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Investigating the Apoptotic Effects of Platinum(II) Amine Complexes With Only One Leaving Ligand on Zebrafish Auditory End Organs

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INVESTIGATING THE APOPTOTIC EFFECTS OF PLATINUM(II) AMINE COMPLEXES WITH ONLY ONE LEAVING LIGAND ON ZEBRAFISH AUDITORY END ORGANS

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I dedicate this thesis to my beautiful wife Hannah, and to my parents Greg and Julie Smith.

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Directed by: Dr. Michael E. Smith, Dr. Jerry D. Monroe, and Dr. Kevin M. Williams

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The FDA-approved platinum compound, cisplatin, is commonly used as a chemotherapy drug to treat many forms of cancer. However, this compound also has several associated side-effects, including ototoxicity. This has made the development of novel platinum compounds that reduce cancer cell viability, while causing fewer and milder side-effects, an area of significant research interest. In the present study, we examined the apoptotic effects that four monofunctional platinum compounds, pyriplatin, phenanthriplatin, Pt(diethylenetriamine)Cl, and Pt(N,N-diethyldiethylenetriamine)Cl, had on zebrafish inner ear auditory epithelial cells. We then compared the apoptotic effects of these compounds to those of cisplatin, which is a bifunctional platinum compound. Our hypothesis was that the four monofunctional platinum compounds would cause reduced inner ear apoptosis compared to cisplatin. Zebrafish were injected with either vehicle solution, cisplatin or with one of the monofunctional compounds. Later, at 24-hour and 48-hour time points, the zebrafish were euthanized, and two of their auditory inner ear endorgans, the utricle and saccule, were dissected out. A terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay was then used to label apoptotic cells, and the inner ear organs were viewed under a microscope. The number of apoptotic cells on each sample was quantified and the data were analyzed for significant differences between

treatments. We found that with the exception of pyriplatin in the saccules, and with the exception of pyriplatin, Pt(N,N-diethyldiethylenetriamine)Cl, and phenanthriplatin in the utricles, the monofunctional compounds and cisplatin did not induce apoptosis in the inner ear of zebrafish at either time point. Based on these results we conclude that the monofunctional platinum compounds largely do not induce zebrafish inner ear apoptosis and if they were to produce ototoxicity, it would not be through an apoptotic mechanism.

Introduction

As 7.6 million people die from cancer each year worldwide, developing novel chemotherapy drugs is of significant research interest (Johnstone et al. 2014). Platinum-based chemotherapy drugs are highly effective against some cancers, e.g., testicular cancer (Feldman et al. 2008), but are ineffective against most cancers and can produce serious side-effects including neurotoxicity, hearing damage (ototoxicity), and kidney damage (nephrotoxicity; Cepeda et al. 2007). The FDA-approved platinum compound cisplatin, has two chloride leaving ligands that become aquated upon entry into cells, allowing them to bind both DNA strands, making cisplatin a bifunctional platinum compound (Knox et al. 1986, Jamieson & Lippard 1999, Raymond et al. 2000). Bifunctional binding typically causes DNA to bend, followed by the activation of apoptotic pathways, and cancer cell death (Jamieson & Lippard 1999, Cepeda et al. 2007, Hellberg et al. 2009, Wang et al. 2010, Alian et al. 2012, Brock et al. 2012, Nitz et al. 2013). However, platinum(II) compounds with only one chloride leaving ligand are monofunctional as they bind to only one strand of DNA and do not cause it to bend. Yet, they can still block RNA transcription in cancer cells and reduce their viability (Park et al. 2012). Because monofunctional compounds can cause cancer cell death potentially through a different mechanism than bifunctional compounds, they may produce reduced side-effects, including decreased ototoxicity.

Cisplatin auditory cell uptake and the mechanisms that cause ototoxicity have been studied in mammalian and zebrafish (*Danio rerio*) models. Mammalian studies show that cisplatin uptake in auditory hair cells is membrane transporter-dependent

(Ciarimboli et al. 2010, More et al. 2010, Ding et al. 2011, Ciarimboli, 2012). These studies implicate specific hair cell plasma membrane transporters, e.g., CTR1 and OCT2. However, the mechanism of cisplatin uptake in zebrafish auditory cells may be different than their mammalian counterparts as zebrafish lateral line hair cells require mechanotransduction for cisplatin uptake and do not express the membrane transporters found in mammalian hair cells (Thomas et al. 2013). At this time, cisplatin uptake in zebrafish inner-ear hair cells has not been characterized.

Studies in both mammalian and zebrafish models have implicated a number of pathways in cisplatin-mediated ototoxicity. In mammals, both DNA dependent and independent pathways of cisplatin ototoxicity have been identified (Karasawa and Steyger 2015). Cisplatin can damage DNA and induce cytotoxicity in cochlear hair cells (Rybak et al. 2007; Slattery et al. 2014). However, reactive oxygen species (ROS) mechanisms independent of DNA damage can cause ototoxicity by ROS targeting of cytoplasmic molecules, e.g., glutathione (Fuertes et al 2003; Karasawa 2015). Less is known about ototoxicity mechanisms in zebrafish, but studies have shown that ototoxicity in zebrafish signal through proteins, e.g., Bax, Bcl2, p38, p53 and cytochrome c, that are implicated in rodent apoptosis (Coffin et al. 2013a,b; Shin et al. 2013).

