DEVELOPMENT OF A CISPLATIN-INDUCED HEARING LOSS (CIHL) RAT MODEL FOR PRECLINICAL EFFICACY ASSESSMENT

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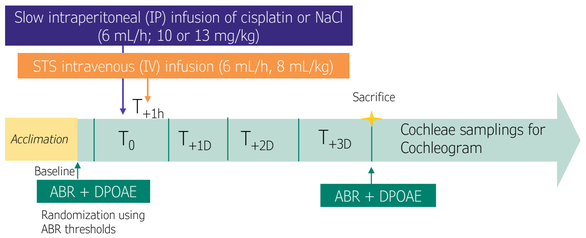
OBJECTIVES

Cisplatin is an antineoplastic drug widely used in the treatment of many cancers. However, its use is often associated with serious side effects, such as a nephrotoxicity and progressive and irreversible hearing loss.
The development of experimental animal models is essential in understanding the mechanisms of cisplatin's ototoxicity and to develop effective treatments. In animals, a single-bolus injection of cisplatin can result in high mortality rates and/or health issues, making it difficult to assess potential therapies.

The goal of these experiments was to develop an acute rat model of cisplatin-induced ototoxicity in a short time frame, to mimic the effects observed in patients. Sodium thiosulfate (STS), a cisplatin chelator under evaluation by the FDA for Cisplatin therapy, was used as an otoprotective reference agent to reverse cisplatin-induced hearing loss in this model.

METHODS

STUDY SCHEME



Study Groups: Male Wistar rats (Janvier Labs) were randomly divided into four groups, receiving single IP infusion at T_0 : vehicle group (NaCl) (group 1), Cisplatin 10 mg/kg group (group 2), Cisplatin 13 mg/kg group (group 3) and Cisplatin 10 mg/kg + STS (2000 mg/kg, IV infusion once at T_{+1HOUR}) group (group 4).
To limit health issues, specific care (hydration, food supplementation) was provided to cisplatin-treated animals.

At baseline (T_0) and T_{+3DAYS}, were measured on both ears:

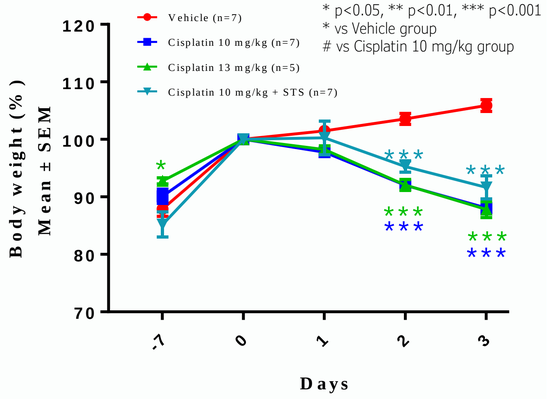
- **DPOAE** (one animal/one ear at a time) at 4, 8, 16, 24 and 32 kHz at an intensity of 63 dB.
- **ABR** (eight animals/one ear) at the same time points at 5 frequencies: 4, 8, 16, 25 and 32 kHz. The signals were amplified (gain 10 000, band pass 100-5000 Hz). The stimuli consisted of tone pips (1 ms linear rise/fall time) presented in 10 dB steps from 90 to 0 dB.

Histological analyses (IHC and OHC count) were performed on cochleae from Groups 1, 2 and 4. At T_{+3DAYS} after sampling, cochleae were fixed overnight in 4% PFA and then microdissected. Hairs cells were immunolabeled with an anti-Myosin-VIIa antibody and images were acquired with a confocal microscope. Hair cell counts were performed using ImageJ.

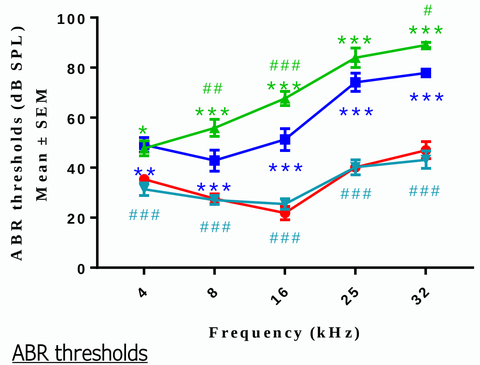
RESULTS

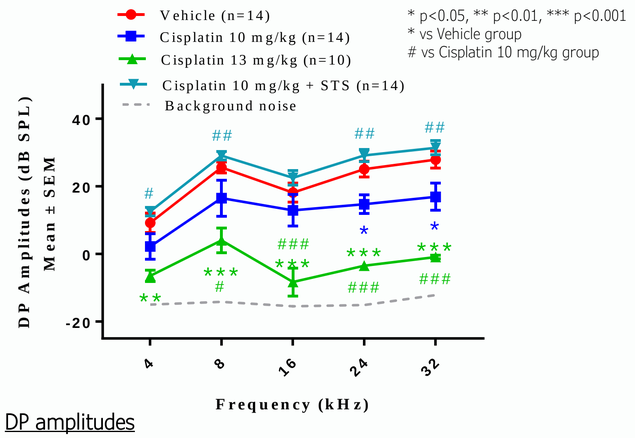
BODY WEIGHT & CLINICAL SIGNS

Although all animals treated with cisplatin exhibited similar and significant body weight loss from T_{+2DAYS}, the Cisplatin 13 mg/kg group suffered severe health issues such as diarrhoea, piloerection, anorexia and dehydration. In the Cisplatin 10 mg/kg treated groups, reduced mobility and piloerection were observed in most animals, but globally no major health issues were noted.



