

Introduction

Cisplatin is a platinum-based chemotherapeutic agent and is widely used in cancer treatment. It is accompanied by **severe side effects**, notably **ototoxicity**. Cisplatin-induced hearing loss (CIHL) results in **permanent cochlear damage** at both **functional and cellular levels**. Despite the extensive use of Cisplatin in clinical treatments, there are currently **few protective strategies and no effective regenerative therapies available**.

Objectives

This study aimed to **optimize a reliable preclinical rodent model of CIHL**.

- Determine the effects of a single exposure to three doses (8, 10 and 13 mg/kg) of Cisplatin at both functional and cellular levels.
- Define the effects of lactated Ringer's (LR) hydration on Cisplatin induced-hearing loss model

Methods

Animals: Male rat Wistar WT at 250 ± 25 g were used.

Cisplatin: Cisplatin (8, 10 and 13 mg/kg) or NaCl were administered by intraperitoneal infusion at 6 mL/hour.

Daily care: Animals received Gerd Diet Breeding placed on the cage floor and Emerald IC Omnivore (PO 5 mL/kg/day). All Cisplatin animals were hydrated twice daily with a subcutaneous injection of PlasmaLyte A or lactated Ringer's (LR) supplemented with magnesium chloride (10 mL/kg).

DPOAEs (Distortion Product Otoacoustic Emissions): DPOAEs were measured with a Otopylab system at Baseline, T_{30DAYS} and T_{45DAYS} (Sham and Cisplatin 8 mg/kg groups only) (one animal/one ear at a time) at 4, 8, 16, 24 and 32 kHz at an intensity of 63 dB.

ABRs (Auditory Brainstem Responses): ABR were measured (up to eight animals/one ear at a time) at Baseline, T_{30DAYS} and T_{45DAYS} (Sham and Cisplatin 8 mg/kg groups only) after the Cisplatin injection at 5 frequencies: 4, 8, 16, 25 and 32 kHz.

Wave I analysis: The files generated with ABR measures were analysed with different proprietary MATLAB scripts to determine the Wave-I peak-to-peak amplitudes from ABR traces. The Wave I amplitudes were determined by an automatic analysis, which was confirmed by a visual reading. This analysis was also used to determine the latency at which the Wave occurred.

Cytochrome c: At T_{3-45DAYS} the cochleae were sampled, perfused in Formalin 10%, then decalcified, dissected and immunostained for anti-Mycosin-VIIa (Proteus Biosciences, cat. No. 25-6790). The fragments were then mounted on a microscope slide and images were acquired with a confocal microscope. Manual cell counts were performed at 4, 8, 16, 25 and 32 kHz, using ImageJ software.

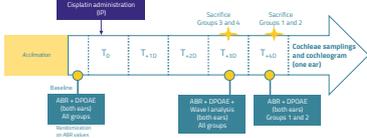


Figure 1: Study Scheme

Results

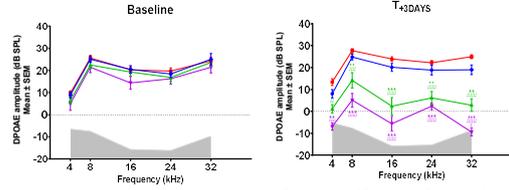
Body weight

Legend:
 • Sham
 • Cisplatin 8 mg/kg
 • Cisplatin 10 mg/kg
 • Cisplatin 13 mg/kg
 • Background noise

Statistical comparisons:
 * vs. Sham
 # vs. Cisplatin 8 mg/kg
 \$ vs. Cisplatin 10 mg/kg

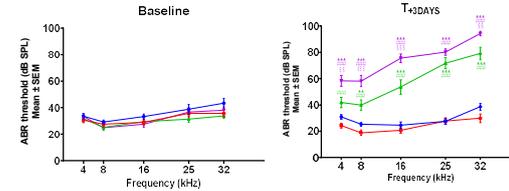
Group	Clinical Signs
Sham	None
Cisplatin 8 mg/kg	Prostrated, lethargic, less grooming with shaggy hair
Cisplatin 10 mg/kg	
Cisplatin 13 mg/kg	

DPOAEs



Analyses performed on both ears. Sham n=14; Cisplatin 8 mg/kg n=14-16; Cisplatin 10 mg/kg n=14 and Cisplatin 13 mg/kg n=14.

ABRs



Analyses performed on both ears. Sham n=14; Cisplatin 8 mg/kg n=14-16; Cisplatin 10 mg/kg n=14 and Cisplatin 13 mg/kg n=14.

