The Use of Pravastatin As An Otoprotective Agent.

Capstone Project

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Audiology
in the Graduate School of The Ohio State University

By

Caroline MacMillan

Graduate Program in Audiology

The Ohio State University

2013

Committee:

Eric Bielefeld, Ph.D., Advisor

Christina Roup, Ph.D.

Gail Whitelaw, Ph.D.

Abstract

Cisplatin is a commonly used anti-neoplastic medication used to treat a variety of cancers. While effective in treatment of cancer, the use of cisplatin causes a variety of side effects including ototoxicity. As such, research has striven to find otoprotective agents in order to prevent these effects. In this study, a cholesterol medication, pravastatin, will be examined for possible otoprotective benefits. Prior research has demonstrated pravastatin's protective effects in the renal system through a decrease in the expression of p53, a protein crucial to process of apoptosis (i.e. cell death). As p53 is also a main component of apoptosis within cochlea during use of cisplatin, pravastatin was deemed a logical treatment. In order to determine the possible benefit of pravastatin, a protocol was developed where hearing thresholds were obtained via Auditory Brainstem Response (ABR) at .5, 10, 15, 20, 30, and 40 kHz for two groups of six Sprague-Dawley rats. Additionally, the weights of each rat were measured as an indicator of general health over the course of the study. Following initial measures, rats were injected intravenously with cisplatin followed by a series of intraperitoneal injections of either pravastatin solution (experimental group) or saline solution (control group) over the course of a week. At the end of this week, hearing thresholds and weights were measured again for all surviving rats. Results revealed no significant differences on either measure between the two groups in question. However, definitive

conclusions on the possible otoprotective effects of pravastatin cannot be made without

further, more comprehensive investigation of the medication's pharmacokinetics.

Vita

2007 ....B.A. Speech and Hearing Science, The Ohio

State University

2010 to 2011 ....Graduate Teaching Assistant, Department of

Speech and Hearing Science, The Ohio State

University

2011 to 2012 ....Graduate Research Assistant, Department of

Speech and Hearing Science, The Ohio State

University

Fields of Study

Major Field: Audiology

Table of Contents

List of Figures

List of Abbreviations

ABR: Auditory Brainstem Response

dB: Decibel(s)

dB SPL: Decibel(s) Sound Pressure Level

D-met: D-Methionine

DPOAE: Distortion Product Otoacoustic Emission

GST: glutathione-S-transferase

CHAPTER 1: INTRODUCTION

Cisplatin is a platinum-based anti-neoplastic drug commonly used to treat solid tumors such as those found in ovarian, testicular, cervical, lung, head and neck, and bladder cancers (Rybak, Mukherjea, Jajoo, & Ramkumar, 2009). While cisplatin is often successful in the treatment of cancer, its clinical utility is frequently limited by unwanted side effects including ototoxicity, nephrotoxicity, neurotoxicity, and nausea. The most life-threatening of these side effects is nephrotoxicity, which can result in renal failure with continued use of cisplatin (Wimmer et al., 2004). While the negative effects on the renal system can be remediated to a certain degree by saline hydration, there are no known cures or preventative treatments available to manage ototoxicity. As such, ototoxicity is currently the main dose-limiting side effect of cisplatin (Rybak et al., 2009).

Damage to the auditory system caused by cisplatin may become present within hours or days of the initial treatment. Such damage generally becomes more severe with cumulative doses of cisplatin (Bertolini et al., 2004). Further, damage is more pronounced with cancers of the head and neck, persons with depleted nutritional status, persons on concurrent ototoxic medications, as well as in the pediatric population (Chen et al., 2006; Kopelman et al., 1988). The greater auditory effect seen in children is especially relevant due to hearing loss' effect on the development of language, which is necessary for academic success. Studies of cisplatin have revealed elevation of hearing

thresholds in approximately 75-100% of patients undergoing treatment (McKeage, 1995).

Hearing losses tend to vary with dosage and duration of treatment; however, hearing

losses generally affect higher frequencies first and are irreversible, bilateral, symmetric,

and sensorineural in nature (Rybak et al., 2009). Moreover, hearing loss associated with

cisplatin is further complicated by cisplatin's interaction with noise exposure. On the

whole, exposure to even moderate levels of noise (80 dB SPL or greater) has been shown

to potentiate cisplatin ototoxicity. This is believed be the result of a synergistic

interaction whereby both cisplatin and noise exposure trigger mechanisms leading to cell

death (Gratton & Kamen, 1990). Therefore, during cisplatin treatment, hearing aids,

which would otherwise be the first form of treatment for hearing loss, may cause

additional hearing loss and are not an option for the patient.

In addition to hearing loss, anywhere between 2 and 36% of patients treated with

cisplatin complain of tinnitus (Reddel, Kefford, Grant, Coates, Fox & Tattersall, 1982).

Cisplatin treatment regimens are variable, based on the type and stage of cancer, and can

range anywhere from under 60 mg/m^2 to as high as 250 mg/m^2 over the course of a three-

week treatment cycle. Administration is completed intravenously over the course of

approximately 30 minutes to 2 hours with 1 mg/minute infusion as the most common rate

of infusion. In general, a patient will receive 1-2 liters of saline intravenously prior to

cisplatin treatment as well as further injections as needed. The patient is also advised to

increase water consumption for a day, all in an attempt to prevent nephrotoxicity (Arora

et al., 2009; National Cancer Institute, 2012).

