TRANSLATIONAL TOOLS TO STUDY OTOTOXIC SIDE EFFECTS OF CANCER AND COVID-19 TREATMENTS

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OBJECTIVES

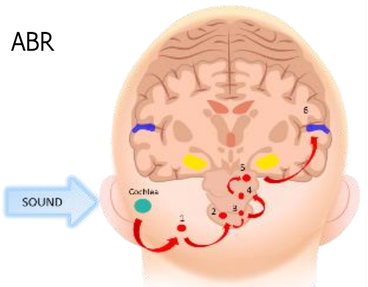
Hearing loss is a major problem that can be caused by diseases, such as NF2 schwannoma, and by ototoxic drugs, the chemotherapeutic cisplatin being the most ototoxic one. Among the medications under investigation as novel therapies to treat Covid-19, Hydroxychloroquine, Azithromycine, and Colchicine have been identified as potentially being ototoxic. The aim of this study was to determine if these drugs, administered similarly to clinical protocol, have any effects on hearing, in comparison to the references, Gentamicin and Cisplatin.

METHODS

Study Groups: Male wistar rats (Janvier Labs) were randomly divided into eight groups: one sham group (no treatment), five groups treated with either Hydroxychloroquine (62 mg/kg, per os once a day for five days), Azithromycin (51.5 mg/kg, per os once a day for five days), Colchicine (0.1 mg/kg, per os once a day for five days), Lopinavir / Ritonavir (41.5 mg/kg / 10.5 mg/kg, per os twice a day for ten days), Ivermectin (0.2 mg/kg, per os once a day for five days), Gentamicine (160mg/kg, repeated IP injections, daily for 5 days or one group treated with Cisplatin (2 mg/kg, 2 cycles of cisplatin injections Daily IP for 4 days).

Auditory Brainstem Response (ABR) & Distortion Product OtoAcoustic Emission (DPOAE) are 2 functional auditory measures that allow differential diagnoses of the sites of dysfunction:

ABR

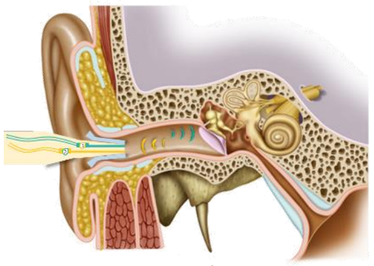


1. Auditory Nerve
2. Cochlear nuclei
3. Olivary nuclei
4. Lateral Lemniscus
5. Inferior colliculus
6. Auditory cortex

ABRs are electric potentials recorded from scalp electrodes, and the first ABR wave represents the summed activity of the auditory nerve fibers contacting **the inner hair cells** (IHC).

ABR (both ears) were measured (eight animals/one ear) at baseline (T0), $T_{+10DAYS}$, $T_{+24DAYS}$ and $T_{+38DAYS}$ 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.

DPOAE



DPOAEs are acoustic signals created and amplified by the cochlear epithelium and measured in the ear canal. DPOAEs depend on the biological motors in **outer hair cells** (OHC) which amplify sound-evoked cochlear vibration.

DPOAE were assessed (one animal/one ear at a time) at 4, 8, 16, 24 and 32 kHz at an intensity of 63 dB at the same time points.

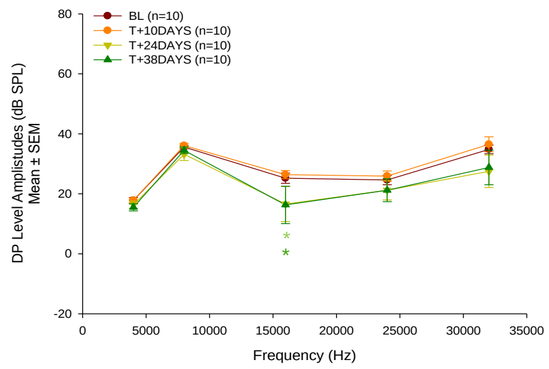
RESULTS

CISPLATIN EFFECTS

DPOAE

Treatment with cisplatin at 2 mg/kg induced a significant difference of the DPOAE amplitudes at 16 kHz from $T_{+24DAYS}$ compared to the baseline values.

