Long-Term Effects of the Synergistic Interaction of Cisplatin and Noise

Honors Research Thesis

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Abstract

As cancer treatments advance, more patients survive each year. Of these survivors, many have experienced treatment with chemotherapeutic drugs like cisplatin that have aided in and enabled their recovery. Cisplatin, a chemotherapeutic drug used to combat head, neck, and urological cancers, is highly ototoxic and causes significant high-frequency cochlear hearing loss, as well as increased susceptibility to noise damage, leading to even more severe hearing losses. The current study assessed whether rats that have been exposed to cisplatin see potentiated noise-induced hearing loss, and determined if the increased susceptibility to noise damage lasts for a period of time after the cisplatin treatment interval. The study found that subjects' susceptibility to noise damage was still increased even four months after the end of cisplatin treatment, suggesting that the effects of this drug can last longer than only the duration of the drug administration itself. Further, the rats involved in this study experienced potentiated losses in frequencies indirectly affected by cisplatin (10, 15, and 20 kHz), indicating that the entirety of the cochlea was affected by the drug, and not merely the high frequencies. Further study is required to evaluate the clinical ramifications of these findings in humans and to develop appropriate counseling strategies to combat these effects, but the results provide an initial indication that clinical patients who have been administered cisplatin need to be educated on the long-term need for hearing protection from noise to avoid further hearing damage.

1. Introduction

The chemotherapy drug cisplatin and its synergistic interaction with noise have been involved in a number of audiological studies spanning from the late 1980's to the present. Cisplatin, also known as Platinol or Cis-Diamminedichloro-Platinum (CDDP), is a chemotherapeutic drug that is often used in the treatment of cancers of the more delicate systems, particularly in testicular, bladder, lung, and ovarian cancers that have spread to other portions of the body, and more contemporarily in head and neck cancers for the elderly or young children (Hitchcock et al., 2009; Knight et al., 2005; Zuur et al., 2007; Zuur et al., 2008). It is a heavy-metal, alkylating, antineoplastic agent that operates by crossing the cell membrane to bind to the DNA-phosphate bases, linking to the DNA double helix and ultimately limiting further DNA synthesis and replication. Its toxic side effects include nephrotoxicity, myleosuppresion, gastrointestinal disturbances, nausea, and, most importantly to the current study, ototoxicity (Boettcher et al., 1987). Ototoxicity is the lethal/destructive action of a drug or substance on the cell systems of the sensorineural auditory system. In this case, cisplatin damages the cochlea, preferentially the first row of outer hair cells (OHCs), beginning at the basal portion of the cochlea and spreading upwards toward the apex and also damaging other rows of hair cells as exposure and dosage is increased. The OHCs are responsible for hearing sensitivity to low-intensity sounds, so loss of OHCs results in a loss of hearing sensitivity. The reports of ototoxicity in patients vary widely across studies and clinical exposures, but an average incidence is reported at 31% (Boettcher et al., 1987). Patients who experience this ototoxic effect were generally seen to experience a symmetrical hearing loss in both ears, with the loss being first detected in the high frequencies, corresponding to underlying damage in the basal regions of the cochlea. Particularly

concerning is that these results were detectable after only the first or second chemotherapeutic treatment, leaving patients who are affected by this hearing loss little time to prepare for or counteract this potentially significant hearing loss (Zuur, 2007). Multiple studies in the pediatric population have found that cisplatin-induced hearing loss continues to grow for several years after the cisplatin treatments have ended (Einarsson et al., 2010; 2011). What was unclear from those studies was if noise exposure after the cisplatin treatment was contributing to the growth of hearing loss. The hypothesis for the current study was that some cisplatin patients are left with a permanent high susceptibility to noise-induced hearing loss due to the accumulation of sub-clinical cochlear damage from their cisplatin treatment. If cisplatin patients do indeed have a permanent susceptibility to noise-induced hearing loss, that hearing loss is almost completely preventable with proper monitoring, education, and protection. Therefore, a more thorough understanding of the relationship between cisplatin exposure and susceptibility to noise-induced hearing loss is crucial for properly counseling patients with regard to preservation of their hearing.