In examining the cisplatin's mechanism of action on the auditory system, it is first important to acknowledge that the toxic effect of cisplatin is somewhat mediated by genetic variations. More specifically, patients with certain polymorphisms of glutathione-S-transferases (GSTs), which are enzymes responsible for detoxifying cisplatin, displayed significantly better thresholds post cisplatin-treatment when controlling for other variables (Oldenburg, Kraggerud, Cvancarova, Lothe, Fossa, 2007). Overall, cisplatin-induced hearing loss is a result of cell death and morphological changes to the organ of Corti as well as stria vascularis. The death of outer hair cells in the organ of Corti prevents the creation of the action potential required for the sensation of sound. Moreover, morphological changes cause the transduction channels of the outer hair cells to become blocked and prevent the restoration of the electrochemical gradient necessary for propagation of a neural impulse leading to auditory sensation (Yamamoto, Kakehata, Sait, Saito & Akaike, 1994). Outer hair cell death is generally restricted to the basal and middle turns and is often combined with substantial damage to supporting cells (Deiters' and Hensen's cells) (Cardinaal, De Groot, Huizing, Smoorenburg, & Veldman, 2004). An example such damage with complete ablation of the outer hair cells is demonstrated in Figure 1.

Moreover, damage to the microarchitecture of the cochlea in the basal and middle turns has been demonstrated in animal studies using higher dosages of cisplatin (1.5-2 mg/kg/day) as shown by the presence of the tunnel of Corti and Nuel's space being completely obscured by supporting cells. Further, within the stria vascularis, blebbing
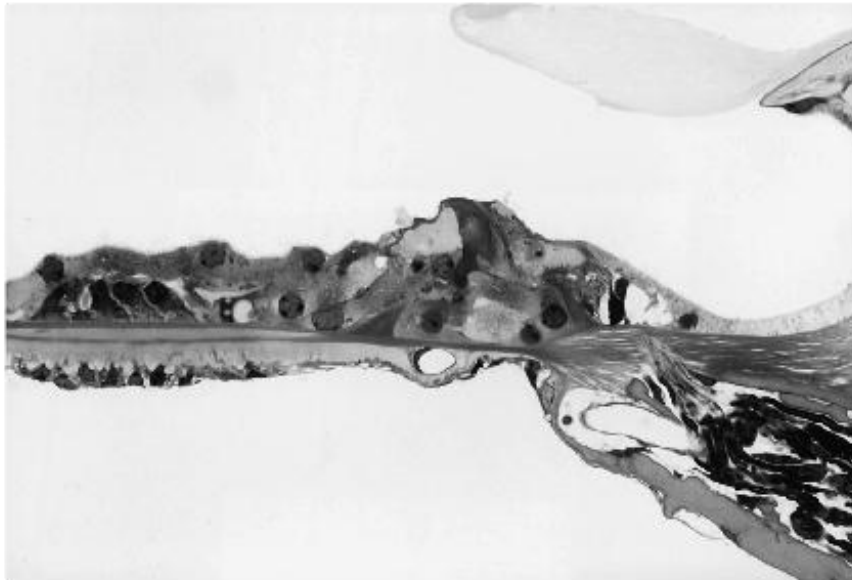
3

Figure 1. Reprinted from Cardinaal et al., 2000. Microscopic still of a guinea pig cochlea after 8 days of treatment with cisplatin at a dose of 1.5 mg/kg. Image displays a complete breakdown of the organ of Corti, including loss of all outer hair cells.

(the breaking apart of cell's as part of apoptosis) and vacuolization of the marginal cells as well as atrophy of the intermediate cells has also been demonstrated. Such damage would be expected to have an impact on the maintenance of the electrochemical gradient of endolymph within scala media. Concurrently, however, there is generally no discernible damage to the inner hair cells or spiral ganglion (Cardinaal et al., 2000).

Looking more specifically at the auditory system's reaction to cisplatin, cisplatin is responsible for interacting with the cells of the organ of Corti, stria vascularis, spiral ligament and spiral ganglion cells to generate an oxidative stress response. As a part of this oxidative stress response, superoxides are generated. Superoxides are essentially molecular oxygen molecules (O_2) with one unpaired electron. They are always present in cells as a result of mitochondrial respiration, but become problematic when they are over abundant, as in the case of cisplatin ototoxicity (Clerici, DiMartino, & Prasad, 1995; Clerici & Yang, 1996). Superoxides, in turn, have four main effects on the cells of the auditory system. Once, they interact with nitric oxide to form peroxynitrites, which are toxic and inactivate healthy proteins (Lee et al., 2004a, b). These toxins cannot be flushed out because the cochlea is a closed system (Rybak et al., 2009). Two, they form free hydroxyl radicals, which with exposure to iron will interact with the bilipid layer of the cell membrane to form aldehyde 4-hydroxynomenal leading to cell death (Lee et al., 2004a, b). Three, they reduce the supply of glutathione, a protein used to deactivate free radicals (Zhang & Lindup, 1993). In addition, cisplatin causes a depletion of the antioxidant enzyme system, which would normally aid in the rebuilding of the supply of glutathione and thereby neutralize the increase in superoxides (Rybak, Husain, Morris,

5

Whitworth, & Somani, 2000). And four, they cause the release of cytochrome c from the mitochondria leading to activation of caspase 3 and 9. The ensuing chain reaction activates deoxyribonuclease, which leads to DNA breakdown and triggers caspase-mediated apoptosis (Wantanabe, Inai, Jinnouchi, Baba, & Yagi, 2003). Apoptosis is essentially a programmed death of the hair cell whereby the cell actively destroys itself. This process involves a condensing of DNA, shrinking of the cell cytoplasm, and eventually blebbing of the cells into smaller apoptotic bodies. As a manner of protecting other healthy cells, these apoptotic bodies are self-contained and not toxic to the surrounding environment. Once the cell is broken down into apoptotic bodies, immune cells, known as phagocytes, which remove the apoptotic bodies from the inner ear, can engulf the remnants (Rybak et al., 2009). A schematic of this process can be found in part b of Figure 2.

