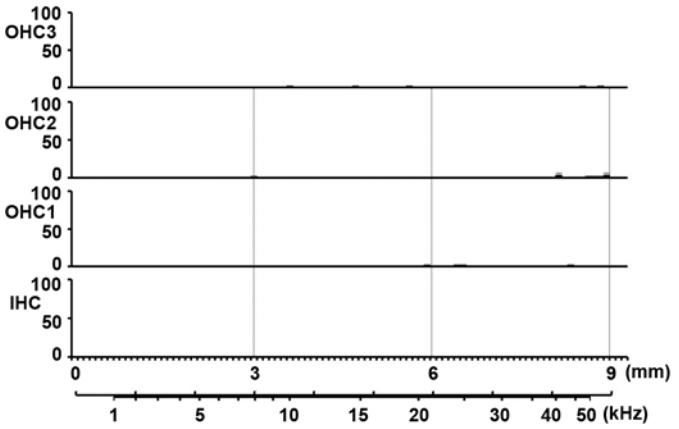

Figure 4.
Average cochleograms of organ of Corti showing hair cell loss in control rats (*n* = 6) that did not receive experimental treatment. Abscissa: upper trace = length (mm) of entire spiral course of organ of Corti from bottom of hook; lower trace = frequency map according to Müller. Ordinate: hair cell loss in percent. IHC = inner hair cells, OHC1 = first row of outer hair cells (OHCs), OHC2 = second row of OHCs, OHC3 = third row of OHCs. Error bars in cochleogram represent standard error. Müller M. Frequency representation in the rat cochlea. Hear Res. 1991;51(2):247–54. [PMID: 2032960]

CO + noise-exposure condition corresponded to the 25.0 to 60.0 kHz range. In this range, the mean OHC loss averaged about 35 percent in the three OHC rows. A microphotograph of a basal turn of a cochlea from a rat that received CO + noise is provided in **Figure 6**. It illustrates the fact that hair cell loss is present in all three OHC rows and that inner hair cells seem rather spared by the exposure. However, at this level of magnification, estimating the physiological state of either the hair cell's soma or stereocilia is very difficult.

Consistent with the physiological results, pretreatment with both LA (**Figure 5(c)**) and 6-FP (**Figure 5(d)**) reduced OHC loss caused by CO + noise in the region corresponding to 25.0 kHz and above. In this area, the cochleae exposed to LA + CO + noise and 6-FP + CO + noise exhibited only an average of 3 and 4 percent OHC loss, respectively, with peak losses not exceeding 10 percent at any given step along the organ of Corti. Consistent with the CAP threshold data, LA was apparently somewhat superior to 6-FP in preserving both normal cochlear function and OHC survival. Further research will be useful in evaluating this apparent difference in drug efficacy.

DISCUSSION AND CONCLUSIONS

The results of this study show pronounced retention of auditory function and hair cells by treating rats either with LA or 6-FP prior to combined treatment with CO + noise. Rats that received CO + noise demonstrated a 10 to 20 dB elevation in CAP thresholds relative to rats that received only noise. However, subjects that received either LA or 6-FP prior to CO + noise had auditory thresholds no higher than rats treated with noise alone and in most instances had thresholds that were more sensitive than the noise-treated rats. Indeed, thresholds determined in the LA and 6-FP groups that received CO + noise were often at or no more than 10 dB above those of untreated control subjects. This study, then, demonstrates the efficacy of LA in treating moderate noise effects when impairment is amplified by an hypoxic stimulus. It further validates the use of 6-FP as a protective agent for sensory cells in a new organ system, the inner ear. The data suggest that these antioxidants might also protect the cochlea from noise effects alone. However, additional studies will be required to assess this possibility. These data suggest that ROS generation is a normal outcome of noise exposure but that the buffering of these oxygen moieties is normally adequate to block oxidative stress at moderate noise levels. Drugs such as LA and 6-FP are able to at least block oxidative damage under conditions in which intrinsic ROS buffering is not adequate to control oxidative stress. While independent measures to assess the effects of LA and 6-FP on oxidative stress were not feasible, previous research has suggested that this mechanism is responsible for the potentiation of NIHL by CO [13,26].