This study used zebrafish inner ear auditory system tissue to investigate the apoptotic effects of four platinum-based compounds with only one leaving ligand. These compounds are the triamine-ligated platinum(II) complexes: Pt(diethylenetriamine)Cl, $[Pt(dien)Cl]^{+}$, and Pt(N,N-diethyldiethylenetriamine)Cl, $[Pt(Et_2dien)Cl]^{+}$, and the monofunctional heterocyclic-ligated platinum(II) complexes:

pyriplatin and phenanthriplatin. These compounds can form monofunctional adducts with DNA nucleotides that could potentially block RNA transcription and induce apoptosis in cancer cells (Park et al. 2012, Monroe et al. 2018). As transcription is increased in cancer cells relative to non-cancerous auditory cells, these compounds might be able to block transcription in cancer cells and cause their apoptotic destruction without causing apoptosis and ototoxicity in auditory hair cells.

The zebrafish inner-ear model has been successfully used to identify and quantify drug-induced apoptosis in auditory sensory tissues using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay (Smith et al. 2006; Sun et al. 2011). Here, the inner-ear model and TUNEL staining was used to determine whether platinum compounds with one leaving ligand caused apoptosis in two zebrafish inner ear auditory sensory structures, the saccule and the utricle.

These experiments investigated the hypothesis: Monofunctional platinum(II) amine complexes that do not distort DNA will cause reduced apoptosis in zebrafish inner-ear tissue compared to the DNA-distorting bifunctional complex, cisplatin. The information obtained from this work should be valuable in the design of new anticancer drugs and the analysis of their ototoxicity and cancer cell cytotoxicity characteristics and mechanisms. Thus, this research could potentially identify novel platinum compounds that are effective, and less ototoxic, chemotherapy drugs.

Materials and Methods

Zebrafish were housed in the animal facility at Western Kentucky University and cared for per the protocol described by (Westerfield 1994). This protocol has been approved by the Institutional Animal Care and Use Committee of Western Kentucky University. A total of 140 zebrafish (from which 68 saccule and 45 utricle samples were recovered) were randomly selected from a population containing both males and females and were injected with one of the four monofunctional compounds, cisplatin, or 0.9% NaCl vehicle control. The fish ranged in mass from 0.1 g to 0.9 g at the time of injection. The mean (± S.E.) mass of fish used in the 24 hour treatments was 0.40 g (± 0.03), and the average mass of fish used in the 48 hour treatments was 0.36 g (± 0.01). 0.015 milligrams of platinum compound or vehicle volume equivalent per gram of body mass was injected into each fish. The volumes injected ranged from 750 nL to 6750 nL. Injections were done using a microinjector (World Precision Instruments, Micro 4, Catalog #: SYS-MICRO4) and according to a protocol adapted from (Kinkel et al. 2010). Then, the fish were returned to the holding tank. After either 24 or 48 hours, the fish were euthanized by exposing them to a lethal dose of MS-222, a common anesthetic used in aquatic research laboratories. Surgical scissors were used to remove the fish heads and then the heads were placed in a vial containing 4% paraformaldehyde at 4^{o} C. After 24 hours, but not more than 6 days later, the heads were removed from the vial and washed in phosphate buffer (PB) three separate times, 15 minutes each time, for a total wash time of 45 minutes. After washing, the fish were dissected and the utricles and saccules removed using microsurgical tools and a Leica dissecting microscope. A TUNEL assay was then performed on the tissue samples (Sun et al. 2011), followed by a

mounting procedure (Olivari et al. 2008), during which the samples were placed in a small pool of Prolong Gold Antifade reagent containing the nuclear stain, 4',6-diamidino-2-phenylindole (DAPI), and mounted on a microscope slide. The samples were secured by carefully depositing a microscope coverslip on top of them and fixing the coverslip in place using nail polish. The slide containing the mounted samples was then quickly placed in a dark box and put in a refrigerator at 4^{o} C until quantification.

A Zeiss Axioplan 2 epifluorescence microscope was used in conjunction with a AxioCam MRm camera to observe the samples and to photograph them at 10X, 20X, and 100X magnification. Each image was taken using the FITC and DAPI camera filters. Zeiss Axioplan software was then used to count the number of TUNEL-labeled apoptotic cells present in each sample (Zeiss Axioplan, Axiovision Release 4.8.2 SP3 08-2013). The specific pathway for quantifying cells was: measure/interactive measurement/start measurement/. Then, the "Event Feature" was used to mark and count apoptotic cells. GraphPad Prism v6 (La Jolla, CA) was used for all statistical analysis. To determine whether there were significant differences in the amount of apoptosis generated by the platinum compounds compared to vehicle, and to compare 24- and 48-hour apoptosis data, Mann-Whitney U tests were used.

Results

This project used TUNEL staining to quantify the apoptotic effects of several monofunctional platinum complexes and the bifunctional compound, cisplatin, in zebrafish auditory tissue. Our analysis of saccular apoptosis at 24 hours revealed that only one of the compounds, pyriplatin, caused a significant effect when compared with vehicle ($p < 0.05$; Figure 2). No significant apoptosis was found in saccules treated with any of these compounds at 48 hours (Figure 3). When we quantified saccular apoptosis, we found the following mean ± S.E. values in apoptotic saccular samples treated for 24 hours: $[Pt(dien)Cl]^+$ (79.0 ± 9.3), pyriplatin (65.8 ± 3.6), cisplatin (57.3 ± 6.2), $[Pt(Et_2dien)Cl]^+$ (56.3 ± 7.4), phenanthriplatin (53.2 ± 12.5), and vehicle (29.7 ± 3.3). The mean values obtained for samples treated for 48 hours were: pyriplatin (82.8 ± 8.0), vehicle (58.3 ± 8.0), $[Pt(Et_2dien)Cl]^+$ (53.4 ± 5.0), $[Pt(dien)Cl]^+$ (47.0 ± 3.7), phenanthriplatin (31.8 ± 5.8), and cisplatin (9.7 ± 1.7). No evident treatment category trend is identifiable in saccules when the mean values are compared across the 24 and 48 hour time points.