Foundational work on the interaction between cisplatin and noise exposure was done by Boettcher and Gratton beginning in 1987, when they first compiled the literature on the synergistic relationship between noise and pharmacological drugs, with particular attention to cisplatin. The interaction between noise and cisplatin ototoxicity is a synergistic one, in which the resultant hearing loss is greater than the sum of the hearing losses that occur from either noise exposure or cisplatin alone (Boettcher et al., 1987). In 1990, Gratton et al. published work that explored the interaction of cisplatin and noise in the peripheral auditory system in a guinea pig model. Earlier research showed a high

affinity of the cochlea for cisplatin, and an apparent lack of an effect of cispatin on the vestibular system. Gratton et al. (1990) created controlled groups to assess the separate effects of cisplatin and of noise on the cochlea to control for a third group that was exposed to both cisplatin and noise concurrently. While this research found no change in hearing sensitivity from either cisplatin or noise exposure alone, substantial hearing loss was seen in the animals that were treated with cisplatin and concurrently exposed to noise at or above 85 dB SPL (Gratton et al., 1990). This study ultimately found that the highest incidence of hearing loss in animal populations was in the group that had been exposed to both cisplatin and moderate or extreme noise, with the greatest loss occurring in the high frequencies. Their conclusion stated, however, that this incidence was only studied in populations receiving cisplatin and noise exposure concurrently, and their study included no observation of populations that had been exposed to noise pre- or post- cisplatin administration (Gratton et al., 1990).

The interaction of cisplatin and noise was studied again in 1992. Laurell (1992) tested the interaction between the timing of cisplatin and noise administration and their effects on cochlear function. His work found that subjects that were exposed to noise before the administration of cisplatin saw significantly more noise-induced hearing loss and cochlear damage than those that were exposed to noise concurrently with cisplatin administration, asserting that the time of introduction of noise was more important than the nature of noise (continuous or interrupted) to which the subjects were exposed (Laurell, 1992).

The current project was built in part on the premise that cisplatin can induce sub-clinical cochlear damage that raises susceptibility to later noise damage. The existing

literature provides ample evidence that cisplatin can inflict sub-clinical damage to the OHCs of the cochlea, even in the absence of measurable hearing loss (Butler, 2011). Most often the damage occurred in the basal cochlear regions that correspond with the high frequencies. This is particularly concerning for human chemotherapy patients. If those patients had experienced cisplatin-induced cochlear damage without measured hearing loss, they may be highly susceptible to the synergistic noise-induced hearing loss. In other words, those patients may undergo hearing loss from noise exposure that would not damage a normal listener's cochlea. Further, there is the potential that this delicate auditory state would last for the rest of the patient's life, if it is the result of sub-clinical OHC loss. While previous studies have asserted the effects of various protective and preventative measures during the treatment, none have satisfactorily assessed the long-term effects of cisplatin on susceptibility to noise-induced hearing loss, nor have any previous studies observed the effects of noise exposure after a defined period of cisplatin administration during which the subjects were not exposed to any higher level noise. Gratton et al. (1990) hypothesized that the synergistic interaction of noise and cisplatin likely occurs because the damage done during exposure to cisplatin causes auditory hair cell death, resulting in a weakened cochlear function. This hypothesis is supported by Boettcher's findings on the effects of sustained noise exposure (1987). When this weakened cochlea is exposed to noise, as was done in both Laurell (1992) and Gratton et al. (1990), the result is sustained and permanent hearing loss.

In the current study, the Fischer 344/NHsd rat was used as the model of cisplatin- and noise-induced hearing loss. The hair cells of the Fischer 344 NHsd rat, like those in the human, do not regenerate after cell death. The working hypothesis for the current

study was that the weakened state of the cochlea at the end of the cisplatin exposure (due to sub-clinical cochlear damage) would be no different than the weakened state of the cochlea four months later. Therefore, if there was increased susceptibility to noise-induced hearing loss immediately after an eight-week cisplatin treatment that increased susceptibility to noise would persist at four months after the cisplatin treatment period. Hypothetically, the increased susceptibility to noise (or any other cause of cochlear hearing loss) would persist either until the subject dies, or until the cochlear damage occurs and hearing loss results. In this hypothesis, there would be no recovery period from cisplatin at which point the cisplatin patient would be equally susceptible to noise as anyone else.

No previous research has been conducted to model the long-term effects of cisplatin on noise-induced hearing loss. With the improving survival rate for chemotherapeutic patients in the United States, there is an ample need for investigation into the long-term effects of these drugs and the subsequent degree of need for hearing protection in these patients. Historically, the ototoxic effect of cisplatin has not been a primary concern for the patients of oncologists, since the cancers had high mortality rates. Therefore, the concern was patient survival, not his or her hearing sensitivity after cancer survival. But with higher survival rates with cisplatin, there is a greater need for determining the patient's hearing health over their life span, especially since noise-induced hearing loss is completely preventable with the proper protection measures and education. If cisplatin patients do indeed have a long-term or permanent susceptibility to noise-induced hearing loss, a more thorough understanding of the relationship between

cisplatin exposure and susceptibility to noise-induced hearing loss is crucial for properly counseling patients with regard to preservation of their hearing.

