ABSTRACT SUBMISSION FOR ICT 2022

Category T14 In-vitro methodologies & screening

Presenter: Marie-Pierre Pasdelou

An in-vitro screening model for ototoxic compounds

Purpose: 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 practices are chemotherapeutics (cisplatin) and aminoglycoside antibiotics (such as gentamicin) of which the incidence in causing ototoxicity is estimated to 23-50% and 63% 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: 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. Furthermore, cisplatin 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 insight into the mechanism of action of drug candidates with activation of apoptosis signaling pathways.

Keywords: Ototoxicity, Auditory Safety, HEI-OC1, cisplatin, in vitro screening

Full author list : Marie-Pierre Pasdelou, Lise BYELYAYEVA, Marie PEYTAVY, Susanna Malmström, Silvia Biggi.

References:

- World report on hearing. Geneva: World Health Organization; 2021. Licence: CC
- BY-NC-SA 3.0 IGO.
- Kalinec, F. et al.,: Audiol Neurootol 2003;8:177–189

ABSTRACT SUBMISSION FOR ICT 2022

Category T04: Biomarkers of adverse effects

Presenter: Lise Byelyayeva

Hearing loss: side-effects of multiple compounds and formulations which can be monitored with functional and histological read-outs

Purpose: Hearing loss is a major global health issue affecting around 1.5 billion people worldwide, with an increasing prevalence. Acquired hearing loss is attributed to different environmental factors including ageing, noise exposure, and the intake of ototoxic medicines. Some commonly used medications can considerably affect the auditory system, resulting in cochlear and central damage that can lead to permanent hearing loss. More than 600 classes of medications are ototoxic. The most used in clinical practice are chemotherapeutics (cisplatin) and aminoglycoside antibiotics (such as gentamicin). Some investigated drugs for Covid-19 treatment (hydroxychloroquine, HCQ) and certain drug delivery agents like cyclodextrins (CD) have also been reported to induce auditory side effects. The aim of these *in vivo* studies was to provide functional and histological data on auditory assessments related to cisplatin, gentamicin, HCQ, and CD, when administered similarly to clinical protocol.

Methods: The studies were conducted in Wistar rats and Albino guinea pigs:

- **Cisplatin** was administered by intraperitoneal route at 10 mg/kg in rats
- **Gentamicin** was administered by intramuscular route at 160 mg/kg for 5 days in rats
- **HCQ was** administered at 62 mg/kg per os daily for five days in rats
- A **cyclodextrin**-based formulation was administered by transtympanic route at 4 mg/mL 1h and 30h after noise exposure in guinea pigs

Hearing was assessed using the techniques of Distortion Product Otoacoustic Emissions (DPOAE) and Auditory Brainstem Responses (ABR) at several timepoints. DPOAE are acoustic signals created and amplified by the cochlear epithelium and measured in the ear canal. DPOAE reflect the activity of outer hair cells (OHC). ABR is an electrophysiological measure of the sensorineural activity of the auditory pathway from the cochlea to the central auditory structures in response to a sound stimulus, recorded as electric potentials from scalp electrodes. A cochleogram, an FDA-recommended histological analysis for hair cell counting, was performed at the end of certain studies.

Results: Results based on ABR thresholds, DPOAE amplitudes, and the cochleogram, showed different patterns of auditory side-effects. Cisplatin induced immediate and permanent hearing loss; gentamicin displayed delayed side-effects on auditory measures; HCQ did not affect Outer Hair Cells but might have had an effect on neurons. CD had an immediate and prolonged effect on hearing.

Conclusion: This short presentation will help you learn the current available methods to measure hearing in preclinical *in-vivo* trials using two complementary functional read-outs and a histological analysis, and to determine the different sites of damage.

Keywords: Ototoxicity, Hearing loss, cochleogram, Auditory assessment, ABR, DPOAE

Full author list: Lise BYELYAYEVA, Susanna MALMSTRÖM, Gaëlle NAERT, Sylvie PUCHEU, Marie PEYTAVY, Marie-Pierre PASDELOU

- Gentamicin administered by Intraperitoneal route at 160 mg/kg daily for 15 days in Long Evans Rats