Zebrafish swimming behavior as a biomarker for ototoxicity-induced hair cell damage: a high-throughput drug development platform targeting hearing loss

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Hearing loss is one of the most common human sensory disabilities, adversely affecting communication, socialization, mood, physical functioning, and quality of life. In addition to age and noise-induced damage, ototoxicity is a common cause of sensorineural hearing loss with chemotherapeutic agents, for example, cisplatin, being a major contributor. Zebrafish (*Danio rerio*) are an excellent model to study hearing loss as they have neurosensory hair cells on their body surface that are structurally similar to those within the human inner ear. Anatomic assays of toxin-mediated hair cell damage in zebrafish have been established; however, using fish swimming behavior—rheotaxis—as a biomarker for this anatomic damage was only recently described. We hypothesized that, in parallel, multilane measurements of rheotaxis could be used to create a high-throughput platform for drug development assessing both ototoxic and potentially otoprotective compounds in real time. Such a device was created, and results demonstrated a clear dose response between cisplatin exposure, progressive hair cell damage, and reduced rheotaxis in zebrafish. Furthermore, pre-exposure to the otoprotective medication dexamethasone, before cisplatin exposure, partially rescued rheotaxis swimming behavior and hair cell integrity. These results provide the first evidence that rescued swimming behavior can serve as a biomarker for rescued hair cell function. Developing a drug against hearing loss represents an unmet clinical need with global implications. Because hearing loss from diverse etiologies may result from common end-effects at the hair cell level, lessons learned from the present study may be broadly used. (Translational Research 2015;166:440–450)

Abbreviations: Cis = cisplatin (following number indicates concentration in micromolars); dpf = days postfertilization; Dex = dexamethasone (following number indicates concentration in micromolars); RI = rheotaxis index; SD = standard deviation

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AT A GLANCE COMMENTARY

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Background

Maki Niihori's previous research focus was on medaka fish embryogenesis and swimming behavior under microgravity in space. At the Japan Aerospace Exploration Agency, she established a swimming analysis technique using high-speed movies, which showed that physical conditions affected swimming ability. Dr Jacob is an experienced Otolaryngology physician and researcher.

Translational Significance

There are no Food and Drug Administration (FDA)-approved pharmacologic treatments for hearing loss. Developing biological models for rapidly assaying drugs to protect or regenerate hearing is an urgent need. Our study demonstrates our validated behavior assay is adaptable to high-throughput methodology allowing for evaluation of agent ototoxicity and potential assessment of otoprotective or otoregenerative effects.

INTRODUCTION

Hearing loss, defined as a partial or complete inability to perceive sound, is one of the most common human sensory disabilities. About 1 in 1000 children are born with severe to profound hearing loss and 3–4 in 1000 develop hearing loss during childhood.[1] Population-based studies in the United States indicate that by the age of 65 years, 42%–47% of individuals are hearing-impaired in one or both ears[2-4] and nearly all persons older than 80 years have some degree of hearing loss.[5] Conservative estimates suggest that hearing impairment affects 32% of Americans aged 20–69 years.[6] Although age-related hearing loss is most common, tinnitus and hearing loss because of noise-exposure now tops the list of war-related health care costs, affecting more than 1.5 million veterans. Ototoxicity has also become a leading cause of hearing loss, with cisplatin being the prototypical drug associated with chemotherapy-induced hearing loss. The link between cisplatin treatment and ototoxicity in both children and adults is well-established,[7-9] with nearly 55% of exposed patients affected.[10] Like noise and aging, chemotherapy causes substantial inner ear damage to cochlear hair cells resulting in permanent hearing loss for cancer survivors.[7-9] Importantly, these hair cells are thought to undergo cell death from noise, ototoxicity, age, and so forth, via final common pathways involving oxidative stress and free radical–mediated damage.[10-12] Therefore, the lessons learned from studying ototoxicity may be more broadly applied to other etiologies of hearing loss.

There are currently no FDA-approved pharmacologic treatments specifically designated for the treatment of hearing loss; consequently, developing biological models for rapidly assaying drugs that might protect or potentially regenerate hearing is an urgent and unmet clinical need. Age-related hearing loss is difficult to model, and research on noise-induced hearing loss typically requires use of higher-order species such as birds or rodents. The latter are expensive and low-throughput biological systems for drug screening. Ototoxicity research, however, lends itself to experimental manipulation using high-throughput biological systems. Also, because a substantial number of patients receive chemotherapy daily at most cancer centers, potential subjects are widely available for translational studies evaluating systemic or intratympanic (local) drug delivery.

Zebrafish (*Danio rerio*) have become a widely used model to study disease and in vivo drug development.[13,14] Fish have neurosensory hair cells on their body surfaces that are aggregated into clumps called neuromasts, which are found at stereotypic positions along their lateral line. These neuromasts and the hair cells contained within them are used to detect changes in movement and vibration from the surrounding water, which plays an important role in schooling behavior, predation, and orientation. The hair cells are structurally similar to those within the human inner ear, making zebrafish an excellent model to study inner ear dysfunction.[15-18] Importantly, because of their superficial location, zebrafish lateral line hair cells can be experimentally damaged by manipulating conditions in their water (eg, adding exogenous toxic agents). For normal zebrafish, the induction of water flow results in a predictable "head-to-current" swimming behavior called "rheotaxis." Work by Suli et al[19] found that adding aminoglycoside toxins to water causes damage to zebrafish lateral line hair cells and negatively impacts rheotaxis behavior in a dose-dependent manner. We hypothesized that the converse was also true—detecting rescue of swimming behavior would serve as a biomarker for anatomic rescue of hair cells. If this hypothesis holds true, swimming behavior could then be assayed as a high-throughput biological platform for screening otoprotective drugs.

To test our hypothesis, we built a novel multichamber, semiautomated behavioral testing apparatus that could measure swimming behavior in up to 6-chambers simultaneously and in darkness. This apparatus was then used to measure rheotaxis at varying doses of cisplatin