2. Methods

Subjects

To test the long-term effects of cisplatin and noise, twenty-five male and female Fischer 344/NHsd rats were used, acquired from Harlan Laboratories at 2-3 months of age. The rats were kept in a quiet colony and all procedures involving the use of animals were approved by The Ohio State University's Institutional Animal Care and Use Committee.

Auditory Brainstem Response (ABR) Testing

The human ABR consists of the first five-seven waves of electrical activity post-stimulus. These five waves reflect the activity of the structures of the cochlea, auditory nerve, and central auditory system as the signal progresses from the cochlea to the brainstem and they typically occur within 10 ms of exposure to a sound stimulus (Yost, 2008). In the rat, the ABR is dominated by two positive and negative peaks. ABRs were recorded and analyzed with BioSigRZ. To determine the threshold of the animals at individual frequencies, the ABRs at each level (dB SPL) were compared. Threshold was defined as the lowest point at which a brainstem response wave could be visually discerned (See Figure 1).

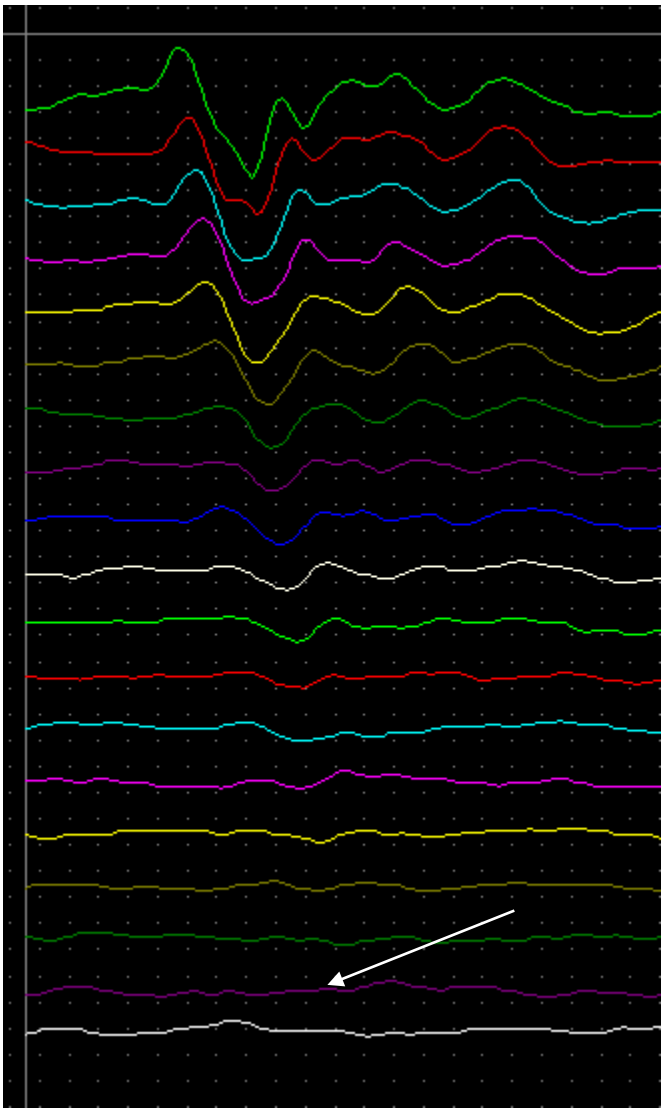

Figure 1. The worksheet view of BioSigRZ showing ABRs from 90 to 5 dB SPL. The arrow indicates where threshold would have been measured for this stimulus group.