Our analysis of utricular apoptosis was largely similar to our findings with saccules. $[Pt(Et_2dien)Cl]^+$ caused a significant increase in apoptosis compared to control at the 24 hour time point ($p < 0.01$; Figure 4). Both pyriplatin and phenanthriplatin caused significantly more apoptosis at 48 hours than control ($p < 0.01$; Figure 5). However, as there was only one data point for the $[Pt(dien)Cl]^+$ treatment at 24 hours, we were not able to comparatively analyze this value. When we quantified utricular apoptosis, we found the following mean values in samples treated for 24 hours: $[Pt(Et_2dien)Cl]^+$ (106.2 ± 4.1), phenanthriplatin (100.5 ± 12.4), pyriplatin (76.8 ± 7.9),

vehicle (57.2 ± 4.0), $[Pt(dien)Cl]^+$ (51.0 ± 0.0) and cisplatin (39.0 ± 9.9). The mean values obtained for utricles treated for 48 hours were: $[Pt(Et_2dien)Cl]^+$ (257.5 ± 103.6), pyriplatin (118.7 ± 15.6), $[Pt(dien)Cl]^+$ (117.0 ± 22.6), phenanthriplatin (94.5 ± 9.5), cisplatin (11.3 ± 3.2), and vehicle (3.2 ± 0.6). As with saccules, no evident treatment category trend is identifiable in utricles when their mean values are compared across the 24 and 48 hour time points.

We also compared the 24 and 48 hour data sets to identify potential temporal effects from treatment with these platinum compounds. Our results showed that in saccules, only cisplatin treatment produced a significantly lower number of apoptotic cells at 48 hours when compared to the 24 hour time point ($p < 0.01$; Figure 6). However, the saccular sensory epithelia for cisplatin samples treated at 48 hours exhibited signs of damage, which may account for the lower apoptotic cell counts at that time point. When we compared utricular samples at 24 and 48 hours, we found that vehicle samples at 48 hours were significantly lower than the samples treated with vehicle at 24 hours ($p < 0.01$; Figure 7). However, as with the 48 hour saccular cisplatin samples, the utricular vehicle samples taken at this time point also exhibited signs of damage, which again could explain the lower apoptotic cell count at 48 hours.

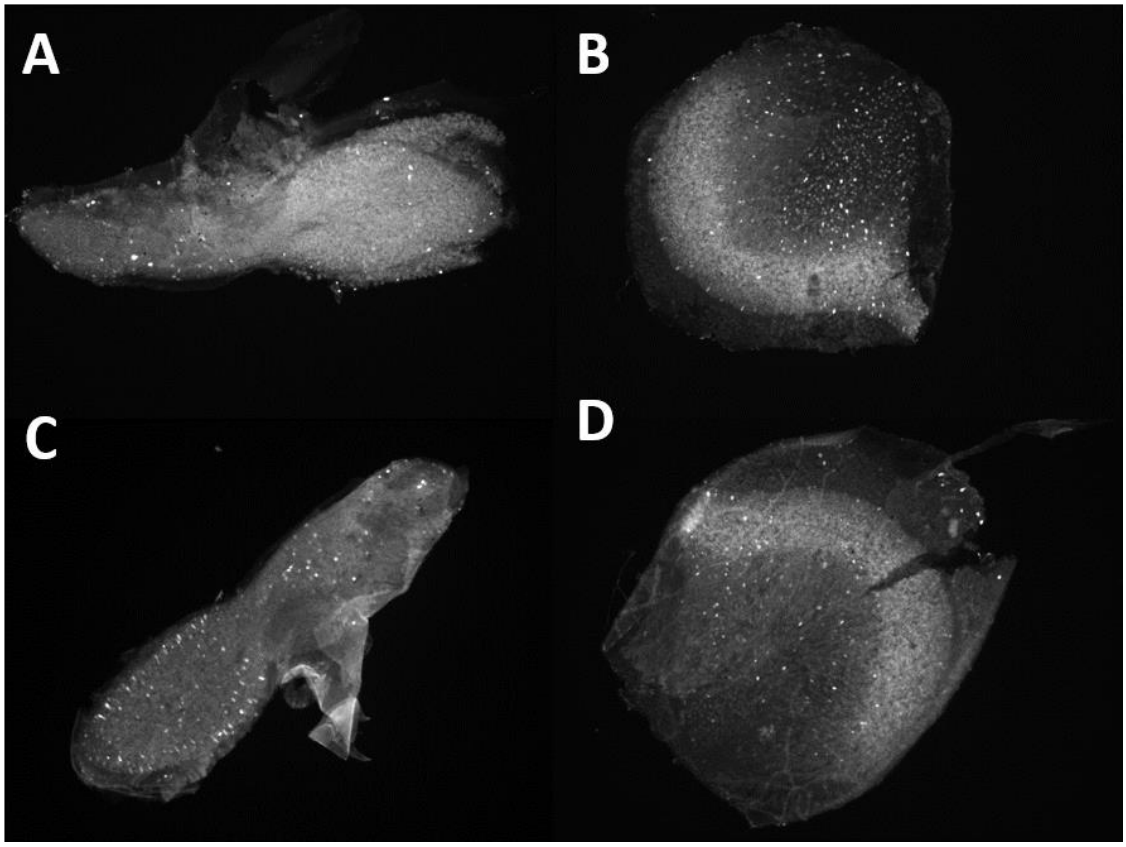

Figure 1. TUNEL-labeled saccule (A) and utricle (B) 24 hours post-vehicle injection. TUNEL-labeled saccule (C) and utricle (D) 24 hours post-cisplatin injection.