Wave I amplitude analysis

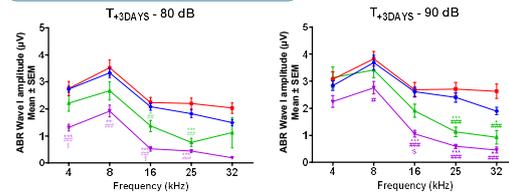


Figure 5: Cisplatin at Doses 10 and 13 mg/kg Decreases Wave I Amplitudes at T_{30DAYS}. Wave I peak-to-peak showed no change for the Cisplatin 8 mg/kg compared to the Sham group at 80 and 90 dB. Cisplatin 10 mg/kg showed a significant decrease at 16 and 25 kHz at 80 dB and at 25 and 32 kHz at 90 dB compared to the Sham and Cisplatin 8 mg/kg groups. At 80 dB, Cisplatin 13 mg/kg showed a significant decrease from 4 to 25 kHz compared to the Sham and Cisplatin 8 mg/kg groups. At 90 dB, from 16 to 32 kHz and from 8 to 32 kHz compared to the Sham and Cisplatin 8 mg/kg groups respectively. Analyses performed on both ears. Sham n=14; Cisplatin 8 mg/kg n=16; Cisplatin 10 mg/kg n=4-14 and Cisplatin 13 mg/kg n=11-13. One-way ANOVA followed by a Tukey's multiple comparisons post hoc test. *p<0.05 **p<0.01 ***p<0.001

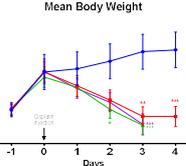


Figure 2: Cisplatin affects general health in rats. All animals injected with Cisplatin at 8, 10 or 13 mg/kg showed similar body weight loss and clinical signs. Sham n=7; Cisplatin 8 mg/kg n=7-8; Cisplatin 10 mg/kg n=7 and Cisplatin 13 mg/kg n=7. 2-way ANOVA followed by a Tukey's multiple comparisons post hoc test. *p<0.05 **p<0.01 ***p<0.001

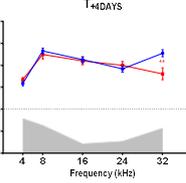


Figure 3: Cisplatin at Doses 10 and 13 mg/kg Induces a Significant Decrease of DPOAE Amplitudes at T_{30DAYS}. DPOAE amplitudes were significantly decreased for the doses 10 and 13 mg/kg compared to the Sham and Cisplatin 8 mg/kg groups at T_{30DAYS} in a dose dependent manner. The Cisplatin 8 mg/kg group showed no change compared to the Sham group at T_{30DAYS} and T_{45DAYS}. 2-way ANOVA followed by a Tukey's multiple comparisons post hoc test. *p<0.05 **p<0.01 ***p<0.001

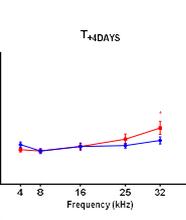


Figure 4: Cisplatin at Doses 10 and 13 mg/kg Induces a Significant Increase of ABR Thresholds at T_{30DAYS}. ABR thresholds were significantly increased for the doses 10 and 13 mg/kg compared to the Sham and Cisplatin 8 mg/kg groups at T_{30DAYS} in a dose dependent manner. The Cisplatin 8 mg/kg group showed no change compared to the Sham group at T_{30DAYS} and only a significant increase for 32 kHz at T_{45DAYS}. 2-way ANOVA followed by a Tukey's multiple comparisons post hoc test. *p<0.05 **p<0.01 ***p<0.001

Wave I latency analysis

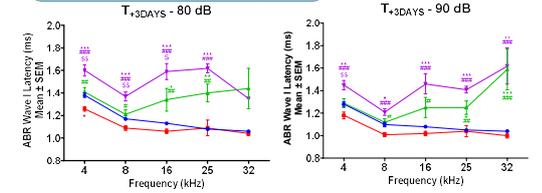
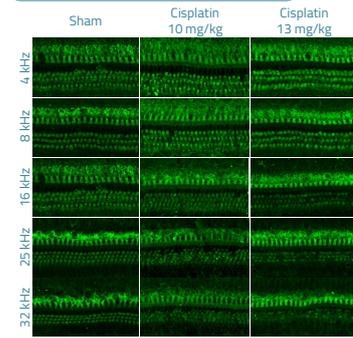