In order to perform ABR tests, the animals were anesthetized with a mixture of gaseous isoflurane and oxygen. For anesthesia induction, 4% isoflurane was used with a 1 L/min O_2 flow rate. Following induction, the animals were placed in a sound booth with a nose cone supplying a mixture of 1.5% isoflurane and oxygen for the duration of the test. In the sound booth, three platinum electrodes were inserted subcutaneously: one at the vertex, one behind the right pinna, and one as a ground near the left rear leg of the animal. All stimuli were generated using Tucker Davis Technologies (TDT, Gainesville, FL) SigGen software. Each tone burst was 1 ms in duration, and had a 0.5ms rise/fall time with no plateau. Stimuli were presented at a rate of 19/sec. Signals were routed to a speaker (TDT Model MF1) positioned at zero degrees azimuth, 6 cm from the vertex of each rat's head. Acoustic stimuli were calibrated prior to each testing session by recording the output of the speaker with a microphone placed at the animal's head level. The rats' evoked responses were amplified with a gain of 50,000, using a TDT RA4LI headstage connected to an RA4PA pre-amplifier, and bandpass filtered from 100-3000 Hz. Two hundred-fifty sweeps were averaged at each stimulus level using TDT BioSigRz software. Six frequencies were tested: 5, 10, 15, 20, 30, and 40 kHz, at decreasing 5 dB SPL intervals from 90 dB SPL to 5 dB SPL.

Cisplatin Treatment

For all rats, initial ABR testing was performed to establish baseline thresholds of normal hearing for each subject. After this testing, the subjects were divided into four groups for exposure: a cisplatin-only group (CDDP), a noise-only group (Noise), a cisplatin plus immediate noise exposure group (Acute), and a cisplatin plus delayed noise exposure group (Delayed). The Noise group did not receive any cisplatin treatments, while the other three groups were enrolled in an eight-week exposure protocol. Each

group underwent a 10 mg/kg total cisplatin exposure over the eight-week period. Subjects were dosed according to the following schedule. Cisplatin was introduced at 2 mg/kg on week one and 3 mg/kg on week three. This cycle was repeated for weeks five and seven, with ABR testing performed during off weeks two, four, six, and eight. Cisplatin was given through intra-peritoneal injections. Upon conclusion of the week eight ABR test, the CDDP group was sacrificed for harvest of the cochleae. Seven days after the conclusion of the cisplatin course, the Acute group was exposed to noise according to the parameters described below. After the week eight measurement, the Delayed group was returned to the housing facility for a four-month recovery period after cisplatin treatment, during which time their hearing was monitored to ensure that no loss occurred during the interim. After the conclusion of this waiting period, this group was exposed to the noise conditions described below and then tested.

Noise exposure and threshold shift measurement

For the noise exposure, the subjects were exposed to a noise of a 110 dB SPL two-octave band (centered at 10 kHz) continuous noise for two consecutive hours. The noise was created on TDT RPvdsEX visual design software and then generated using a TDT RP2 Real time signal processor, amplified by a Marathon DJ-5000 power amplifier (New York, NY). The noise signal was then delivered to a speaker driver (JBL Model 2446H, JBL Inc., Northridge, CA) and acoustic horn (JBL Model 2380A) placed to the side of a wire cage (28″ × 30″ × 36″) in which the animals were held for the noise exposure. The noise level was calibrated at the level of the animals' heads utilizing a calibrated LxT1 sound level meter (Larson Davis Inc., Depew, NY) and a 1/2″ condenser microphone (Model 377B02, PCB Piezotronics, Inc., Depew, NY). Following the

exposure, subjects underwent ABR threshold testing at 24 hours, 7 days, and 28 days to monitor temporary threshold shift (TTS) and permanent threshold shift (PTS).

Statistical Analyses

Thresholds were recorded and collated in a Microsoft Excel spreadsheet to aid in analysis. Pre-exposure thresholds were analyzed with a two-factor (group x frequency) analysis of variance (ANOVA), with frequency treated as a repeated measure. To assess threshold changes from the eight-week cisplatin treatment interval in the CDDP, Acute, and Delayed groups, a three-factor ANOVA (group x frequency x day) was used with frequency and day treated as repeated measures. Thresholds in the Noise, Acute, and Delayed groups from the post-noise exposure 24-hour, 7-day, and 28-day testing were compared with a three-factor ANOVA (group x frequency x day) with frequency and day treated as repeated measures. For all significant main effects of group, Tukey-A post hoc testing was used to delineate any differences between specific experimental groups.

3. Results

Pre-exposure thresholds

Mean pre-exposure thresholds for the four experimental groups (CDDP, Noise, Acute, and Delayed) are displayed in Figure 2. The two-factor ANOVA (group x frequency) was performed for pre-test thresholds, finding no significant main effect of group ($p=0.543$) or group x frequency interaction ($p=.108$). There was a significant main effect of frequency, but that was expected since thresholds in dB SPL are higher for 30 and 40 kHz than the other frequencies in the Fischer 344/NHsd rat. The lack of differences particularly between the CDDP, Acute, and Delayed groups indicated that any threshold changes demonstrated during the eight-week cisplatin exposure interval would be due to the cisplatin exposures.