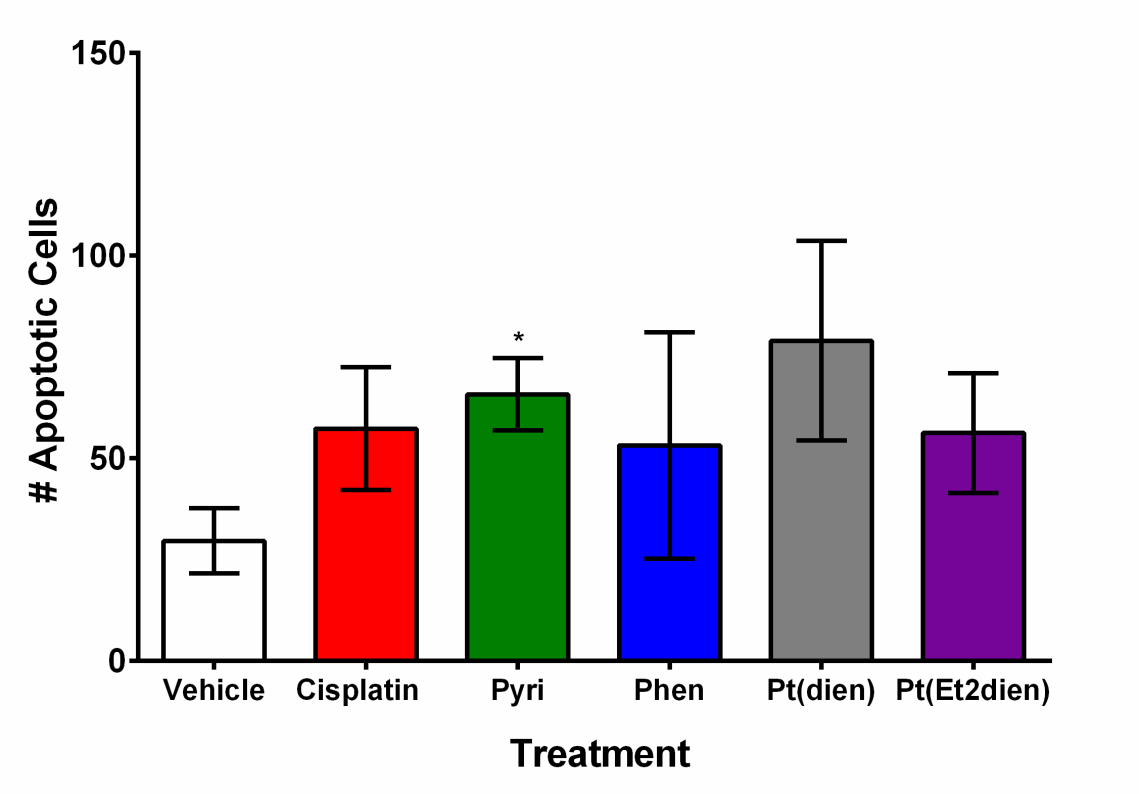

Figure 2: Pyriplatin treatment increases apoptosis in zebrafish saccules 24 hours post-injection. Data indicate the mean (± S.E.) number of apoptotic cells counted in saccular tissue treated with vehicle, cisplatin, and four monofunctional platinum compounds. Pyri = pyriplatin, Phen = phenanthriplatin, Pt(dien) = Pt(diethylenetriamine)Cl, Pt(Et2dien) = Pt(N,N-diethyldiethylenetriamine)Cl. N = 4-7; * $p < 0.05$.

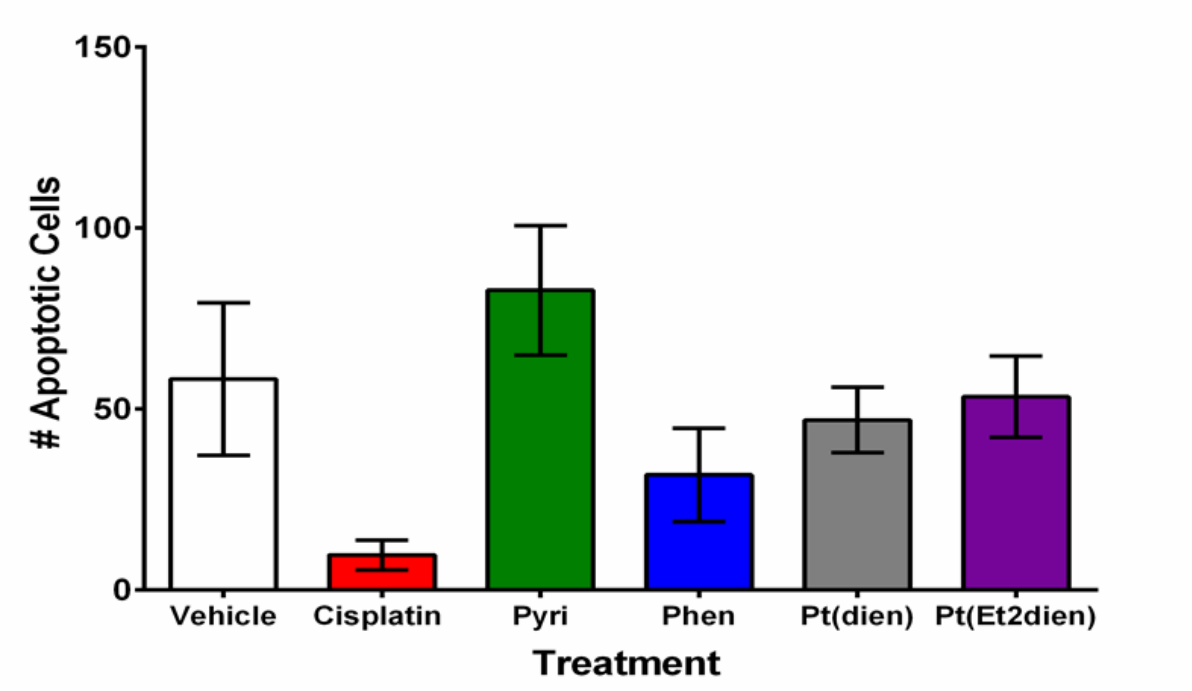

Figure 3: Platinum compound treatment does not affect apoptosis in zebrafish saccules 48 hours post-injection. Data indicate the mean (± S.E.) number of apoptotic cells counted in saccular tissue treated with vehicle, cisplatin and four monofunctional platinum compounds. Pyri = pyriplatin, Phen = phenanthriplatin, Pt(dien) = Pt(diethylenetriamine)Cl, Pt(Et2dien) = Pt(N,N-diethyldiethylenetriamine)Cl. N = 5-7; $p > 0.05$.

