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What is This?
Targeted Amelioration of Cisplatin-Induced Ototoxicity in Guinea Pigs

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract
This pilot study compared otoprotection provided by trans-tympanic formulations and systemic intraperitoneal administration of L-N-acetylcysteine from cisplatin-induced cochlear oxidative stress. Protection was assessed by measures of hearing loss and cochlear glutathione levels. All groups received an equivalent single dose of L-N-acetylcysteine followed by cisplatin.

Cisplatin was administered subcutaneously for 3 days (5.5 mg/kg/day). Two hours prior to day 1 cisplatin, L-N-acetylcysteine was administered either intraperitoneally (250 mg/kg), trans-tympanic as 2% L-N-acetylcysteine in gel, or trans-tympanic as L-N-acetylcysteine–loaded nanocapsules in gel. Hearing was assessed prior to and 3 days after cisplatin followed by microdissection of cochlear tissue. The levels of reduced (GSH) and oxidized (GSSG) glutathione in homogenized tissue supernatants were determined via luminometry.

Intraperitoneal L-N-acetylcysteine administration preceding cisplatin resulted in less hearing loss and a higher GSH/GSSG ratio than either trans-tympanic formulation. This suggests that for equivalent doses of L-N-acetylcysteine, systemic rather than targeted cochlear delivery provides increased otoprotection from cisplatin ototoxicity.

Keywords
otoprotection, drug delivery, ABR, L-N-acetylcysteine, nanocapsules, trans-tympanic, systemic, glutathione, ROS

Introduction
Cisplatin, a chemotherapeutic agent, induces cell death by binding to DNA up-regulating reactive oxygen species (ROS), therefore depleting endogenous antioxidants, ultimately leading to apoptosis.1 As free radicals accumulate, cochlear oxidative damage ensues, resulting in ototoxicity.1,2

The ototoxic potential has sparked the search for otoprotective agents that may limit the impact of cisplatin at a cochlear level. A promising agent is the antioxidant L-N-acetylcysteine (LNAC), which has been shown to protect auditory neurons and hair cells from cisplatin ototoxicity in vitro and in vivo.2,3 The low molecular weight of LNAC allows it to permeate the round window, giving the option of trans-tympanic (TT) administration; however, maintaining adequate concentrations of LNAC in the middle ear while using a liquid suspension has proven to be a clinical dilemma.2,3 Nanocapsules have proven to be an effective vehicle for drug delivery to the inner ear, and a gel suspension would theoretically maintain higher LNAC concentrations in the middle ear for a longer duration.4-9 Systemic administration of LNAC has been shown to provide otoprotection without limiting the chemotherapeutic efficacy of cisplatin.10,11

No prior study has compared TT and systemic delivery of LNAC. This pilot project aimed to determine which LNAC delivery method resulted in less hearing loss and a higher GSH/GSSG ratio.

Methods
Subjects/Drug Administration
Young Hartley female albino guinea pigs (GP, about 300 g, n = 45) divided into 9 groups received LNAC and/or cisplatin (Figure 1). The Saint Louis University Institutional Animal Care and Use Committee approved this study.

Auditory Brainstem Response
Hearing loss (HL) was assessed before and 3 days after cisplatin treatment. Some GPs in the LNAC-NC group underwent additional testing at 1 and 3 weeks post treatment. Auditory brainstem response (ABR) waves were recorded in

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anesthetized GPs (ketamine, 40 mg/kg, ip) from tone pips (2, 4, 8, 16, and 20 kHz) using the ABR workstation (Tucker Davis Technologies, Alachua, Florida). The lowest intensity required to elicit a characteristic waveform defined threshold.

**Tissue Collection**

After ABR testing, cochlear tissue including the modiolar portion of the eighth nerve was microdissected in Hanks Buffered Salt Solution. Cochlear tissue from each temporal bone was individually flash-frozen and stored (–70°C) until assay.

