Title: Effects of Dexamethasone on Cisplatin Ototoxicity In Vitro

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Background

Cisplatin is a chemotherapeutic agent used in the treatment of solid organ tumors such as those seen in head and neck, ovarian, testicular, cervical, and lung malignancies. This drug can produce dose-limiting, bilateral, and often irreversible hearing loss in a majority of patients. Currently, there is no treatment for the prevention of cisplatin ototoxicity, which occurs in 75-100% of patients that receive cisplatin chemotherapy\(^1\). The molecular mechanisms associated with cisplatin ototoxicity include generation of reactive oxygen species, activation of NAPDH oxidase 3 (NOX-3), and production of inflammatory cytokines such as TNF\(\alpha\), which can initiate caspase activation, breakdown of DNA, and apoptosis of auditory HCs\(^1\). There are several therapeutic drugs that can protect against cisplatin-induced hearing loss; however, these agents are given systemically and can interact with the chemotherapeutic properties of cisplatin and affect cancer survival.

Steroid use has been shown to protect against ROS, NAPDH oxidase, TNF\(\alpha\) production, and programmed cell death in other inner ear disorders and is a promising agent for the treatment of cisplatin ototoxicity\(^2,3\). Dexamethasone (DXM) is a glucocorticoid that has been used as a local intratympanic (IT) agent with minimal local/systemic side effects and can potentially protect against the pro-inflammatory and pro-apoptotic effects of cisplatin in the inner ear. Due to its molecular properties and size, it can diffuse through the round window membrane into the inner ear and potentially block the ototoxic effects of cisplatin without systemic side effects.

The effects of DXM on cisplatin-injured auditory hair cells (HC) have not been previously studied on a molecular level. There are only a few published studies of small sample size that suggest DXM can abrogate hearing threshold shifts in high frequencies following cisplatin exposure in vivo, and there is only one human clinical trial published that demonstrates some protection with DXM\(^4-11\). This in vitro study performed in rat organ of Corti (OC) explants provides some scientific molecular background and evidence to support further research on the effects of intratympanic DXM for the prevention of cisplatin ototoxicity in human clinical trials.

Hypothesis

Central Hypothesis: DXM treatment protects against cisplatin-induced loss of auditory HCs in vitro by reducing oxidative stress, NOX-3, and TNF expression levels.

Specific Aim 1: Examine the effects of cisplatin on oxidative stress, NOX-3, TNF, HC viability and apoptosis in rat OC explants in vitro
**Specific Aim 2:** Examine the effects of DXM on oxidative stress, NOX-3, TNF, HC viability and apoptosis in cisplatin-injured rat OC explants in vitro

**Study and Results:**

OC explants obtained from 3-day old rats were cultured with no treatment or various concentrations of cisplatin (2, 5, 10 µM) and DXM (75, 150, 300 µg/ml) for 48 or 96 hrs in vitro. HC viability and TUNEL assay for apoptosis levels were performed after 96 hrs in vitro. Expression levels of oxidative stress, NOX-3, and TNF were examined with confocal microscopy after 48 hrs in vitro. Six OC were used for each experiment and treatment condition. ANOVA and Tukey post hoc testing were used to analyze the results.

Cisplatin initiated a dose-dependent loss of outer HCs in the basal turn of the OC explant; however the outer HCs of the middle and apical turns were not significantly affected except at 10 µM cisplatin (p < 0.05) (Figure 1). The inner HCs were resilient to the effects of cisplatin in all turns of the cochlea except at the highest dose of cisplatin (10 µM, p < 0.05) (Figure 2). Lower dose DXM (75 µg/ml) did not protect against cisplatin induced inner or outer HC loss (Figure 3); however, DXM protected against cisplatin (2 µM) initiated outer HC loss of the basal turn in a dose-dependent manner with complete protection achieved at 300 µg/ml DXM (Figure 4).

![Graph](image)

Figure 1. Cisplatin initiated a dose-dependent loss of auditory HCs in vitro primarily in the basal turn of the cochlea. *p<0.05
Figure 2. Inner HCs are resilient to cisplatin ototoxicity in vitro in all turns of the cochlea except at a high dose (10 µM). *p<0.05

Figure 3. Low dose DXM (75 µg/ml) did not protect against cisplatin ototoxicity in vitro.
Figure 4. DXM protected against cisplatin induced HC loss in vitro in a dose dependent manner with complete protection seen at 300 µg/ml DXM. *p<0.05

OC explants exposed to cisplatin (2 µM) were associated with higher levels of oxidative stress, NOX-3, TNF, and apoptosis in the basal turn compared to control explants. DXM (150 µg/ml) treatment significantly reduced cisplatin induced increases in oxidative stress, NOX-3, TNF, and apoptosis in the basal turn at 48 hrs in vitro (Figure 5, select images shown).
Figure 5. DXM abrogates cisplatin induced oxidative stress, NOX-3, TNF, and apoptosis (conglomerates of pink) in the auditory HCs of the basal turn of rat OC explants in vitro. Blue represents cell nuclei.

The results of this study show that DXM protects against cisplatin ototoxicity in vitro by reducing levels of oxidative stress, NOX-3, and TNF that promote inflammation and apoptosis of auditory HCs important for hearing.

**Significance:**
Systemic steroids remain the mainstay of treatment for many disorders of the inner ear but this class of drugs can induce apoptosis resistance of tumor cells toward cancer therapy and have limited utility in cancer patients receiving cisplatin\textsuperscript{12}. DXM can be administered as a local IT injection through the tympanic membrane into the middle ear, where it can diffuse through the round window membrane and into the cochlea without systemic effects. IT DXM has demonstrated protective effects against cisplatin induced hearing loss in some in vivo animal studies and one human trial; however, levels of protection with DXM vary significantly between studies\textsuperscript{4-11}. Our study demonstrated complete protection against cisplatin induced auditory HC loss only with higher doses of DXM. DXM can abrogate HC losses by reducing cisplatin induced up regulation of oxidative stress, NOX-3, TNF, and apoptosis. One major reason for the variability in published data on IT DXM against cisplatin ototoxicity is the amount of DXM that actually traverses the round window membrane and enters the inner ear to exert its effects. Our results support further research in IT DXM against cisplatin induced hearing loss as well as other modalities to improve deliver of DXM into the cochlea.

**Plan:**
High doses of DXM significantly protect against cisplatin ototoxicity in vitro. Variability in the effects of IT DXM on cisplatin induced hearing loss in vivo and in human trials may be due to dosage, duration, and degree of passive diffusion into the inner ear. Future studies consist of studying other mechanisms behind cisplatin induced death of auditory HCs, whether DXM can protect against other pro-inflammatory and pro-apoptotic pathways associated with cisplatin ototoxicity, combination drug regimens that may better inhibit these pathways, and the development of different modalities for DXM delivery into the inner ear (such as DXM-eluting nanoparticles that are biodegradable, deliver prolonged levels of steroid in the cochlea, and can be directly inserted into the inner ear). The effects of cisplatin and DXM on the supporting cells of the OC are also important to investigate as well.

The results of this study will be submitted as a poster presentation to the American Otologic Society to be presented at the Combined Otolaryngological Spring Meeting in April of 2015. Findings will be submitted as a manuscript to the associated journal (Otology & Neurotology).
References:
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