AUDITORY FUNCTIONS AT T_{+3DAYS}





<u>ABR thresholds</u>

Cisplatin 13 & 10 mg/kg : Significant increase at all frequencies compared to vehicle group. Thresholds were higher in 13 mg group than in 10 mg group (89 vs 78 dB SPL at 32 kHz).

Cisplatin 10 mg/kg + STS: ABR thresholds between 25 & 43 dB SPL: normal hearing.

<u>DP amplitudes</u>

Cisplatin 13 mg/kg:
Significant decrease at all frequencies compared to vehicle group. Amplitudes significantly lower than in 10 mg/kg group (-0.9 vs 16.9 dB SPL at 32 kHz).

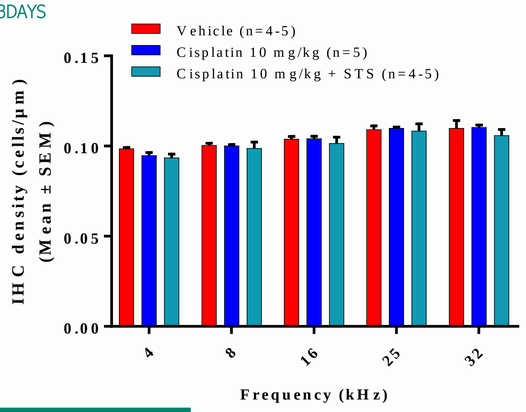
Cisplatin 10 mg/kg: Significant decrease at 24 & 32 kHz compared to vehicle group.

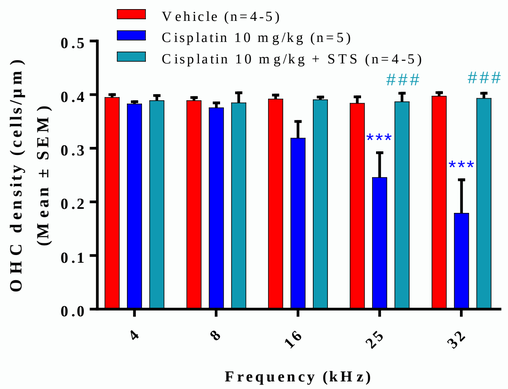
Cisplatin 10 mg/kg + STS: DP amplitudes between 12.6 & 31.4 dB SPL: normal hearing.

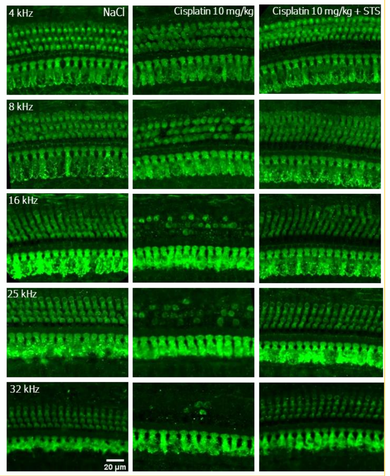
CYTOCOCHLEOGRAM AT T_{+3DAYS}

Cisplatin 10 mg/kg:
No IHC loss at any frequencies, but significant OHC loss at 25 & 32 kHz compared to vehicle group (>50 % at 32 kHz).

Cisplatin 10 mg/kg + STS:
No IHC & OHC loss at any frequency.







CONCLUSION

Both doses, 10 and 13 mg/kg, induced significant hearing loss, demonstrated by a substantial increase of ABR thresholds and a decrease of DPOAE amplitudes. However, health issues were observed in animals at the higher dose, while animals treated with the lower dose showed no clinical signs. Therefore, no further analyses were performed on the 13 mg/kg treated group, and the 10 mg/kg treated group was selected. In this group, a loss of outer hair cells (OHC) was observed only at the base of the cochlea, with no IHC loss. The administration of STS at T_{+1HOUR} completely reversed hearing and cellular loss. ABR, DP level and cochleogram results were all perfectly correlated, and demonstrated STS-induced protection of the sensory organ of the cochlea.
Acute administration of cisplatin at 10 mg/kg induced no deleterious effects on health, reflecting CIHL observed in clinic, and consisting in a suitable model to test the protective effects of drugs against cisplatin's ototoxicity.