**Glutathione Assay**

The cochlear tissue was resuspended (200 μl PBS-EDTA, pH 7.4), micro-homogenized, and pelleted. The supernatant was assayed for reduced (GSH) and total (GSHt) glutathione using the GSH-Glo Glutathione Assay (Promega, Madison, Wisconsin). Oxidized glutathione (GSSG) levels were calculated: (GSSG = [GSHt – GSH]/2). Cochlear glutathione levels were referenced to protein (BCA assay, Pierce, Fisher Scientific, Waltham, Massachusetts).

**Statistics**

Statistical analysis was performed using SigmaStat (SYSTAT Software, San Jose California). Group differences were evaluated using ANOVA, with post hoc analysis (Holm-Sidak pairwise multiple comparisons) or Kruskal-Wallis followed by a post hoc Bonferroni-Dunn test. Significance was set as \( P \leq .05 \).

**Results**

Significantly less HL occurred in the Cisplatin-LNAC IP group (Table 1). Low frequency threshold elevations in the Cisplatin-LNAC TT and Cisplatin-LNAC NC groups are most likely from gel in the middle ear since low frequency thresholds in the LNAC NC subgroup tested beyond 3 days post cisplatin returned to baseline 1 week post TT administration (data not shown).

The Cisplatin-LNAC IPo combination induces a significant elevation in GSH levels (Kruskal-Wallis, \( H = 29.047, df = 5, P \leq .001 \)). The GSH/GSSG ratio in this group is 2-fold greater than other cisplatin-treated groups and more than 5-fold above the control and LNAC-only groups (Figure 2).

**Discussion**

Concerns that systemic administration of antioxidants might limit antitumor efficacy have encouraged research into local delivery methods.\(^3\)\(^,\)\(^11\) TT delivery of LNAC has proven to be difficult in the clinical setting due to inflammation of the middle ear for concentrations exceeding 2% and the drainage of the liquid formulation down the Eustachian tube.\(^3\)\(^,\)\(^12\)\(^-\)\(^15\) The obstacles encountered with TT delivery of liquid suspensions of LNAC and recent research reporting no significant decrease in antitumor efficacy from systemic antioxidant supplementation prompted this investigation of alternative methods of administration.\(^11\)

LNAC delivery methods were assessed using the ABR and an assay of the GSH/GSSG ratio 3 days after cisplatin administration. Otoprotection was determined from higher frequency threshold shifts only because tympanic membrane perforation and residual gel in the middle ear within the LNAC-TT groups contributed a conductive component to the lower frequency hearing loss. The 16 and 20 kHz data revealed that GPs pretreated via the LNAC-IP method had significantly better hearing than the other groups. Supplementing the physiologic finding were increased levels of glutathione and a higher GSH/GSSG ratio in this group,
lending support to the theory that oxidative stress is the mechanism for cisplatin-induced hearing loss. Combined, the ABR and glutathione assay data suggest that systemic delivery of LNAC (250 mg/kg) provided protection against cisplatin ototoxicity more effectively than either TT method. Administration of LNAC TT whether loaded in nanocapsules or in a gel suspension provided limited otoprotection. This was possibly due to the single dosing, the 2-hour time interval between LNAC and cisplatin dosing, insufficient LNAC round window absorption, or poor LNAC formulation bioavailability that resulted in inadequate cochlear concentrations of LNAC. Future directives include histological studies that demonstrate LNAC-loaded nanocapsules penetrate the inner ear, analysis of LNAC concentration in the perilymph, and studies that titrate systemic LNAC dosing upwards.

Conclusion

In this first comparison of local and systemic LNAC administration, systemic LNAC preceding cisplatin resulted in less hearing loss and a higher GSH/GSSG ratio than either TT formulation. Further study of the cochlear and systemic LNAC/cisplatin pharmacokinetics is needed to determine the administration formulary, route, and dosage of LNAC that provides maximal otoprotection in a clinically efficient model.

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Author Contributions

Shaulnie Mohan, auditory brainstem response, microdissection, analysis, presentation; Brendan J. Smyth, glutathione and protein assay, analysis, presentation; Arya Namin, auditory brainstem response, drug administration, presentation; Grady Phillips, sacrificed guinea pigs, drug administration, presentation; Michael Anne Gratton, design, analysis, presentation.

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References