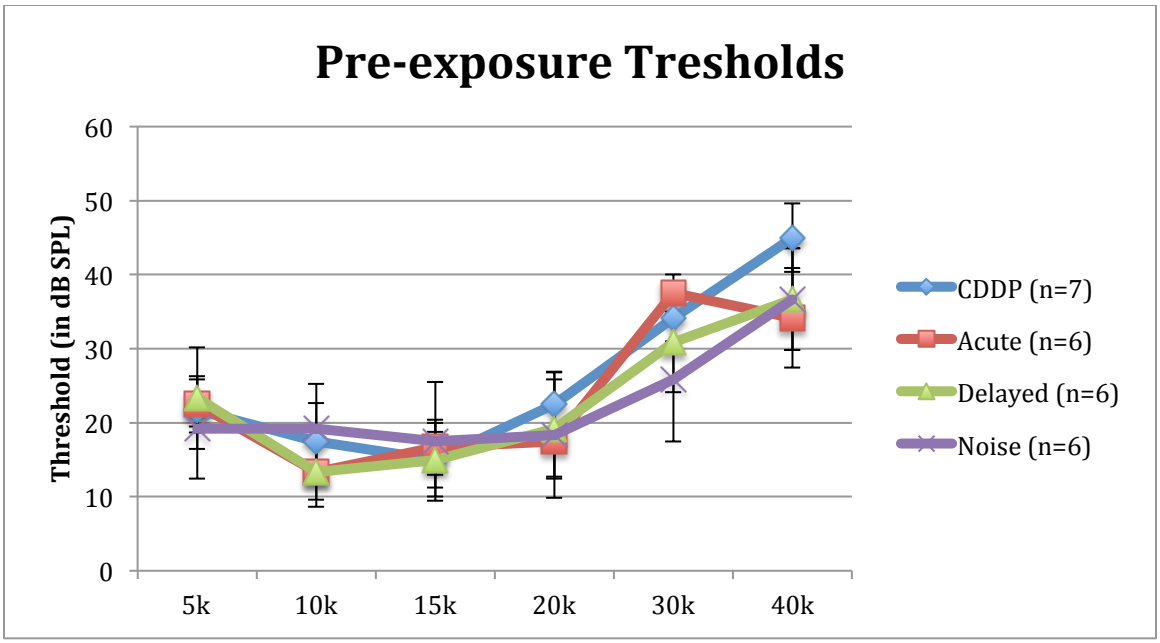

Figure 2. This figure compares pre-exposure thresholds across all tested groups. Tested frequencies are presented across the x axis, with thresholds in dB SPL increasing along the y axis. Pre-exposure ABR threshold test results revealed no significant difference between any of the four groups for thresholds 5-40 kHz. Error bars are +/- 1 s.d.

Threshold shift from the cisplatin exposures

A three-factor (group x frequency x day) ANOVA was used to assess threshold changes in the CDDP, Acute, and Delayed groups that were exposed to the eight-week cisplatin exposure interval. A significant three-way interaction was detected ($p=0.048$). To further analyze that interaction, a series of two-factor (group x day) ANOVAs were run at each frequency. Significant two-way interactions were detected at 10 kHz ($p=0.045$), 15 kHz ($p=0.027$), 20 kHz ($p=0.008$), 30 kHz ($p=0.015$), and 40 kHz ($p=0.0.19$). To analyze those interactions, a series of one-factor ANOVAs (group) was performed for each of those frequencies at each test day. This analysis found no statistically significant differences between groups through week four of testing. At week six, a significant difference was seen for the Acute group at 30 kHz, with Tukey A testing indicating that the Acute group had higher thresholds than the CDDP group. This difference did not persist through week eight. At week eight, a significant threshold elevation was seen in the Delayed group at 10 and 20 kHz compared to the Acute and CDDP groups. While statistically significant, the mean differences between these groups was under 5 dB at 10 kHz, and under 10 dB at 20 kHz.