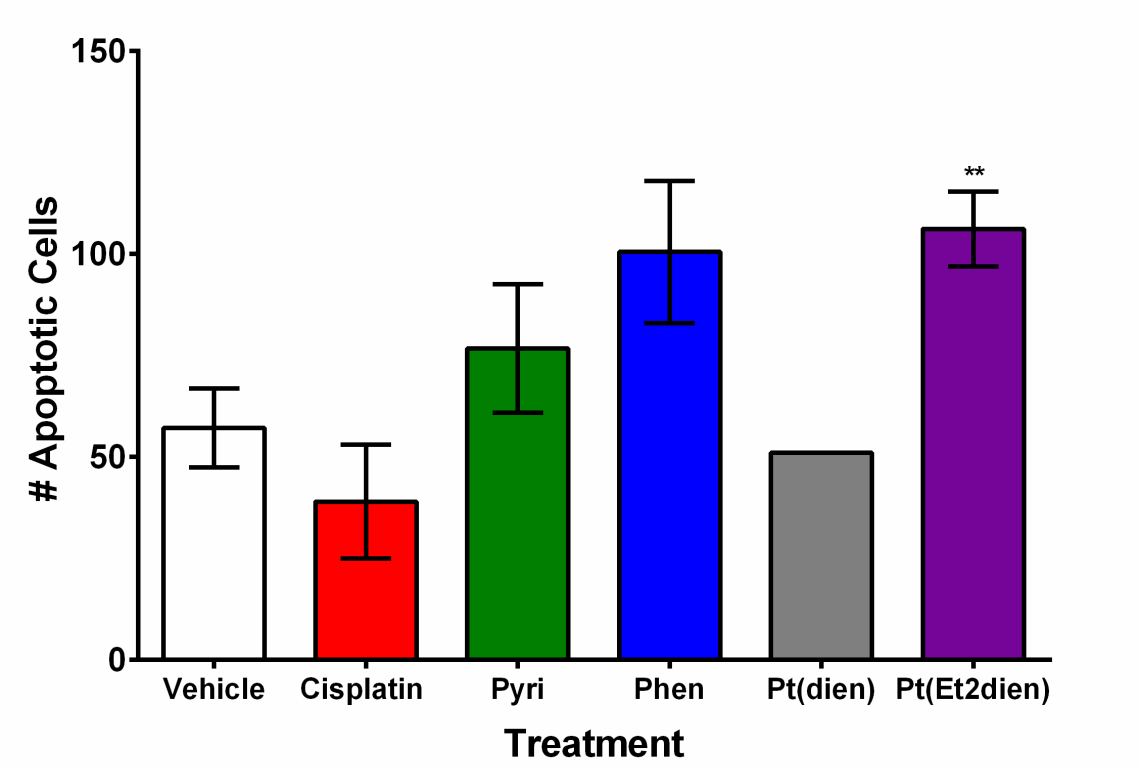

Figure 4: Pt(Et_2dien)Cl treatment increases apoptosis in zebrafish utricles 24 hours post-injection. Data indicate the mean (± S.E.) number of apoptotic cells counted in utricular tissue treated with vehicle, cisplatin and four monofunctional platinum compounds. Pyri = pyriplatin, Phen = phenanthriplatin, Pt(dien) = Pt(diethylenetriamine)Cl, Pt(Et2dien) = Pt(N,N-diethyldiethylenetriamine)Cl. N = 1-6; ** $p < 0.01$.