Past research regarding prevention of ototoxicity has focused largely on antioxidant agents. One of the most researched otoprotective agents is D-Methionine (D-Met), a protein found naturally in many foods such as cheese and yogurt and considered safe for consumption (Campbell et al., 2007). Studies have revealed that when administered prior to treatment with cisplatin, D-Met provides significant protection to outer hair cell function as measured by distortion product otoacoustic emissions (DPOAEs) (Wimmer et al., 2004), auditory thresholds as measured by ABR (Campbell, Rybak, Meech, & Huges, 1996), and damage to outer hair cells and stria vascularis as shown by microscopic investigation (Lockwood et al., 2000; Giordano, Lorito, Ciorba, Martini, & Hatzopoulos, 2006). D-Met is thought to provide this protective effect by

6

working as antioxidant scavenging for free radicals produced by cisplatin's toxic

interactions with the cochlea (Vogt, 1995). More specifically, D-Met protects critical

enzymes such as glutathione (by aiding in the recycling of its reduced form, glutathione

disulfide back into usable glutathione), which is used to deactivate free radicals, thereby

up-regulating antioxidant pathways (Campbell, Meech, Rybak, & Huges, 2003). Further,

D-Met has been shown to have similar protective effect regarding nephrotoxicity (Jones

& Basinger, 1989). Another promising otoprotective agent can be found in the selenium-

containing compound, ebselen. Like D-met, ebselen functions as a part of the glutathione

peroxidase pathway (i.e. glutathione peroxidase is an enzyme that catalyzes a reaction in

which glutathione neutralizes the ROS hydrogen peroxidase by converting it into water

molecules); however, in this case, ebselen works by mimicking glutathione within the

cochlea (Lynch, Gu, Pierce, & Kil, 2005). While use in humans is still in the early

stages, animal studies have demonstrated significant reductions in cisplatin-related

ototoxicity without reduction in cisplatin's anti-neoplastic properties (Rybak & Somani,

1999; Lynch et al., 2005). Different agents such as neurotrophic factors and sodium

thiosulfate have been examined for otoprotective value with little success in protecting

hair cells from cisplatin. Neurotrophic factors are proteins that signal cells to grow and

differentiate, but have shown no positive effect. Sodium thiosulfate, on the other hand, is

a compound that reacts with cisplatin to form a complex that can be excreted by the

kidney. In doing so, some protective effect was seen. However, this positive impact was

outweighed by its negative effect on the potency of chemotherapy medication in treating
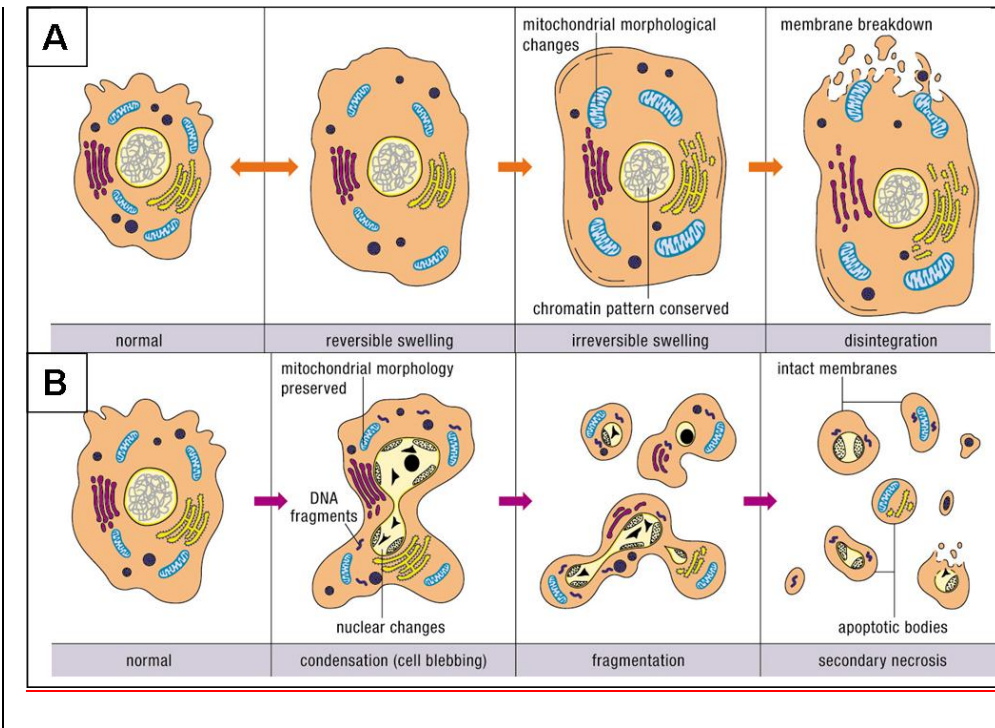
7

Figure 2. Reprinted from Henderson, Bielefeld, Harris, & Hu, 2006. Part B of this schematic displays the gradual breakdown of a cell through the process of apoptosis described previously.

cancerous cells (because the cisplatin was excreted, as stated above) (Wimmer et al., 2004).

For the purposes of the current study, the cholesterol-management medication, pravastatin, was examined for possible otoprotective effects. Pravastatin has been shown to have some nephro-protective effects as shown by an increase in antioxidant levels and more importantly decreases in renal damage, p53 expression, and apoptosis (Fujieda et al., 2010). p53 is a protein that is activated by cisplatin-induced damage to a cell's DNA. Once activated, p53 has been shown to up-regulate another pro-apoptotic molecule known as Bax. The increase of Bax, in turn, leads to the release of caspase proteins, which are key to the execution of cell apoptosis (including outer hair cell apoptosis) (Fujieda et al., 2010). Therefore, since p53 activation and a decrease in antioxidant activity are both key parts of the cell death process in cisplatin ototoxicity, pravastatin is logical candidate to attempt to ameliorate these effects. Thus, the purpose of this study was to investigate for an otoprotective effect of pravastatin mediated by a decrease in p53 levels. If pravastatin produces a decline in p53 levels such as those in the kidney, then one would expect an otoprotective effect.