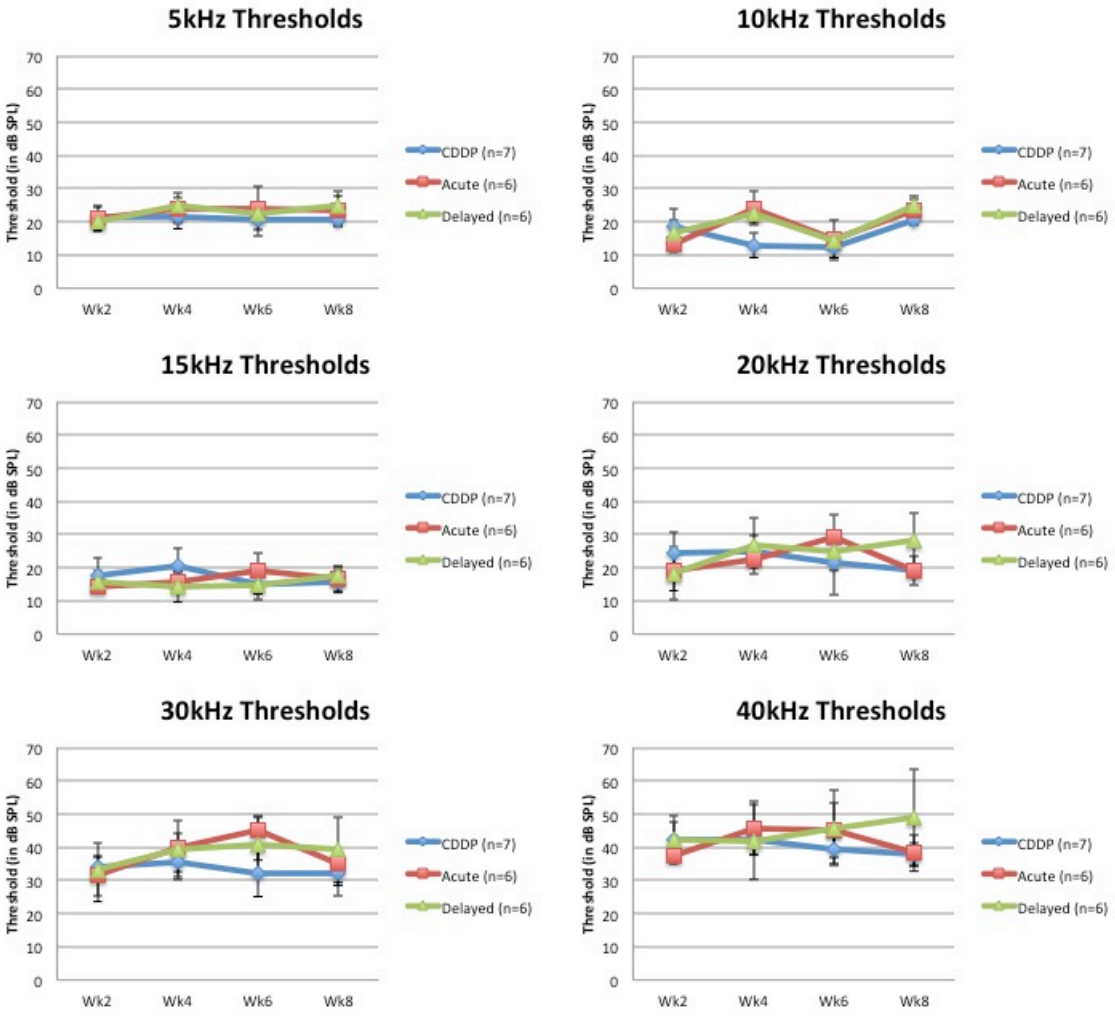

Figure 3. Mean thresholds of the three experimental groups (CDDP, Acute, Delayed) at the ABR tests taken during weeks 2, 4, 6, and 8 of the eight-week cisplatin exposure period. Time lapse between the weeks is represented along the x axis while thresholds are again represented on the y axis. Error bars are +/- 1 s.d.

Noise-induced threshold changes

Mean thresholds in the three groups exposed to noise (Noise, Acute, and Delayed) are displayed in Figure 4. Statistically significant results again emerge for the groups at post-noise testing, with the three-factor (group x frequency day) ANOVA revealing significant two-way interactions of group x day ($p=0.031$) and day x frequency ($p=0.01$). In order to break down the group x day interaction, thresholds were collapsed across frequency. Then a series of one-factor (group) ANOVAs were run at each day. At Day 28, a significant difference between the Delayed and Noise groups was detected, indicating that there was a synergistic interaction between cisplatin and noise, leading to a potentiated loss from noise.