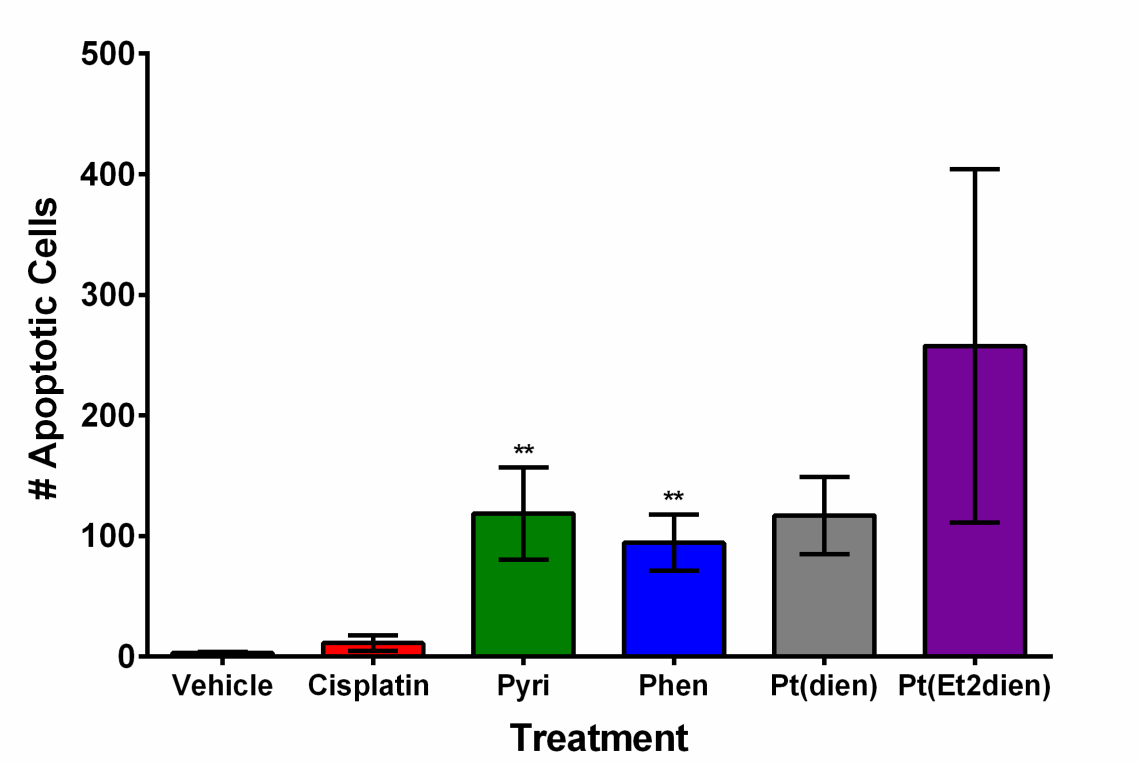

Figure 5: Both pyriplatin and phenanthriplatin treatment increases apoptosis in zebrafish utricles after 48 hours exposure. Data indicate the mean (± S.E.) number of apoptotic cells counted in utricular tissue treated with vehicle, cisplatin and four monofunctional platinum compounds. Pyri = pyriplatin, Phen = phenanthriplatin, Pt(dien) = Pt(diethylenetriamine)Cl, Pt(Et2dien) = Pt(N,N-diethyldiethylenetriamine)Cl. N = 2-6. ** $p < 0.01$.

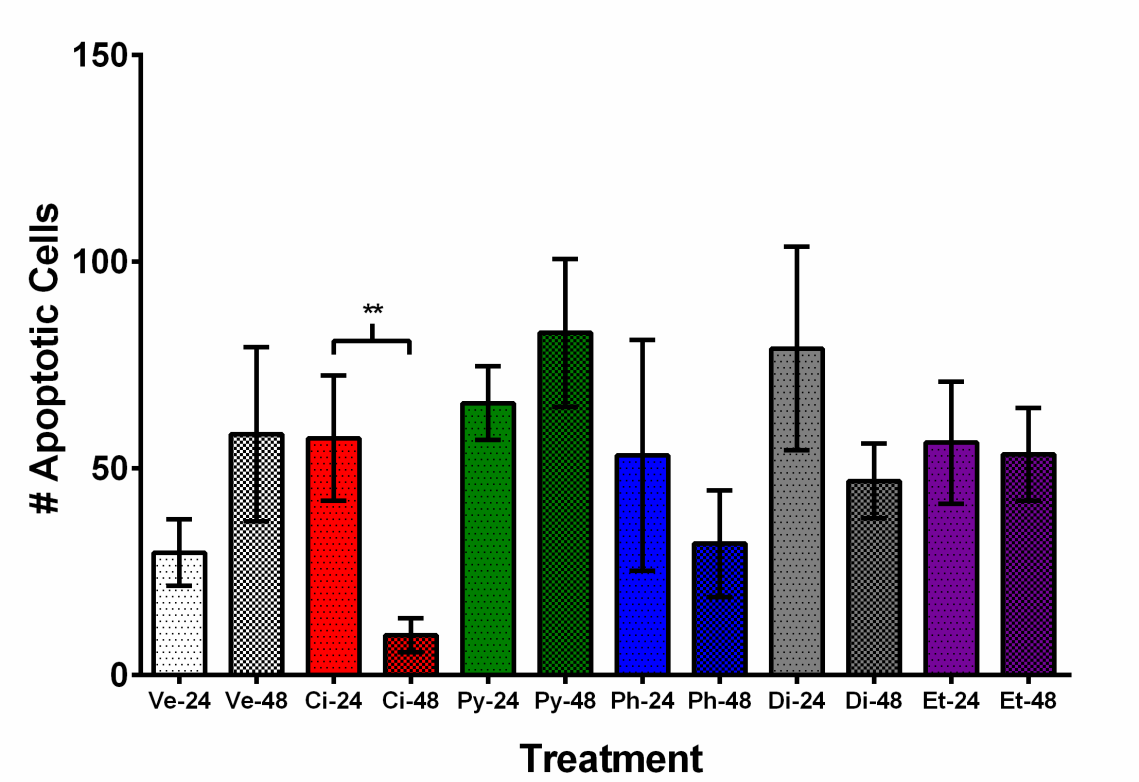

Figure 6: Comparison of 24- and 48-hour saccular apoptosis data for each treatment. Ve-24 = Vehicle at 24 hours, Ve-48 = Vehicle at 48 hours, Ci-24 = Cisplatin at 24 hours, Ci-48 = Cisplatin at 48 hours, Py-24 = Pyriplatin at 24 hours, Py-48 = Pyriplatin at 48 hours, Ph-24 = Phenanthriplatin at 24 hours, Ph-48 = Phenanthriplatin at 48 hours, Di-24 = Pt(diethylenetriamine)Cl at 24 hours, Di-48 = Pt(diethylenetriamine)Cl at 48 hours, Et-24 = Pt(N,N-diethyldiethylenetriamine)Cl at 24 hours, Et-48 = Pt(N,N-diethyldiethylenetriamine)Cl at 48 hours. ** $p < 0.01$.