CHAPTER 2: METHODS

Study Parameters:

Twelve male Sprague-Dawley rats (253 to 271 g) were randomly divided into two

groups of six. Each rat was initially sedated using the inhaled anesthetic, isoflurane (4%

concentration for induction, 1.5% for maintenance, 1 L/min O_2 flow rate).

Hearing thresholds were obtained via ABR with tone-bursts at 5, 10, 15, 20, 30, and 40

kHz. Each burst was approximately 1 msec in duration and was gated through a

Blackmann window with a 0.5 ms rise/fall time and no plateau. Stimuli were produced at

a rate of 21/s. Stimuli were emitted from a speaker placed at zero degrees azimuth, 17

cm from the vertex of the rat's head. Evoked responses were obtained using TDT BioSig

software, which averaged two hundred-fifty sweeps at each stimulus level. Responses

were amplified with a gain of 50,000, using a TDT Headstage-4 bioamplifier and

bandpass filtered from 100-3000 Hz. In order to ascertain threshold, the level of the

stimulus at each frequency was decreased in 5 dB steps from 90 dB pSPL to 0 dB pSPL.

Threshold was marked at the lowest level displaying a repeatable response for wave V.

An example of an ABR threshold series elicited with a 5 kHz tone burst stimulus is

presented in Figure 3. ABR was performed using needle electrodes placed at the vertex

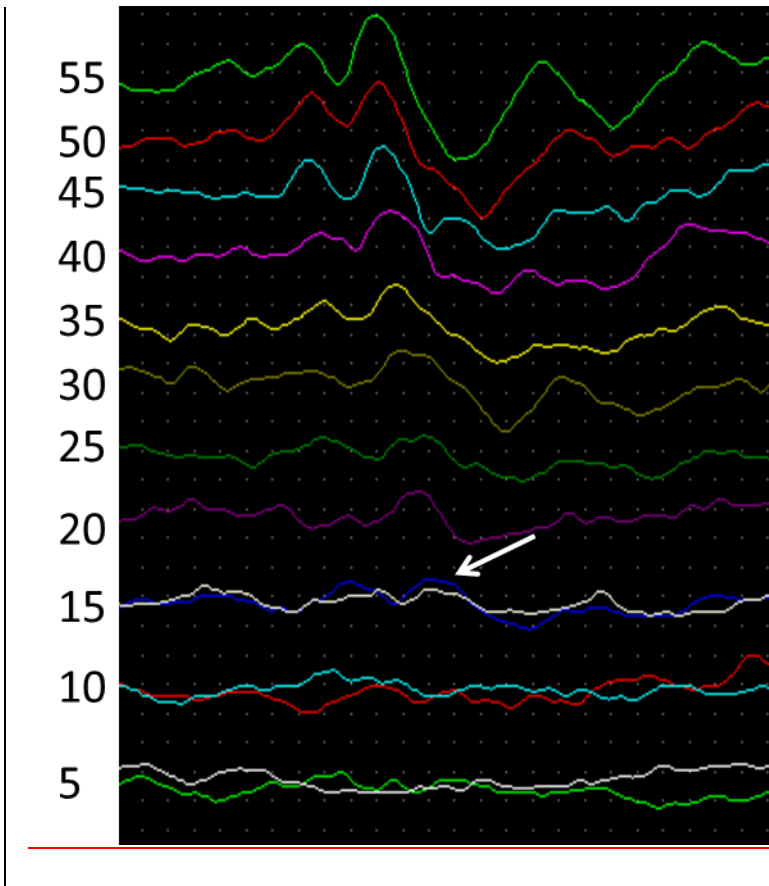(non-inverting), below the left pinna (inverting), and below the right pinna (ground).

Figure 3. An ABR threshold series for 5 kHz as the intensity of the stimulus is decreased from 55 to 5 dB SPL. Note that the last visible, repeatable finding of wave V (the threshold) was at 15 dB SPL.

Cisplatin Exposure:

All rats received a single dose of cisplatin dissolved in saline solution at a concentration of 1 mg/ml. Dosage was determined based on rat weight at a ratio of 8 mg/kg. This equates to approximately 2-2.5 mg per injection. The solution was slowly infused subcutaneously at a rate of 8 ml per hour (typical duration of 15-20 minutes). Rats for anesthetized for infusion using the inhaled anesthetic, isoflurane (4% concentration for induction, 1.5% for maintenance, 1 L/min O_2 flow rate).

Rats in the experimental group received injections of pravastatin. The pravastatin was dissolved in saline at a ratio of 1 mg/ml. Dosage was determined based on rat weight at a ratio of 2.3 mg/kg. This equates to approximately 0.8-1.0 mg per injection. Pravastatin was administered via intraperitoneal injection one hour before and after cisplatin infusion as well as once in the morning and once in the evening for the four days following, resulting in a total of 10 injections. Rats in the control group received equivalent injections of physiologic saline on the same schedule.

In order to flush cisplatin from the renal system and prevent premature death, all rats in the study also received 5 ml subcutaneous injections of physiologic saline at the same intervals at which they received pravastatin injections. To ascertain any effect of pravastatin on cisplatin-related weight loss, rat weight was measured pre-administration as well as on days 1, 2, 4, and 7 post-administration. Finally, 7 days post-administration of cisplatin, hearing thresholds of all rats were obtained again via anesthetized ABR.

Statistical Analysis:

　　　Pre-exposure thresholds were analyzed between the control and experimental groups using a two-factor ANOVA (group x frequency) to examine for the presence of any initial differences between the two groups across frequencies. Pre-exposure differences in weight between the two groups were analyzed using student's t-tests. Following exposure to cisplatin, a second two-factor ANOVA was administered to analyze for significant differences regarding the variables of group, frequency, and group x frequency for threshold shift (this was only performed using surviving subject data). A repeated-measure two-factor ANOVA was performed following exposure to analyze for significant differences in animals' weights at Days 1, 3, 4, and 7 of the week long study.