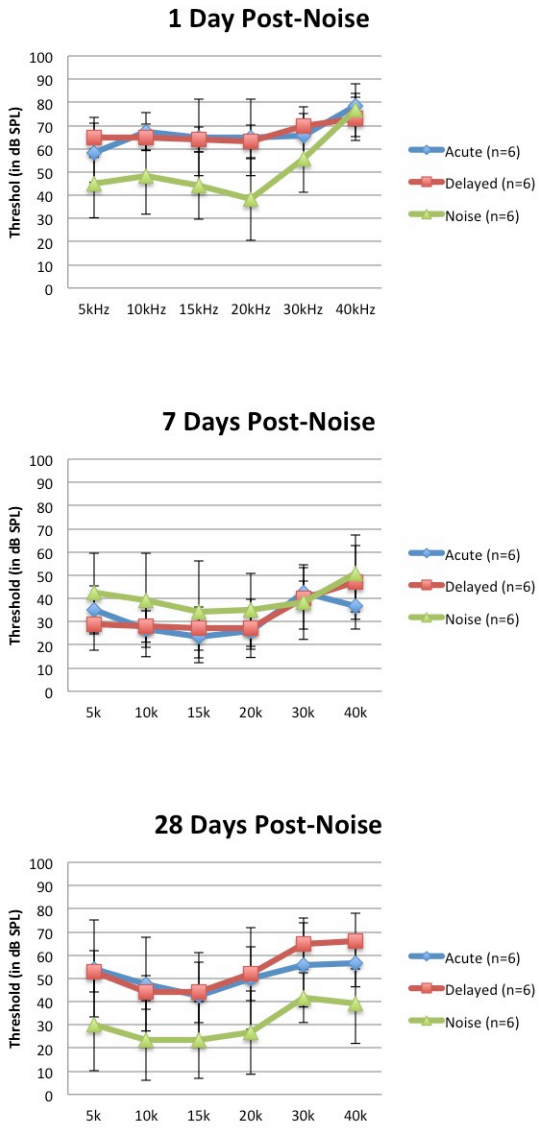

Figure 4. Mean thresholds after the noise exposure. All groups saw temporary and permanent threshold shifts as can be seen in the Day 1 and 28 results above. For both days, the most significant hearing losses were seen in the cisplatin-treated Acute and Delayed groups, demonstrating a synergistic effect that continues even after a sheltered period following cisplatin administration.

4. Discussion

The current study sought to evaluate the risk for long-term interaction of the chemotherapy drug cisplatin and noise in a rodent model. The key questions were whether the eight-week cisplatin exposure period created a synergistic interaction with noise, and if that synergistic interaction would persist with several months in between the cisplatin exposure and the noise exposure. Upon evaluation of the results, there is evidence of a statistically significant synergistic cisplatin-noise interaction in the Delayed group, but no statistically significant cisplatin-noise interaction in the Acute group. These results serve to both confirm and refute the hypotheses for the study under these experimental conditions, and create interesting implications for future studies of the interaction between this drug and the auditory system.

Upon analyzing the study's results, it became clear that despite the low cisplatin dose, subjects in both the Acute and Delayed groups saw minor hearing loss in the high frequencies (see Figure 3 above) in addition to the subclinical damage done at the lower frequencies. This loss is reflected in Figure 5 below, which displays actual mean thresholds of the Acute and Delayed groups at Day 28 post-noise compared to the predictions for them based on adding their Week 8 of cisplatin measurements and adding the threshold shifts from the Noise only group, thus creating a noise plus cisplatin threshold estimate. As can be seen in the figure, the actual mean thresholds in both the Acute and Delayed groups are 15-20 dB higher than their predictions, reflects that a significant synergistic loss was seen across all frequencies tested. This finding also provides evidence that the threshold shifts at Week 8 of cisplatin in the Delayed group did not have significant impact on the study. When analyzing the synergistic results of

this loss paradigm, however, no statistically significant synergy was found in the Acute group when comparing thresholds in the Acute group to thresholds in the noise group, this despite the observed synergy in the Delayed group. As significant synergy can be observed between the Acute and Noise group means, it is likely that the lack of statistical significance between these groups is the result of the high variability in the Acute and Delayed groups and that this synergy would be observed in a group with higher statistical power from larger sample sizes.

The human OHCs do not regenerate, and as such it was hypothesized that any damage to these cells that persisted after treatment would continue for the duration of the subjects' life. In the rodents tested, the four-month interval was chosen so that the auditory system could be appropriately assessed independently of the onset of presbycusis in the subjects (Bielefeld, 2013), since they would still be young enough (< 9 months old) that they would have no age-related hearing loss. Upon evaluation of the results, it was seen that the hypothesis was supported. By the conclusion of the experiment, subjects in the Delayed group were independently more impaired than both the Noise or CDDP groups, and their thresholds were higher than what would have been predicted by adding the CDDP group's threshold shift to the Noise thresholds on Day 28, demonstrating a synergistic effect.

