# AN IN-VITRO SCREENING MODEL FOR OTOTOXIC COMPOUNDS

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## INTRODUCTION

More than **600 categories of drugs** have the potential to cause **ototoxicity** (WHO 2022). Some commonly used medications can seriously affect the auditory system and lead to **permanent hearing loss.**
The most commonly used in clinical practice are **chemotherapeutics** (cisplatin) and **aminoglycoside** antibiotics (such as gentamicin) of which the incidence in causing ototoxicity is estimated to reach up to 60% and 47% respectively. Loop diuretics, quinine-based medications, NSAIDs, and **excipients** such as **cyclodextrin** derivatives, are also known as ototoxic substances.
Development of novel drugs and formulations should include **auditory safety assessments** to prevent hearing loss. The aim of these studies was to provide an efficient and rapid screening method for ototoxic compounds.

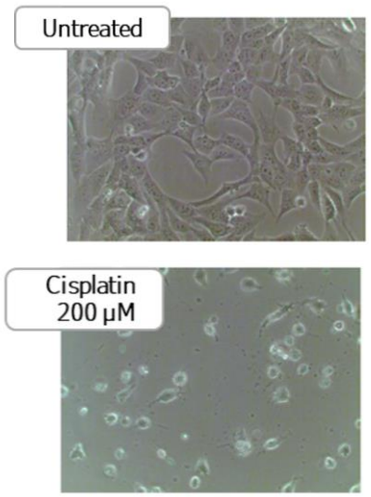
## METHODS

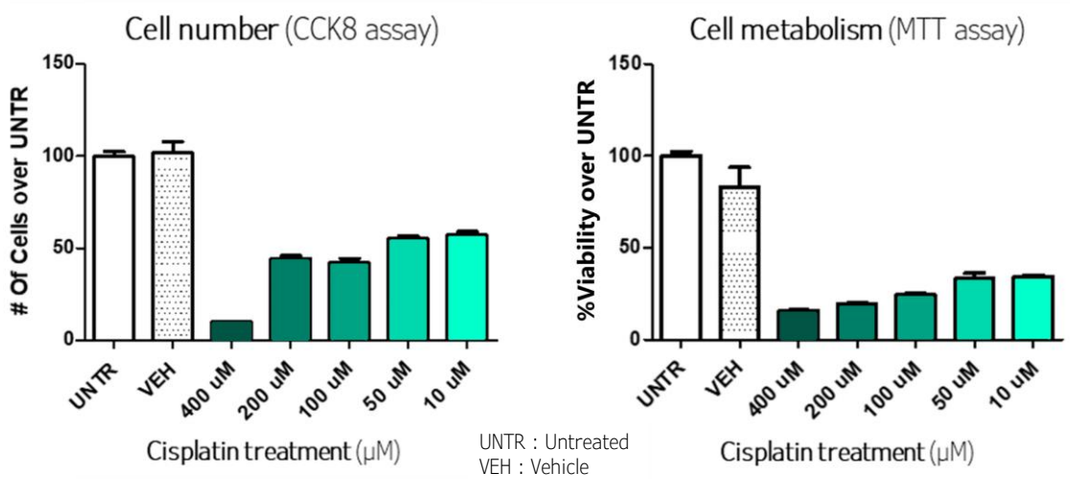
The immortalized **mouse cell line** HEI-OC1 derived from the mouse's organ of Corti was used (Kalinec et al., 2003). These cells are derived from early stage (post-natal day 7) mouse cochleae and express **specific markers** including those of **cochlear sensory cells** and **supporting cells.**

Cells were treated with different concentrations of cisplatin and evaluated with the MTT assay for cell metabolism and CCK8 assay for cell numbers to select the optimal cisplatin dose.

Additional assays for apoptosis, with Annexin, caspase-3/7- and -8 activities, and for ROS production were also performed, and validated with a reference otoprotective compound, N-Acetylcysteine (NAC).

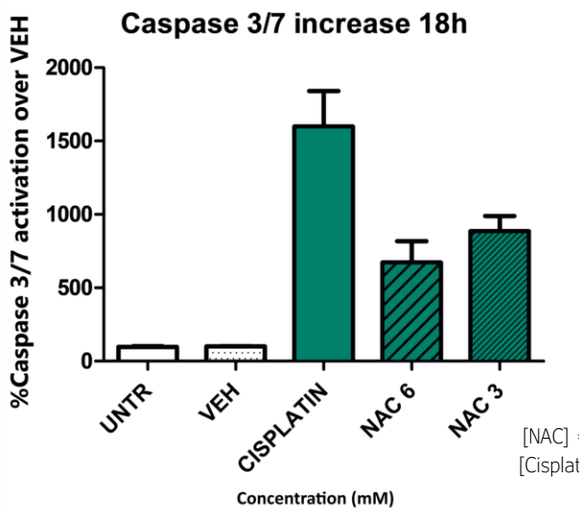
## RESULTS



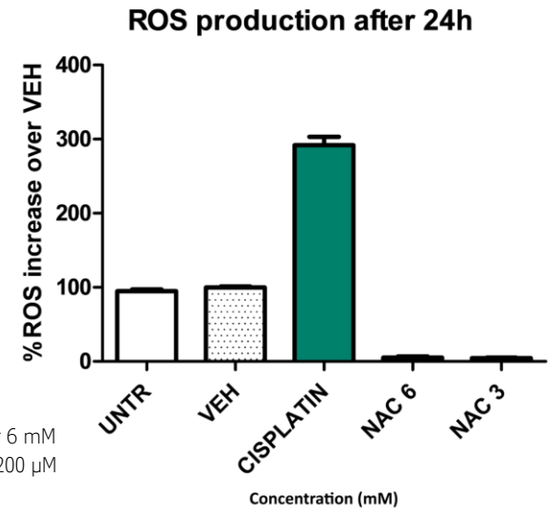




UNTR : Untreated
VEH : Vehicle

Cisplatin induced significant cell death, with a dose-effect ranging from 400 µM to 10 µM. The dose of 200 µM was chosen for further experiments. In the Annexin assay, cisplatin did not induce necrosis after 24h but only apoptosis (data not shown).




[NAC] = 3 or 6 mM
[Cisplatin]= 200 µM




- Cisplatin 200 µM activated both caspase 3/7 and caspase 8 with the strongest activation for caspase 3/7 and induced significant ROS production.
- NAC partially prevented caspase activation in a dose-dependent fashion and completely abrogated the ROS production.

CONCLUSION: This cochlear cell line allows to study and screen the potential ototoxicity of a large library of drug candidates in a short timeframe. Compounds can be assessed, and their ototoxicity compared to a reference agent such as cisplatin. This reliable and high throughput assay can provide some insights into the mechanism of action of drug candidates with the activation of apoptosis signaling pathways.