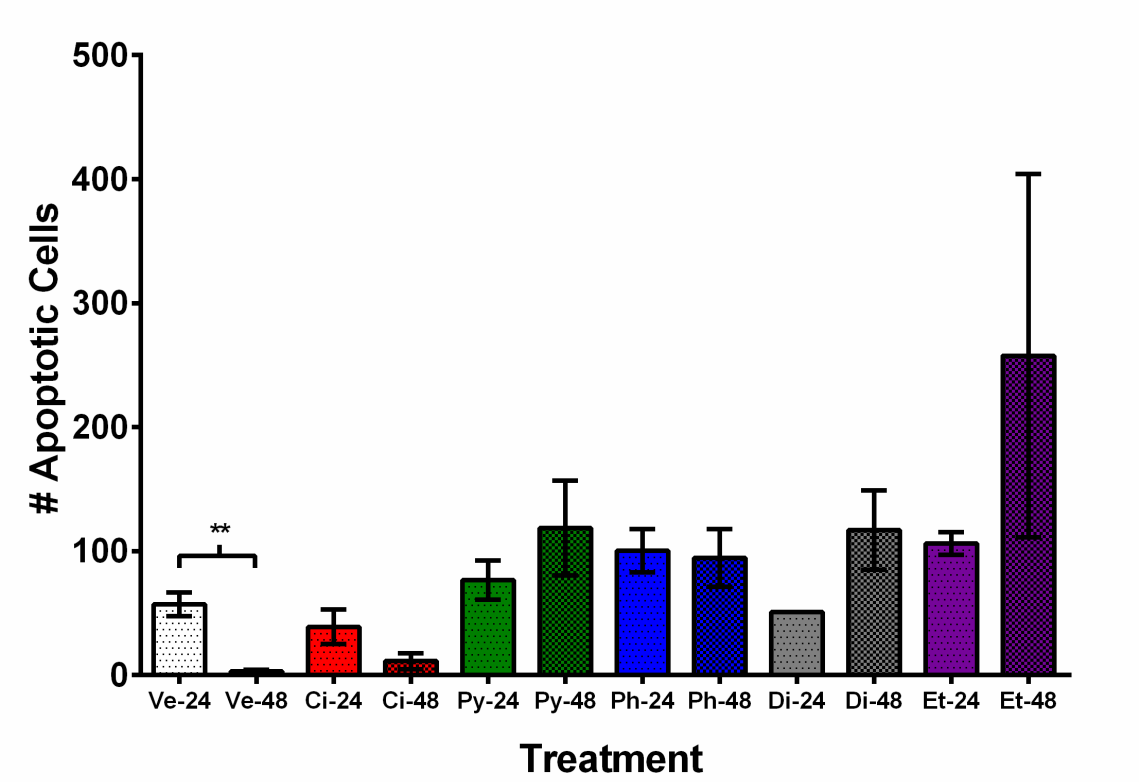

Figure 7: Comparison of 24- and 48-hour utricular apoptosis data for each treatment. Ve-24 = Vehicle at 24 hours, Ve-48 = Vehicle at 48 hours, Ci-24 = Cisplatin at 24 hours, Ci-48 = Cisplatin at 48 hours, Py-24 = Pyriplatin at 24 hours, Py-48 = Pyriplatin at 48 hours, Ph-24 = Phenanthriplatin at 24 hours, Ph-48 = Phenanthriplatin at 48 hours, Di-24 = Pt(diethylenetriamine)Cl at 24 hours, Di-48 = Pt(diethylenetriamine)Cl at 48 hours, Et-24 = Pt(N,N-diethyldiethylenetriamine)Cl at 24 hours, Et-48 = Pt(N,N-diethyldiethylenetriamine)Cl at 48 hours. ** $p < 0.01$.

Discussion

Platinum compounds that bind to DNA differently than cisplatin could cause reduced apoptosis in auditory tissue. Cisplatin has two leaving ligands that become aquated after entering cells, which enables the ligands to bind to both DNA strands (Jamieson et al. 1999; Knox et al. 1986; Cepeda et al. 2007). Binding to both strands causes DNA to bend and the subsequent recruitment of apoptotic proteins that activate cell death pathways and the termination of RNA transcription (Jamieson et al. 1999; Knox et al. 1986; Cepeda et al. 2007). Unfortunately, cisplatin typically targets non-cancerous cells, including auditory hair cells, where it can cause cell death by activating apoptotic mechanisms and releasing reactive oxygen species (Hellberg et al. 2009; Karasawa and Steyger 2015). Recently, monofunctional platinum compounds with only one leaving ligand have been proposed as chemotherapy drug candidates (Lovejoy and Lippard 2009; Park et al. 2012; Johnston et al. 2014). As these compounds have only one leaving ligand, they only bind one DNA strand and do not cause the DNA to bend (Park et al. 2012; Johnston et al. 2014). Instead, these monofunctional compounds are thought to cause apoptosis by blocking RNA polymerase II and preventing transcription (Park et al. 2012; Johnston et al. 2014). As monofunctional compounds do not cause DNA distortion like cisplatin, this suggests that they could modulate cancer cell cytotoxicity differently than their bifunctional counterparts. Specifically, monofunctional compounds could impair transcription more strongly in cancer cells, where mRNA production is upregulated, and have more limited impact on non-cancer cells, e.g., auditory hair cells, where fewer genes would be undergoing transcription.

Therefore, monofunctional compounds might produce fewer side-effects in auditory tissue than in cells treated with cisplatin.

