Monitoring ototoxicity in drug development: Robust *in vitro* & *ex vivo* models

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INTRODUCTION AND OBJECTIVES

Ototoxicity is a leading cause of acquired hearing loss and more than 200 drugs on the market induce ototoxicity. Ototoxicity testing programs should be adapted to the type of therapy, its indication (targeting the ear or potentially ototoxic), and the number of assets to test. For multiple molecules and/or multiple concentrations, screening options are available: *in vitro* (otic cell assays) and *ex vivo* (cochlear explant). Besides assessing the ototoxicity of a drug, it may also be useful to compare its ototoxicity to that of a well-known drug of a similar class. Screening assays provide a streamlined and rapid method to assess whether a drug is safe for inner ear structures. Here, we describe the establishment of robust *in vitro*, and *ex vivo* **cisplatin** models to rapidly evaluate ototoxicity of drugs and chemical compounds.

METHODS

1 - *In vitro*

HEI-OC1 otic cells were exposed to 5 different concentrations of cisplatin, a sham group was treated only with the vehicle, and one group was untreated. Cell metabolism (MTT assay) and cell numbers (CCK8 assay) were evaluated. A reference compound, N-acetylcysteine (NAC), was used as a positive control, testing for Caspase 3/7 activity and ROS production.
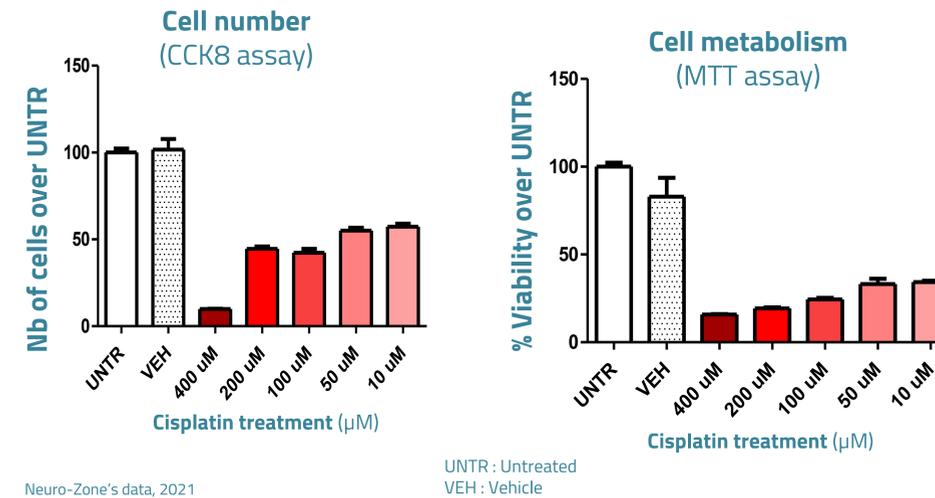
2 - *Ex vivo*

Rodent cochlear explants were incubated and treated with 5 µM of cisplatin (same as used in clinical setting; Viatris 400005334) and for different exposure durations, then fixed and labeled with Myosin 7a and phalloidin. The extent of damage inflicted on the Organ of Corti was evaluated with hair cell quantification and a disorganization score.

CONCLUSIONS
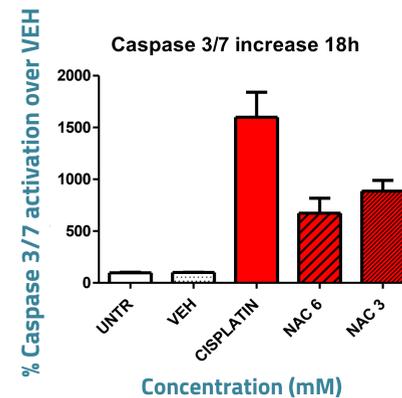
In the **HEI-OC1 model**, a concentration of 100 – 200 µM of cisplatin induced adequate cytotoxicity and demonstrated a clear dose effect on the survival of the HEI-OC1 cells as well as on the cell's metabolic activity. The ototoxic effect of cisplatin was partially rescued by the antioxidant NAC.
In the **cochlear explant model**, the strongest effect both on hair cell loss and disorganization was observed with 9h of cisplatin exposure, with similar results after 16h of exposure.
We successfully established robust, rapid, and cost-effective cisplatin-based *in vitro* and *ex vivo* models for ototoxicity screening. Their implementation early in the drug development process can **reduce the risk of late-stage auditory safety concerns** and supports **better selection of compounds and doses** before initiating costly GLP-compliant ototoxicity studies.

RESULTS

1 - *In vitro*

Cisplatin induced significant HEI-OC1 cell death

Cell number (CCK8 assay) — Nb of cells over UNTR; Cisplatin treatment (µM): UNTR, VEH, 400 µM, 200 µM, 100 µM, 50 uM, 10 uM

Cell metabolism (MTT assay) — % Viability over UNTR; Cisplatin treatment (µM): UNTR, VEH, 400 µM, 200 µM, 100 µM, 50 uM, 10 uM

Neuro-Zone's data, 2021

UNTR : Untreated
VEH : Vehicle

NAC partially rescued caspase activation in a dose-dependent manner

Caspase 3/7 increase 18h — % Caspase 3/7 activation over VEH; Concentration (mM): UNTR, VEH, CISPLATIN, NAC 6, NAC 3

Neurozone's data

NAC completely abrogated ROS production

ROS production after 24h — % ROS increase over VEH; Concentration (mM): UNTR, VEH, CISPLATIN, NAC 6, NAC 3

[Cisplatin]= 200 µM

2 - *Ex vivo*

Exposure to 5 µM cisplatin for 9h or 16h, but not for 6h, induced a significant increase of hair cell death, while the disorganization score was increased in all treated groups.

Control, Cisplatin (6h), Cisplatin (9h), Cisplatin (16h); Apex, Middle, Base

Myosin 7a
Phalloidin
VGlut3

HC density — HCs/µm Mean +/- SEM; 1- Sham (n=8); 2- Cisplatin 6H (n=6); 3- Cisplatin 9H (n=6); 4- Cisplatin 16H (n=6); Apex, Middle, Base

HC disorganization score — 1- Sham (n=8); 2- Cisplatin 6H (n=6); 3- Cisplatin 9H (n=6); 4- Cisplatin 16H (n=6); Apex, Middle, Base