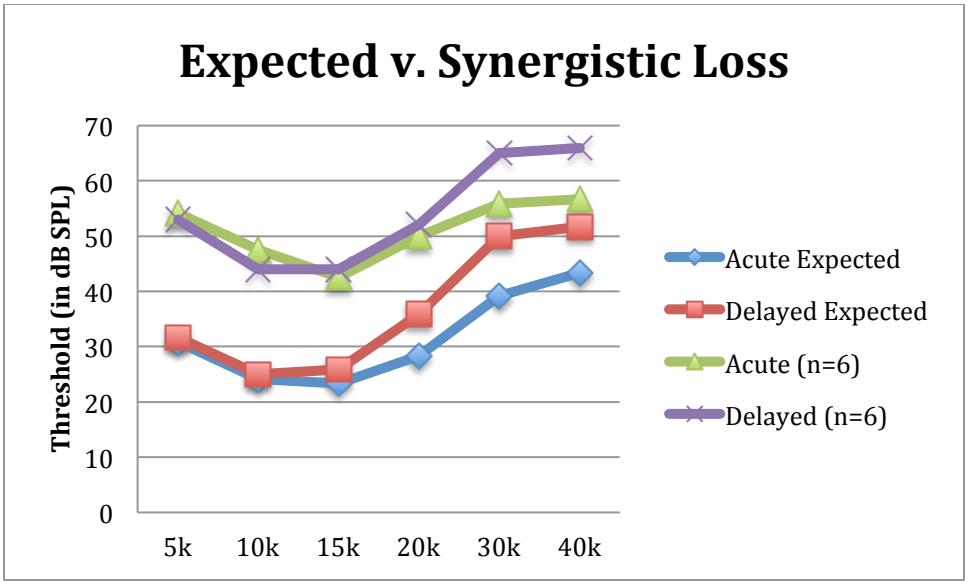

Figure 5. Acute and Delayed groups saw potentiated mean hearing loss. At 28 days post-noise, the groups saw thresholds 20-25 dB SPL higher than cisplatin and noise hearing loss alone, as is shown by the expected lines above. These results suggest that the sheltered period for the delayed group had no protective effect for the synergy seen in cisplatin-related noise-induced hearing loss. Further study is necessary to determine the clinical impact on human patients.

Should this effect be observed in human patients, it would create a need for continued protective monitoring of the auditory system to avoid synergistic damage to the OHCs. These results are merely suggested in this study, and further investigation would be required to establish such an effect on the human auditory system.

As reported in the results above, a statistically significant shift is seen in week eight of cisplatin treatment for the Delayed group. These results demonstrate the individualized effect of cisplatin upon the auditory system, reflecting that treatment does not damage all patients to the same degree throughout treatment. This effect is well documented throughout the literature (Knight et al., 2005; Boettcher et al., 2007; Butler, 2011) and was expected during the course of the experiment. It is not disproportionately reflected in the results post-noise and does not interfere with interpretation of the synergistic interaction seen between the Acute and Delayed subject groups.

As expected, the study suggests that the delicate state of the auditory system caused by cisplatin treatment is synergistically affected by noise-exposure, causing a potentiated hearing loss in all subjects observed. Due the high variability and weak statistical power of the study, due largely to sample sizes of six animals per group, statistical evaluation of the results did not yield the compelling results that the mean data (depicted in Figure 4) suggested.

Interestingly, the current study was unable to isolate specific frequencies which were affected by this loss, indicating that though cisplatin's subclinical effect is most notable in the high frequencies, the auditory system may sustain damage across multiple frequencies, and that damage manifests in threshold shift when combined with noise exposure. This indicates the potential for a more serious concern than that which would

be posed by a loss only in the supra-speech frequencies (>6 kHz in the human). Further study is required to determine the degree of this permeation and if this effect carries beyond rodents and into clinical populations.

As this study evaluated the treatment and effects upon the rodent auditory system, further study is necessary to determine the effects on the human auditory system and whether these results persist in human patients. Should these results be seen in human populations, questions concerning treatment side-effects should be incorporated into patient education and there is potential that otoprotective drugs should be explored to be concurrently administered with cisplatin.

Ultimately, the results of the current study suggest interesting new fronts of study for the already rich cisplatin-related literature and expose new concerns for clinical counseling and the ever-changing treatment of cancers in today's medical landscape.

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