Monofunctional platinum compounds that work by blocking transcription would be expected to produce less apoptosis in auditory tissue than cisplatin. However, our results suggest that the amount of apoptosis caused by these monofunctional compounds is not different than that of cisplatin. In saccules, cisplatin treatment at 24 hours produces a very similar mean number of apoptotic cells as the monofunctional compounds (Figure 2), and, surprisingly, at 48 hours, produces the lowest mean number of apoptotic cells of any treatment category (Figure 3). The low level of apoptosis for cisplatin observed in saccular samples at 48 hours may be due to sensory epithelial damage sustained during the dissecting process. Our utricle data at 24 hours shows that cisplatin treatment caused the lowest mean number of apoptotic cells and that $[Pt(Et_2dien)Cl]^+$ caused significantly more apoptosis than control (Figure 4). Similarly, our utricle data at 48 hours shows that cisplatin treatment again caused the lowest mean number of apoptotic cells and that both pyriplatin and phenanthriplatin caused significantly more apoptosis than control (Figure 5). These results could be interpreted to mean that both the monofunctional compounds and cisplatin cause auditory tissue apoptosis primarily by blocking mRNA transcription, and that cisplatin-modulated apoptosis from DNA distortion is negligible. Alternatively, it could be that the apoptotic mechanisms activated by cisplatin and the monofunctional compounds signal initially through distinct molecular components, but that subsequently these different signals become integrated into a common downstream pathway. This interpretation is suggested by research showing that zebrafish and mammalian ototoxins signal through

common molecular effectors, e.g., Bax, Bcl2, p38, p53 and cytochrome c (Coffin et al. 2013a,b; Shin et al. 2013). Therefore, cisplatin and monofunctional apoptotic signaling could proceed initially mainly through a common pathway or be integrated later in another pathway to produce similar apoptotic effects.

Cisplatin and the four monofunctional compounds can affect auditory tissue and function in distinct ways. Electron microscopy studies have shown that cisplatin can cause extensive damage to zebrafish inner ear tissue (Giari et al. 2012). Further, a recent study of these four monofunctional compounds and cisplatin found that at 48 hours all of the monofunctional compounds, with the exception of pyriplatin, caused significantly higher temporary threshold shifts than cisplatin (Monroe et al. 2018). This study also found that cisplatin reduced saccular hair cell density to a much greater extent than the monofunctional compounds, but that none of the compounds reduced hair cell density in the utricle (Monroe et al. 2018). As the saccule is the primary zebrafish auditory endorgan (Popper et al. 2003), our data suggests that the number of apoptotic cells are not well correlated with hearing threshold shifts associated with these compounds. Our utricle data is generally more in conformity with the results reported in Monroe et al. (2018), although pyriplatin, phenanthriplatin, and $[Pt(Et_2dien)Cl]^+$ were found to increase the number of apoptotic cells compared to control at one of the two time points (Figures 4-5). However, our control data set at 48 hours is difficult to interpret due to the presence of damage to the sensory epithelia of some of the samples. Taken together, our saccular and utricular data indicate that the mean number of apoptotic cells and hair cell density are not particularly correlated.

The apoptotic action of cisplatin and the monofunctional compounds could depend upon distinct time courses. Our results showed that apart from cisplatin treatment in the saccule and vehicle treatment in the utricle, where damage in the 48 hour sensory epithelial samples was present, all treatments caused similar levels of inner ear apoptosis at the 24- and 48-hour time points in both saccules and utricles (Figure 6-7). It is possible that peak apoptosis for either cisplatin or the monofunctional compounds occurs at a time point either before or after the 24 and 48 hour time points examined in this study. Cisplatin can cause hearing deficits in zebrafish after 48 hours exposure but not after only 24 hours exposure (Monroe et al. 2018). Similarly, goldfish (*Carassius auratus*) exposed to white noise for 48 hours experienced temporal shifts in apoptosis in the inner ear but these shifts differed by auditory endorgan. In the saccule, apoptosis was greatest immediately after the acoustical trauma, but in the utricle, apoptosis peaked 24 hours later (Smith et al. 2006). Since the saccule is thought to be the primary endorgan for hearing and the utricle the primary vestibular endorgan (Kwak et al. 2006), an acoustical stimulus might promote apoptosis sooner in the saccule than in the utricle. These studies suggest that drug-induced apoptosis could also follow different time courses in either the saccule or utricle. Our results indicate that cisplatin and the monofunctional compounds largely do not have this effect in either endorgan. However, it is possible that at other time points than the ones studied here, these compounds could begin to exhibit significantly lower or higher numbers of apoptotic cells.

In these experiments, we analyzed significance by quantifying and comparing the number of apoptotic cells observed in individual saccules and utricles. If these

values were instead reconsidered in terms of a percent of the total number of cells present in the sensory epithelia, it could be that apoptosis is an insignificant phenomenon at either 24 or 48 hours. This interpretation could mean that the auditory threshold shifts caused by the monofunctional compounds in Monroe et al. (2018) might be from a transient physiological effect and that recovery of auditory function could occur at a later time point. Additional study is required to evaluate whether cisplatin or the monofunctional compounds cause reversible or irreversible physiological damage to the saccular and utricular cells and the time point range in which significant apoptosis occurs.

In conclusion, only one of the compounds, pyriplatin, caused significant apoptosis in the saccules of zebrafish at either time point, and $[Pt(Et_2dien)Cl]^+$, pyriplatin, and phenanthriplatin caused significant apoptosis in the utricles at one of the two points. However, this conclusion is somewhat tentative as some of the treatments require additional data from higher quality samples which would allow us to perform a more powerful analysis. This information is particularly needed for the cisplatin 48-hour saccule and vehicle 48-hour utricle data sets. Evidently, additional investigation of how cisplatin and these monofunctional compounds function temporally, mechanistically and physiologically is needed. Acquiring this information should allow a more precise determination of the relationships between apoptosis, hair cell density and auditory function in the zebrafish inner ear model during platinum(II) compound treatment. These future studies should allow us to establish whether these monofunctional compounds can act as effective chemotherapy agents with reduced ototoxicity.

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