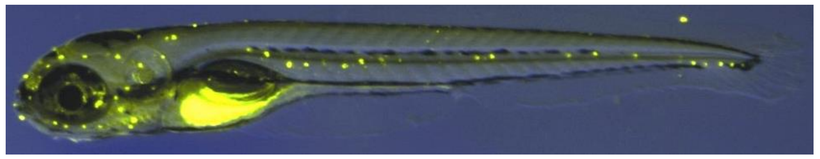
Exploring Antioxidant Drug Therapies for Noise-Induced Hearing Loss

Abigail Jiang

Overview

Noise-induced hearing loss (NIHL) is commonly caused by exposure to excessive noise from occupational or recreational sources. Sensory hair cells are damaged by this acoustic trauma, and NIHL is permanent in humans. One known mechanism of noise-induced hair cell damage is oxidative stress and the accumulation of **reactive oxygen species (ROS)** in hair cells.

We aim to identify antioxidant compounds that attenuate noise-induced hair cell damage by limiting ROS production, utilizing zebrafish (Danio rerio) as a model organism.



Lateral line labeled with DASPEI dye (neuromasts are yellow spots).

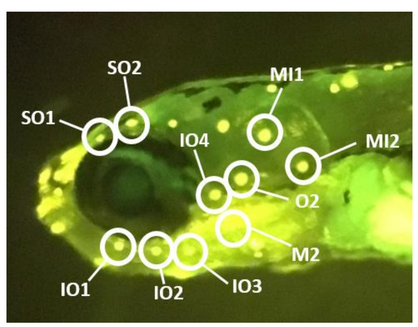
The **hair cells** of zebrafish closely mimic human hair cells of the inner ear in function and structure. These cells are accessible for *in vivo* experimentation in the **lateral line**, which is the external arrangement of neuromasts, or clusters of hair cells, along the head and torso of the zebrafish.

Materials & Methods

A novel **acoustic device** (left) was developed on-site in order to administer acoustic trauma to the larval zebrafish. Ultrasonic waves oscillate microbubbles of dissolved gases in the water, which cause the microbubbles to implode in a process known as cavitation, producing broadband shockwaves. Shockwaves (intensity: 1.7V) caused consistent acoustic damage to hair cells of 5 dpf (days past fertilization) zebrafish.

Immediately following acoustic trauma, fish were transferred into various **antioxidant solutions** for up to 72 hours post-noise exposure. Antioxidants were initially assessed from a larger **redox library,** and compounds that protected hair cells against noise damage were further analyzed through dose-responses.

Vital dye **DASPEI** was used to stain hair cells for imaging and evaluation of 10 pre-selected neuromasts on a 20 point scale per fish (right).



ROS production will be evaluated with an **ROS Assay**, with CellROX Green as an indicator of ROS presence.

Results

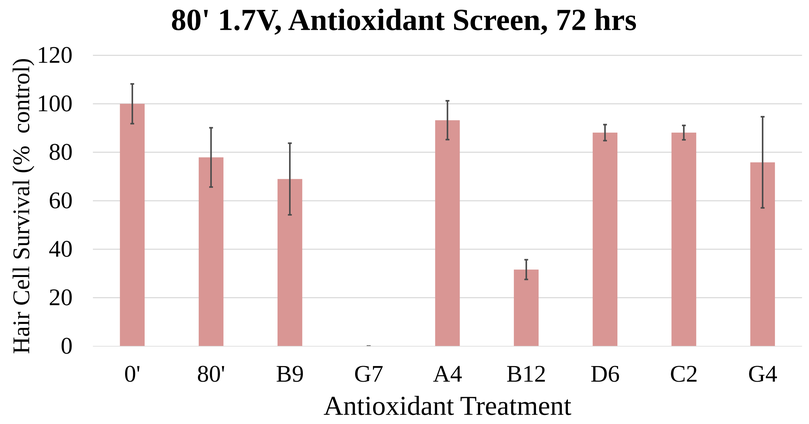


Figure 1. Example of a blinded antioxidant drug screen, where several unknown compounds were tested in one experiment. Compounds A4, D6, and C2 were chosen for further investigation because their hair cell survival was higher than the 80' noise only control group, suggesting that those antioxidants may protect hair cells against noise-induced damage.