CHAPTER 3: RESULTS

As seen in Figure 4, pre-exposure thresholds displayed no significant differences between control and experimental groups. Average thresholds for each group never varied by more than 5 dB for any of the tested frequencies. This lack of significant difference also held true when analyzing weights of rats in each group. Mean weights for each group were within approximately 3 grams of each other (first two columns of Fig. 6), and the student's t-test revealed no significant differences in weights between the two experimental groups. The lack of difference in pre-exposure thresholds and weights provides assurance that any differences detected after cisplatin exposure would be the result of the experimental treatments.

Following cisplatin exposure, each group displayed significant elevations in hearing thresholds at all frequencies. Further, as expected, cisplatin caused a significantly greater degree of hearing loss in the higher frequencies for both groups in question, as detected by a significant main effect of frequency in the two-factor ANOVA testing for differences in threshold shift (0.014; $p < 0.05$). Tukey A post-hoc testing revealed a significant difference between threshold shifts at .5 and 30 kHz (0.048; $p < 0.05$) and approaching significant between threshold shifts at .5 and 40 kHz (0.057; $p > 0.05$). However, the treatment group did not differ significantly from the control group in

terms of cisplatin-induced threshold shift at any frequency (0.327; p > 0.05), nor was there any evidence of a group x frequency interaction. The experimental group never showed greater than a 1 dB decrease in threshold shift by comparison to the control group. Figure 5 displays these results showing similar mean threshold shifts for the control and treatment groups at tested frequencies.

As a measure of general health, weight loss during the course of this study was examined. As evidenced by the results of a two-factor (group x day post exposure) ANOVA, both groups displayed significant declines in weight over the course of the study, resulting in a significant main effect of day in the analysis (0.000; p < 0.05). However, there was no significant difference between groups with both losing approximately 17% of their body mass (0.698; p > 0.05). Figure 6 displays similar mean weights both pre and post-exposure to cisplatin. Moreover, in terms of general health, cisplatin-induced mortality was experienced in both experimental and control groups with a total loss of 3 subjects over the course of the experiment, two from the pravastatin-treated group and one from the control group.
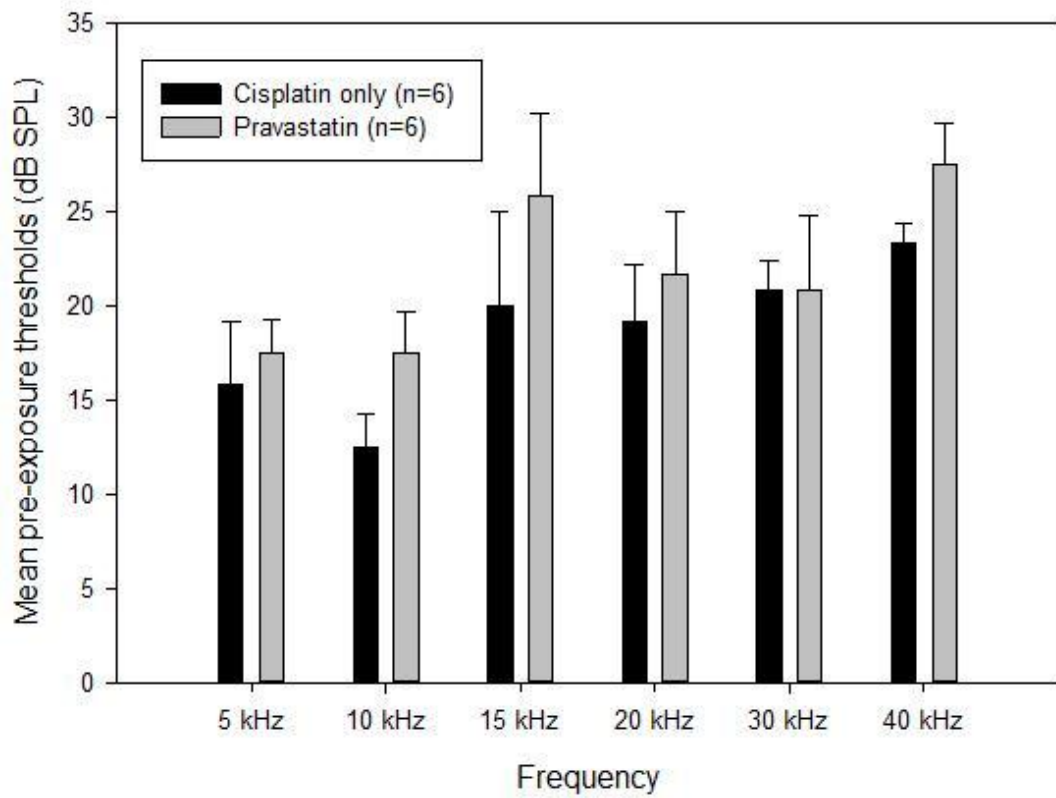
Figure 4. Initial, pre-exposure mean thresholds in dB SPL. Error bars are +1 SEM.