Several laboratories have demonstrated that intense and prolonged noise exposure may generate ROS. First, genetic studies have shown that animal models with reduced intrinsic antioxidant potential are more susceptible to NIHL than wild-type subjects [31–32]. Second, pharmacological studies have reported the ability of antioxidants to block or reduce NIHL [4,33–37]. Finally, a limited number of reports have documented direct evidence of oxidative stress or increased ROS in subjects that have been exposed to noise [32,38–40]. The noise exposures used in these studies were indeed severe, and so despite these different lines of evidence indicating that oxidative stress is involved in NIHL, essential questions regarding the amount of ROS generation as a function of noise intensity remain. We propose that even moderate noise

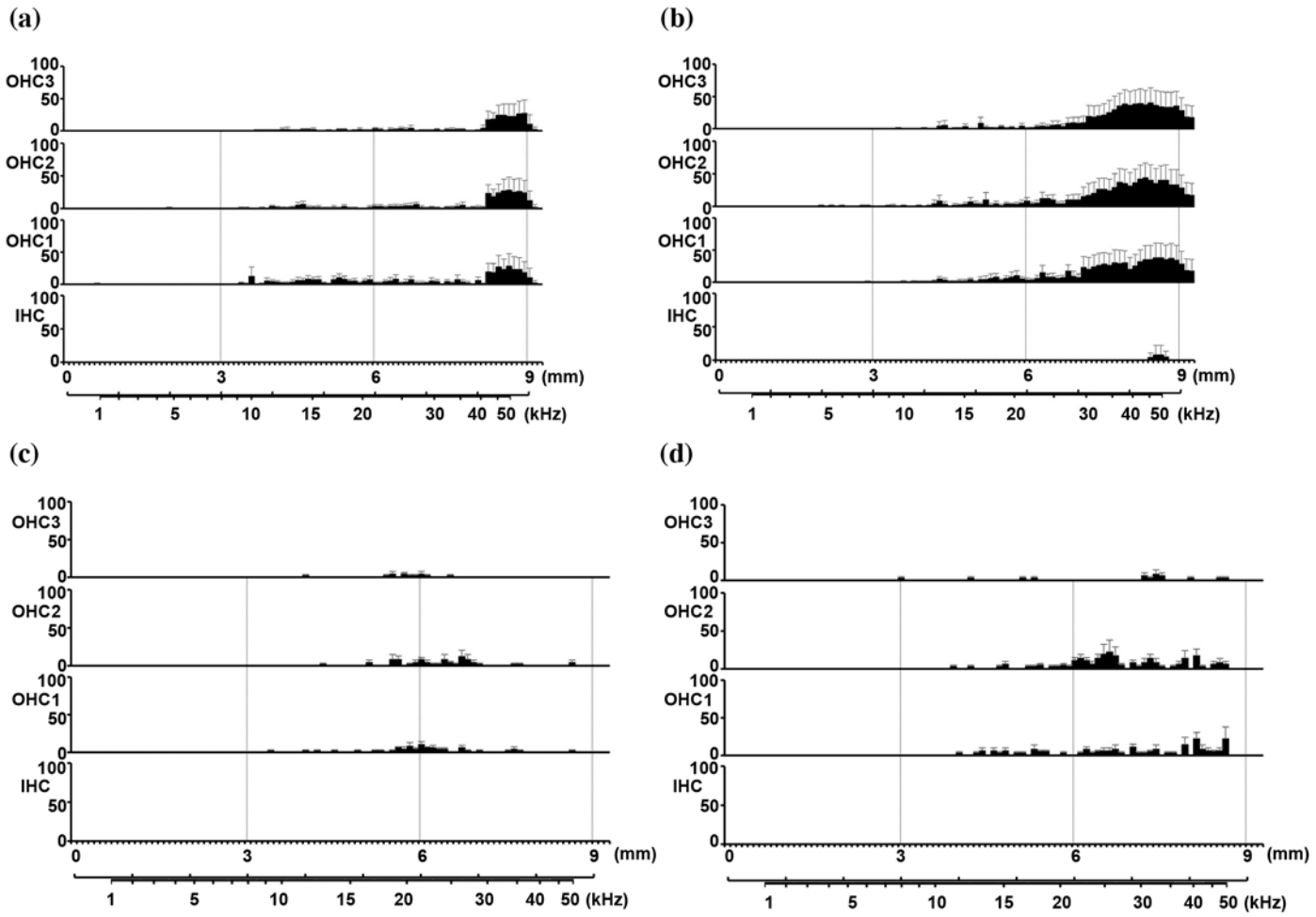

Figure 5.
Average cochleograms of organ of Corti showing hair cell loss in groups of rats exposed to **(a)** noise alone (n = 10), **(b)** carbon monoxide (CO) + noise (n = 12), **(c)** lipoic acid + CO + noise (n = 4), or **(d)** 6-formylpterin + CO + noise (n = 3). Treatment doses and schedules are detailed in the **Table**. Abscissa: upper trace = length (mm) of entire spiral course of organ of Corti from bottom of hook; lower trace = frequency map according to Müller. Ordinate: hair cell loss in percent. IHC = inner hair cells, OHC1 = first row of outer hair cells (OHCs), OHC2 = second row of OHCs, OHC3 = third row of OHCs. Error bars in cochleogram represent standard error. Müller M. Frequency representation in the rat cochlea. Hear Res. 1991;51(2):247–54. [PMID: 2032960]

(close to the conditions that can be encountered at the workplace, for instance) can initiate ROS generation and that under normal conditions intrinsic antioxidant pathways are sufficient to prevent oxidative stress. We hypothesize that compounds that disrupt antioxidant defenses can render the inner ear vulnerable to ROS generated by noise.