Figure 6: Cisplatin at Doses 10 and 13 mg/kg Decreases Wave I Latencies at T_{30DAYS}. No change was observed in the Cisplatin 8 mg/kg compared to the Sham group excepted a significant decrease at 80 dB at 4 kHz. At 80 dB, Cisplatin 10 mg/kg showed a significant increase of the wave latency at 16 and 25 kHz compared to the Sham and from 4 to 25 compared to the Cisplatin 8 mg/kg. At 90 dB, Cisplatin 10 mg/kg showed a significant increase of the wave latency at 25 and 32 kHz compared to the Sham and from 8 to 32 compared to the Cisplatin 8 mg/kg. Cisplatin 13 mg/kg showed a significant increase of the wave latency at 4 to 25 kHz compared to the Sham and Cisplatin 8 mg/kg groups at 80 and 90 dB. A dose dependent effect can be observed between the groups Cisplatin 10 mg/kg and Cisplatin 13 mg/kg. Analyses performed on both ears. Sham n=14; Cisplatin 8 mg/kg n=16; Cisplatin 10 mg/kg n=4-14 and Cisplatin 13 mg/kg n=11-13. One-way ANOVA followed by a Tukey's multiple comparisons post hoc test. *p<0.05 **p<0.01 ***p<0.001

Hair Cell Quantifications



Legend:
 • Sham (n=7)
 • Cisplatin 10 mg/kg (n=7)
 • Cisplatin 13 mg/kg (n=7)

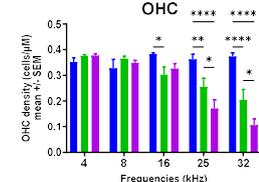
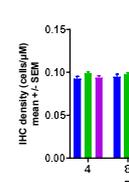


Figure 7: Cisplatin at Doses 10 and 13 mg/kg Induces Cellular Loss. Immunolabelling of Myosin-VIIa showed no change in IHC density but a significant decrease of OHC density at high frequencies for doses 10 and 13 mg/kg compared to the Sham group, with a dose res. 2-way ANOVA followed by a Tukey's multiple comparisons post hoc test. *p<0.05 **p<0.01 ***p<0.001

Legend:

• Sham (LR)
 • Cisplatin 10 mg/kg (LR)
 • Background noise

Group	Clinical Signs
Sham (LR)	None
Cisplatin 10 mg/ml (LR)	Prostrated, lethargic, less grooming with unkempt hair

Body weight

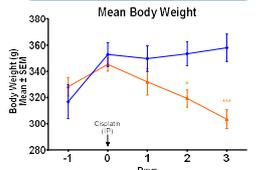


Figure 8: Lactated Ringer's had no effect on Cisplatin health status. Cisplatin animals showed similar clinical signs and body weight loss despite LR rehydration.

DPOAEs

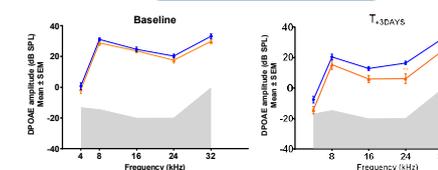


Figure 9: Lactated Ringer's protects against DPOAE amplitude decrease. No significant change were reported on DPOAE amplitude between Sham and Cisplatin 10 mg/kg rehydrated with LR at T_{30DAYS} with the exception of a significant decrease at 24 kHz. Statistical analysis: 2-way ANOVA followed by a Sidák's multiple comparisons post hoc test. *p<0.05; **p<0.01; ***p<0.001

ABRs

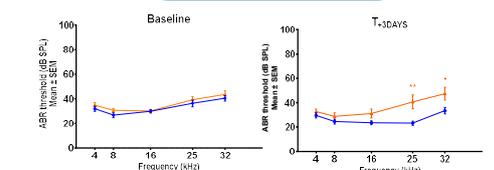


Figure 10: Lactated Ringer's protects against ABR threshold increase. ABR thresholds in Cisplatin 10 mg/kg rehydrated with LR were similar to Sham, except a slight significant increase at 25 and 32 kHz

Conclusion

Cisplatin at a dose of **8 mg/kg does not induce a functional hearing loss, neither a cellular damage**. However, Cisplatin at doses **10 & 13 mg/kg** causes body weight loss, substantial clinical signs, and induces a dose response hearing loss as our results demonstrated: i) a **decrease in DPOAE amplitudes**, ii) an **increase in ABR thresholds**, iii) a **decrease in wave I amplitude** and iv) an **increase in wave I latency**. This loss of function occurred alongside a **decrease in OHC density**. **Daily care**, including Lactated Ringer's, replacing PlasmaLyte A, showed no improvement of the general health, but **protected the hearing functions on DPOAE amplitudes and ABR thresholds in rats treated with Cisplatin at 10 mg/kg**.