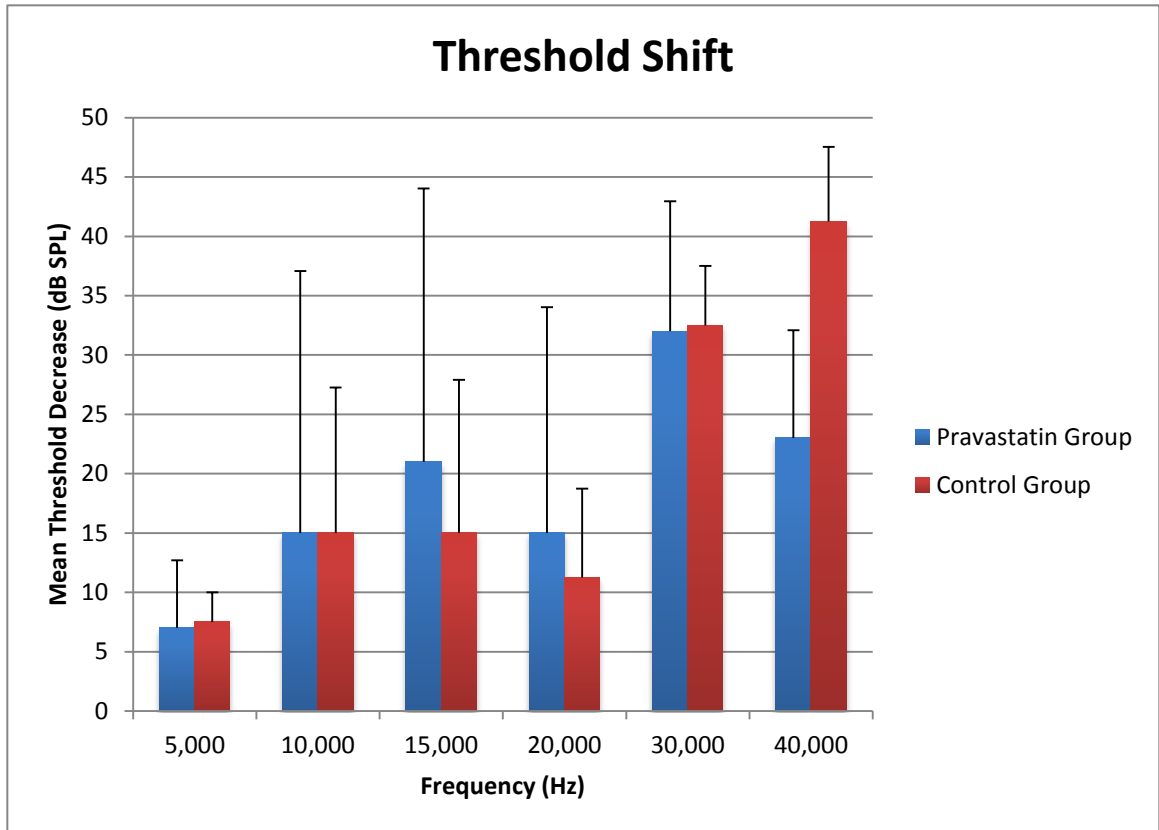Figure 5. Mean threshold shift 1 week after exposure to cisplatin. Error bars are +1 SD.
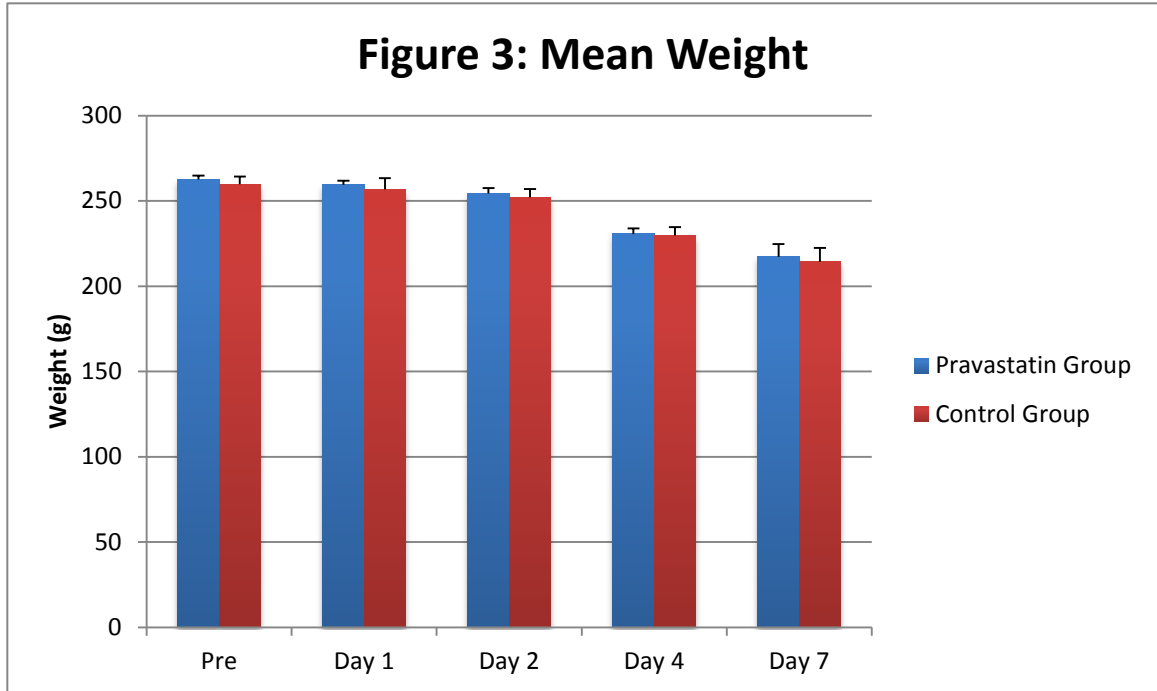
Figure 3: Mean Weight

Figure 6. Mean weight (in grams) of the control group and group receiving pravastatin across the duration of the study. Error bars are +1 SEM.