While the occurrence of combined exposure to noise and “pro-oxidant” chemicals is quite rare in humans [3], research on these kinds of interactions in laboratory animals can help us determine by which mechanisms the cochlea can defend itself against oxidative stress and in which cases it cannot. The determination of such mechanisms can in turn suggest potential treatments: 6-FP and LA are among those.

The observations that a diversity of antioxidant compounds can protect the cochlea, at least partly, either against high-level noise exposures or against combined exposures to moderate noise and chemicals, drugs, or asphyxiants strongly suggest that all these conditions share common mechanisms and are therefore susceptible to being treated with the same compounds. The ideal

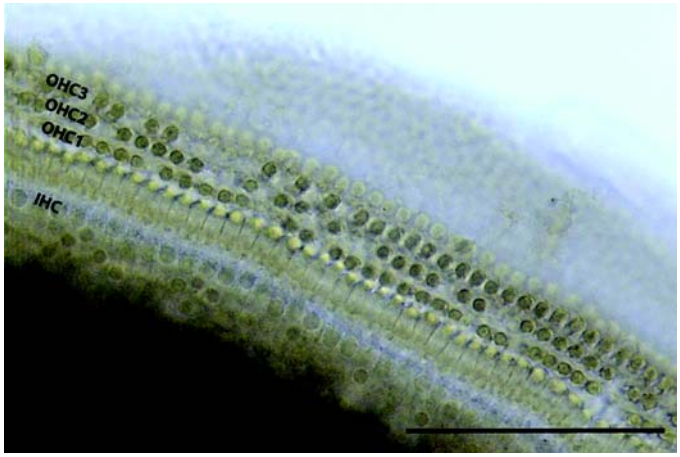

Figure 6.
Microphotograph of organ of Corti showing hair cell loss in rat exposed to carbon monoxide and noise. IHC = inner hair cells, OHC1 = first row of outer hair cells (OHCs), OHC2 = second row of OHCs, OHC3 = third row of OHCs. Picture was taken in 20.0 to 30.0 kHz region where damage was maximal. Scale bar represents 100 μm.

treatment would therefore maintain oxidative homeostasis by (1) chelating ROS; (2) replenishing intrinsic antioxidant enzymes like glutathione, superoxide dismutase, or cysteine; and (3) preventing lipid peroxidation and DNA oxidation, as well as be devoid of side effects. Today, this ideal ubiquitous treatment does not yet exist. However, it is not unlikely that a combination of various known antioxidant compounds, possibly including 6-FP or LA or both, could have all these properties.

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