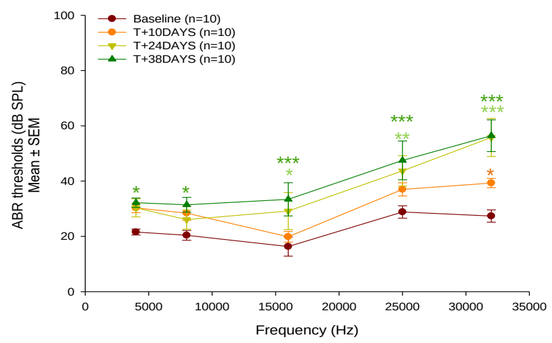
Cisplatin effects on DPOAE amplitudes



ABR

Concerning ABR, slight hearing loss at high frequencies was demonstrated by a significant increase of ABR thresholds from $T_{+10DAYS}$ compared to baseline values.

Cisplatin effects on ABR Thresholds



OTHER COMPOUNDS EFFECTS

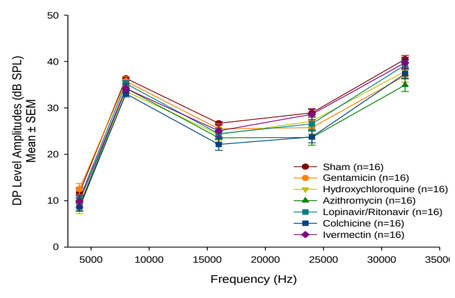
DPOAE

DPOAE amplitudes decreased in the Azithromycin treated group only at 32 kHz at $T_{+38DAYS}$ compared to the Sham group, but no difference was observed in the Hydroxychloroquine, Colchicine, Lopinavir / Ritonavir and Ivermectin treated groups throughout the study. In the Gentamicin treated group, a significant decrease of DPOAE amplitudes measured at 45 dB and 63 dB was observed at 32 kHz at $T_{+38DAYS}$ compared to the Sham group

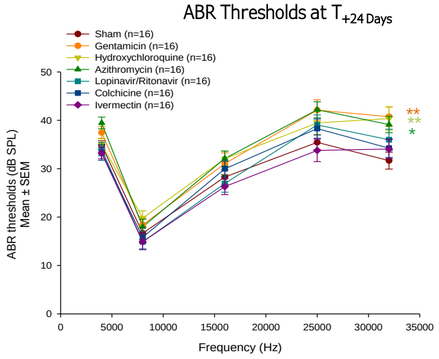
ABR

In the Hydroxychloroquine, Azithromycin and Colchicine treated groups, a significant increase of ABR thresholds was observed for at least one frequency from $T_{+24DAYS}$ compared to the Sham group. Conversely, in the Lopinavir / Ritonavir and Ivermectin treated groups, no significant increase of ABR thresholds was observed. In the Gentamicin treated group, a significant increase of ABR thresholds was observed at high frequencies compared to the Sham group from $T_{+24DAYS}$.

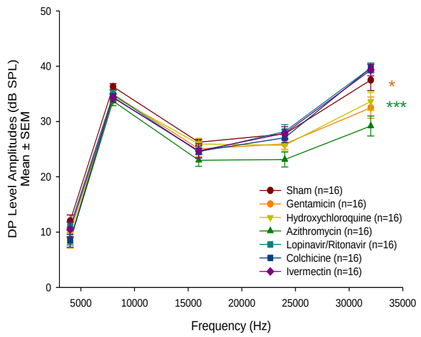
DPOAE Amplitudes at $T_{+24\ Days}$



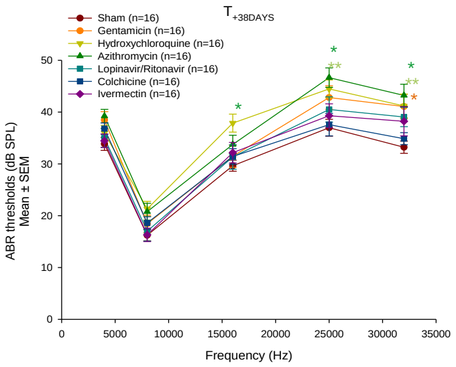
ABR Thresholds at $T_{+24\ Days}$



DPOAE Amplitudes at $T_{+38\ Days}$



ABR Thresholds at $T_{+38\ Days}$



CONCLUSION

The **hearing loss** observed in the groups treated with Hydroxychloroquine and Azithromycin was **progressive** and more pronounced than in the other treated groups.
The effects on hearing of Colchicine, Lopinavir/Ritonavir and Ivermectin in our experimental conditions were negligible (mean loss <10 dB) which suggests they are not ototoxic.
The **long-term ototoxicity** of these compounds remains to be studied. **Cisplatin** and **Gentamicin**, known to be **ototoxic compounds**, induced, at the tested dose and treatment regimen, a **slight hearing loss at the higher frequencies**.
ABR and DPOAE techniques are **highly translational and applicable to other pathologic conditions such as NF2 schwannoma**, and can be combined with histological read-outs.