CHAPTER 4: DISCUSSION

While common and effective in the treatment of cancer, the use of the medication

cisplatin is accompanied by a variety of side effects. At present, the main dose-limiting

side effect associated with cisplatin is ototoxicity, specifically death of the outer hair cells

within the cochlea. This toxic effect on the cochlea is a result of production of

superoxides and consequential oxidative stress. The increase in levels of superoxides

coupled with a decrease in antioxidant levels leads to a series of reactions that degrade

both the cell membrane and DNA of the outer hair cells. The result of these reactions is

apoptosis, the programmed death of a cell (Rybak et al., 2009). One important factor in

the process leading to apoptosis is the protein p53, which is activated by cisplatin-

induced damage to the cell's DNA (Fujieda et al., 2010). Once activated, p53 has been

shown to up-regulate another pro-apoptotic molecule known as Bax. The increase of Bax

then leads to the release of caspase proteins, which are key to the execution of cell

apoptosis (Fujieda et al., 2010).

With p53 being an important piece in the process leading to apoptosis, researchers

have focused on agents that may be used to decrease this protein's expression. In a study

by Fujieda et al., the use of the cholesterol medication, pravastatin, was demonstrated to

decrease the expression of p53 and subsequent apoptosis in the renal systems of subjects

being treated with cisplatin. As such, pravastatin was deemed a logical candidate to

attempt to decrease the level of hearing threshold shifts associated with cisplatin-induced

ototoxicity. Nevertheless, following this study, no significant difference hearing

threshold decline was noted between subjects receiving pravastatin versus those that did

not.

However, the results of this experiment do not definitively indicate lack of effect

when using pravastatin in conjunction cisplatin. The absence of significant findings

could be the result of a variety of factors. First of all, the dosing of pravastatin used in

this protocol may have been at insufficient levels in order to produce a significant effect.

In order to examine the impact of different levels of pravastatin, in future research, it

would be necessary to increase the number of animal subjects used in the study and to

complete a multi-treatment protocol. Furthermore, the injection of pravastatin

intraperitoneally may not be the ideal route in order for the drug to reach the cochlea.

Other options would be using an intravenous injection or directly placing the solution on

the round window of the cochlea. Additionally, the use of saline hydration in order to

flush out the kidneys and prevent renal failure may have also removed the pravastatin

from the subject's bloodstream and prevented any significant impact. However, a

protocol without saline hydration would likely result in a high mortality rate due to

cisplatin-induced renal failure. Ultimately, it would be beneficial to assess the

pharmacokinetics of pravastatin to determine how much of the drug is able to enter the

cochlea with any given injection route and dose, and to determine how long the

pravastatin stays in the cochlea in order to maximize the potential for a protective effect.

While further research will be necessary to determine whether pravastatin has otoprotective properties against cisplatin-induced hearing loss, the need for such agents continues to be crucial for cancer patients. The American Cancer Association estimates that approximately 1.5 million are newly diagnosed with cancer each year and approximately 1 million of these persons will receive some sort of chemotherapy treatment (ACA, 2009). With many chemotherapy medications, including cisplatin, possessing ototoxic properties, an unknown but undoubtedly large number of these persons will experience some degree of hearing loss. Thus, the discovery of useful otoproective agents would remove the threat of a lifelong side effect to cisplatin and other chemotherapy medications.

CHAPTER 5: CONCLUSIONS

As one of the main side effects of cisplatin treatment is ototoxicity, this study sought to find a possible otoprotective agent in the cholesterol medication, pravastatin. Following administration of pravastatin to an experimental group and saline solution to a control group, both being treated with cisplatin, no significant differences were found either in cisplatin-induced hearing threshold shift or decline in overall weight. While this experiment did not reveal significant findings, it should be noted that more comprehensive research would be required to draw definitive conclusions on any possible otoprotective benefits.

References

American Cancer Society. *Cancer Facts & Figures 2009.* Atlanta: American Cancer Society; 2009.

Arora, R., Thakur, J.S., Azad, R.K., Mohindroo, N.K., Sharma, D.R., & Seam, R.K. (2009). Cisplatin-based chemotherapy: Add-frequency audiometry in the regimen. *Indian Journal of Cancer, 46*(4), 311-317.

Bertolini, P., Lassalle, M., Mercier, G., Raquin, M.A., Izzi, G., Corradini, N., & Hartmann, O. (2004). Platinum compounding-related ototoxicity in children: Long-term follow-up reveals continuous worsening of hearing loss. *J. Pediatr. Hematol. Oncol., 26,* 649-655.

Campbell, K.C., Meech, R.P., Klemens, J.J., Gerberi, M.T., Dyrstad, S.S.W., Larsen, D.L., … Huges, L.F. (2007). Prevention of noise- and drug-induced hearing loss with D-methionine. *Hearing Research, 226,* 92-103.

Campbell, K.C., Meech, R.P, Rybak, L.P., & Huges, L.F. (2003). The effect of D-Methionine on cochlear oxidative state with and without cisplatin administration: Mechanisms of otoprotection. *JAAA, 14*(3), 144-156.

Campbell, K.C., Rybak, L.P., Meech, R.P, & Huges, L.F. (1996). D-Methionine provides excellent protection from cisplatin ototoxicity in the rat. *Hearing Research, 102,* 90-98.

Cardinaal, R.M., De Groot, J.C.M.J, Huizing, E.H., Smoorenburg, G.F., & Veldman, J.E. (2004). Ultrastructural changes in the albino guinea pig cochlea at different times following cessation of 8-day cisplatin administration. *Acta Otolaryngol, 124,* 144-154.

Cardinaal, R.M., De Groot, J.C.M.J, Huizing, E.H., Smoorenburg, G.F., Veldman, J.E., & Smoorenburg, G.F. (2000). Dose-dependent effect of 8-day cisplatin administration upon the morphology of the albino guinea pig cochlea. *Hearing Research, 144,* 135-146.

Clerici, W.J., DiMartino, D.L., & Prasad, M.R. (1995). Direct effects of reactive oxygen species on cochlear outer hair cell shape in vitro. *Hear. Res., 84,* 30-40.

Clerici, W.J., Yang, L. (1996). Direct effects of intraperilymphatic reactive oxygen species generation on cochlear function. *Hear. Res., 101,* 14-22.