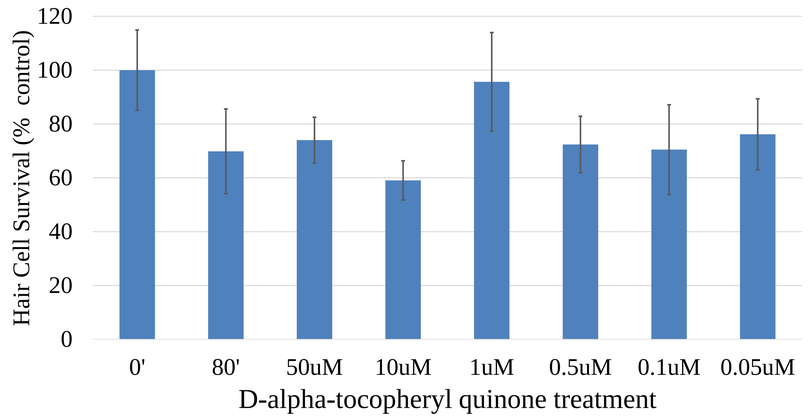


Figure 2. Certain compounds most effectively protect hair cells from acoustic damage at certain concentrations. As seen above, D-alpha tocopheryl quinone, an antioxidant derivative of vitamin D, offers the most hair cell protection at a concentration of 1 μM.

Antioxidant compound	Most effective concentration(s)
D-alpha-tocopheryl quinone	~ 1μM
D-alpha-tocopheryl succinate	~ 1μM and below
Resveratrol	~ 10μM
2-oxo-4-thiazolidinecarboxylic acid	~ 10μM
Ferulic acid ethyl ester	~ 0.1μM and below
Picetannol	~ 10μM

Figure 3. Through many dose-response experiments, six different antioxidant compounds were determined to protect lateral line hair cells from noise-induced damage at various concentrations.

Discussion

Although several antioxidants were identified to protect lateral line hair cells against noise-induced damage, some compounds showed **inconsistent protection** across experiments. For example, 10 μM baicalein increased hair cell survival in the initial blind screen, yet in the dose response, the same concentration of baicalein did not protect hair cells at all.

Poor zebrafish survival also led to data variation. In order to limit loss of zebrafish larvae across the course of the noise damage and treatment, several experiments were assessed 48 hours after noise exposure as opposed to a previous standard of 72 hours. There was no significant difference between the control groups for 48 and 72 hour recovery times, as the 80' noise exposure still caused 25-35% hair cell loss. However, zebrafish uptake and response to the treatments may have differed with the shorter 48 hour recovery and exposure time.

Conclusion

Antioxidant compounds including ferulic acid ethyl ester, D-alpha-tocopheryl quinone, D-alpha-tocopheryl succinate, resveratrol, 2-oxo-4-thiazolidinecarboxylic acid, capsaicin, and picetannol protect hair cells of the zebrafish lateral line from noise-induced hair cell damage when administered as a post-noise exposure treatment for 48 to 72 hours. These compounds are strong potential candidates for noise-induced hearing loss drug treatments than can be used in a clinical setting.

Future Directions

The efficacy of these various antioxidant treatments still must be re-evaluated with an **ROS Assay**. Zebrafish hair cells will be analyzed under a fluorescent microscope, where higher fluorescence indicates production of ROS in the cell. To confirm The identified compounds should show a decrease in ROS production (less fluorescence) in order to confirm that they act as antioxidants to protect the hair cells.

These compounds are strong potential candidates for **clinical, post-exposure NIHL treatments** in order to attenuate hair cell damage and better preserve hearing. FDA approval must be granted in order to move these compounds into clinical trial.

Acknowledgements

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