Chen, W.C., Jackson, A., Budnick, A.S., Pfister, D.G., Kraus, D.H., Hunt, M.A., Stambuk, H., Levegrun, S., & Wolden, S.L. (2006). Sensorineural hearing los in combined modality treatment of asopharyngeal carcinoma. *Cancer, 106,* 820-829.

Fujieda, M., Morita, T., Naruse, K., Hayashi, Y., Ishihara, M., … Wakiguchi, H. (2010). Effect of pravastatin on cisplatin-induced nephrotoxicity in rats. *Human and Experimental Toxicology, 000*(00), 1-13.

Giordano, P., Lorito, G., Ciorba, A., Martini, A., Hatzopoulos, S. (2006). Protection against cisplatin ototoxicity in a Sprague-Dawley rat animal model. *Acta Otorhinoloaryngol. Ital., 26,* 198-207.

Gratton, M.A., & Kamen, B.A. (1990). Potentiation of cisplatin ototoxicity by noise. *J Clin Oncol, 8*, 2091-2092.

Henderson, D., Bielefeld, E.C., Harris, K.C., & Hu, B.H. (2006). The role of oxidative stress in noise-induced hearing loss [review]. *Ear Hear, 27,* 1-19.

Jones, M.M., & Basinger, M.A. (1989). Thiol and thioester suppression of cisplatin-induced nephrotoxicity in rats bearing the Walker 256 carcinosarcoma. *Anticancer Res., 9*, 1937-1942.

Lee, J.E., Nakagawa, T., Kim, T.S., Endo, T., Shiga, A., Iguchi, F., Lee, S.H., & Ito, J. (2004a). Role of reactive radicals in degeneration of the auditory system of mice following cisplatin treatment. *Acta Otolaryngol., 124,* 1131-1135.

Lee, J.E., Nakagawa, T., Kita, T., Kim, T.S., Endo, T., Shiga, A., Iguchi, F., Lee, S.H., & Ito, J. (2004b). Mechanisms of apoptosis induced by cisplatin in marginal cells in mouse striavascularis. *ORL J. Otorhinolaryngol. Relat. Spec., 66,* 111-118.

Lockwood, D.S., Ding, D.L., Wang, J., et al. (2000). D-Methionine attenuates inner hair cell loss in carboplatin-treated chinchillas. *Audiol. Neurootol., 5,* 263-266.

Lynch, E.D., Gu, R., Pierce, C., & Kil, J. (2005). Reduction of acute cisplatin ototoxicity and nephrotoxicity in rats by oral administration of allopurinol and ebselen. *Hearing Research, 201,* 81-89.

McKeage, M.J. (1995). Comparative adverse effect profiles of platinum drugs. *Drug Saf., 13,* 228-244.

Oldenburg, J., Kraggerud, S.M., Cvancarova, M., Lothe, R.A., & Fossa, S.D. (2007). Cisplatin-induced long-term hearing impairment is associated with specific glutathione-s-transferase genotype in testicular cancer survivors. *J. Clin. Oncol.,* 25, 708-714.

Pigeolet, E., Corbisier, P., Houbion, A., Lambert, D., Michiels, C., Raes, M., Zachary, M.D., & Remacle, J. (1990). Glutathione peroxidase, superoxide dismutase, and catalase inactivation by peroxides and oxygen derived free radicals. *Mech. Ageing Dev., 51,* 283-297.

Reddel, R.R., Kefford, R.F., Grant, J.M., Coates, A.S., Fox, R.M., & Tattersall, M.H. (1982). Ototoxicity in patients receiving cisplaint: Importance of dose and method of drug administration. *Cancer Treat. Rep., 66,* 19-23.

Rybak, L.P., Husain, K., Morris, C., Whitworth, C., & Somani, S. (2000). Effect of protective agents against cisplatin ototoxicity. *Am. J. Otol., 21,* 513-520.

Rybak, L.P., Mukherjea, D., Jajoo, S., & Ramkumar, V. (2009). Cisplatin ototoxicity and protection: Clinical and experimental studies. *Tohoku J. Exp. Med., 219,* 177-186.

Rybak, L.P., & Somani, S. (1999). Ototoxicity amelioration by protective agents. *Ann. N.Y. Acad. Sci. 884 (Nov 28),* 143-151.

Vogt, W. (1995). Oxidation of methionyl residues in proteins: Tools, targets, and reversal. *Free Radic. Biol. Med., 18,* 93-105.

Wantanabe, K., Inai, S., Jinnouchi, K., Baba, S., & Yagi, T. (2003). Expression of capaseactivated deoxyribonuclease (CAD) and capase 3 (CCP32) in the cochlea of cisplatin (CDDP)-treated guinea pigs. *Auris Nasus Larynx, 30,* 219-225.

Wimmer, C., Mees, K., Stumpf, P., Welsch, U., Reichel, O., & Suckfull, M. (2004). Round window appication of D-methionine, sodium thiosulfate, brain-derived neurotrophic factor and fibroblast growth factor-2 in cisplatin-induced ototoxicity. *Otol. Neurotol., 25,* 33-40.

Yamamoto, T., Kakehata, S., Sait, T., Saito, H., & Akaike, N. (1994). Cisplatin blocks voltage-dependent calcium current in dissociated outer hair cells of guinea-pig cochlea. *Brain Research, 648,* 296-298.

Zhang, J.G., & Lindup, W.E. (1993). Role of mitochondria in cisplatin-induced oxidative damage exhibited by rat renal cortical slices. *Biochem. Parhmacol., 45,* 2215-2222.

Zhang, M., Liu, W., Ding, D., & Salvi, R. (2003). Pifithrin-alpha suppresses p53 and

protects cochlear and vestibular hair cells from cisplatin induced apoptosis. *Neuroscience, 120,* 191-205.