The Ototoxic Chemotherapeutic Drug Cisplatin is Retained in the Human Inner Ear Indefinitely

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cisplatin is an effective and widely used anti-cancer drug. It’s used to treat a variety of solid tumors in both adult and pediatric cancer patients. It is also the most ototoxic drug in clinical use, with 40 to 80 percent of patients treated with it experiencing significant permanent hearing loss.1,2 We sought to examine cisplatin pharmacokinetics as a means of understanding the unique susceptibility of the inner ear to cisplatin-induced damage.3

At the core of the cisplatin molecule is the metal platinum, and we utilized inductively-coupled plasma mass spectrometry (ICP-MS) to measure platinum levels in human and mouse tissues. ICP-MS is a sensitive technique for detecting metals that can measure platinum levels as low as parts per quadrillion.4,5 Since there is no platinum in normal biological tissues, this method allows us to detect cisplatin with high sensitivity and with no background signal.

We began our studies in adult (12 weeks of age) CBA/CaJ mice, which received three cycles of cisplatin administration. Each cycle consisted of four days of cisplatin administration (3.5 mg/kg/day), followed by ten days of recovery (Figure 1A). This protocol
approximates multi-cycle cisplatin protocols used clinically. Hearing testing was conducted before and after cisplatin administration by recording auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs). Together, these tests measure hearing sensitivity and the function of the sensory cells in the inner ear.

After three cycles of cisplatin, mice had moderately-severe to severe hearing losses (approximately 35 to 55dB threshold shifts) across frequencies (Figure 1B), similar to cisplatin-induced hearing loss in humans. Mice were euthanized after the recovery period of the third cisplatin cycle, and cisplatin levels in their tissues were measured using ICP-MS. Most organs (liver, kidney, lung, and heart) showed increasing platinum levels during each cisplatin administration period, followed by rapidly declining platinum levels during the recovery period (Figure 1C), indicating that these tissues efficiently cleared cisplatin after cessation of drug treatments. Platinum levels in the brain remained low, which is consistent with the fact that cisplatin does not cross the blood-brain barrier. In contrast to these tissues, the cochleas of these mice showed platinum uptake during cisplatin administration, but they did not show declining platinum levels during the recovery periods (Figure 1C). Instead, platinum levels progressively increased in the inner ear during successive cycles of cisplatin administration. These data indicate that while other organs efficiently clear platinum, the cochlea instead retains it. When we looked again 60 days later (in a separate cohort of animals), platinum levels in most tissues were returning to baseline, while those in the cochlea had not decreased, suggesting that platinum may be retained in the cochlea indefinitely.

In order to determine if platinum is also retained in the human cochlea, we collaborated with the NIDCD National Temporal Bone, Hearing and Balance Pathology Resource Registry at Massachusetts Eye and Ear to obtain donated temporal bones from persons who had been treated with cisplatin. The National Institute of Health’s Office of Human Subjects Research Protections determined that this study was exempt from Institutional
Insufficient clearance of cisplatin from the cochlea may explain the progression of cisplatin-induced hearing loss after cessation of cisplatin therapy, and it likely contributes to the unique susceptibility of the inner ear to cisplatin-induced damage. In addition, our data predict that therapeutic strategies aimed at reducing cisplatin uptake (or increasing cisplatin clearance) from the cochlea should reduce cisplatin-induced hearing loss in cancer patients.

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Sensorineural hearing loss (SNHL) is the most common sensory deficit in the world. Disabling SNHL affects 466 million people globally and is projected to affect 900 million people by the year 2050.\(^1\) It is characterized by damage to the mosaic of delicate, micron-scale mechanosensory cells and auditory nerve fibers that comprise the sensory epithelium of the cochlea, the tiny, snail-shaped structure that facilitates hearing from within the densest bone in the body, the petrous portion of the temporal bone (Figure 1). These inner ear microstructures are known to be involved in human SNHL from decades of research conducted in extracted cadaveric human temporal bone specimens.

Indeed, post-mortem human temporal bones remain our only source of information on the cellular signatures of human SNHL. The gold standard method for studying human temporal bones has been hematoxylin and eosin (H&E)-based histological preparation for the past 125 years (Figure 2).\(^2\) This technique involves harvesting the temporal bone at patient autopsy and subsequently fixing, decalcifying, dehydrating, manually sectioning, staining, and mounting the tissue on glass slides for evaluation using light microscopy.\(^3\) While this technique has
improved our understanding of human SNHL in critical and profoundly impactful ways, it has several limitations: 1) it is
extremely laborious and time-consuming, requiring up to a year
of preparation before analysis is possible in a single specimen, 2)
it introduces irreversible artifacts to the tissue at every stage in
processing; and 3) it reduces the level of study possible to what is
visible along a single orientation plane.

Despite major advances in biomedical and clinical imaging
and microscopy over the past several decades, the cochlea has
historically evaded attempts at visualizing its interior in situ
because of its small size, fragility, complex three-dimensional
configuration, and nested location within extremely dense bone.
However, recent advances in x-ray and phase contrast imaging
techniques have enabled, for the first time to our knowledge,
cellular-level visualization of the sensory cells and neurons
implicated in SNHL in three-dimensionally intact human
temporal bones (Figure 3). Referred to as synchrotron radiation
phase contrast imaging (SR-PCI), this novel imaging technique
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leverages extremely high energy x-ray radiation generated using a synchrotron particle accelerator to penetrate through the dense bone surrounding the cochlea and utilizes sophisticated phase detection algorithms to resolve the cellular features of the cochlea's thin, membranous sensory epithelium. Importantly, these features are typically undetectable with standard x-ray-based imaging techniques (Figure 2).

For the present experiments, harvested human temporal bones were first fixed to maintain the sensory epithelium's integrity and then drilled to a size of roughly three cubic centimeters, ensuring that both the cochlea and the vestibular end organs, which are also located in the petrous portion of the temporal bone, remained intact. Specimens were then transported to the Canadian Light Source synchrotron imaging facility in Saskatoon, SK, Canada, where they were positioned on an imaging stage located 55 meters from the synchrotron x-ray source and two meters from the detector for imaging. The imaging stage was then rotated slowly to enable tomographic cross-sectional imaging (3,000 projections acquired over 180° of rotation). Total imaging time was roughly two hours per specimen. Image reconstruction, stitching, and virtual dissection analyses were all performed using commercial software programs.

Three-dimensional virtual sectioning of volumetric image stacks revealed the cochlea's interior in striking levels of detail—structures visualized included those known to be damaged in the progression of SNHL, such as hair cells and auditory nerve fibers (Figure 3). In addition, SR-PCI was able to detect differences between healthy tissue and tissue that was damaged to varying degrees due to natural or unnatural causes (Figure 4). It also enabled visualization of the vestibular end organ sensory epithelia, which, when damaged in humans, can cause debilitating balance disorders.

Taken together, these findings suggest that SR-PCI has the potential to revolutionize and accelerate the study and diagnosis of human inner ear diseases, promising to serve as a future alternative to standard histological processing of human temporal bones that is both faster and easier to implement.

As the field of human temporal bone pathology approaches extinction⁴, the present and related studies promise to rejuvenate this critically important branch of translational research. These results motivate further investigation into how SR-PCI can be specifically optimized for human temporal bone imaging through improvements in resolution and contrast. Importantly, reducing the amount of x-ray radiation required to achieve the results presented here would motivate swift translation of this technique to the clinic for in vivo, objective diagnosis of SNHL, and identification of its specific underlying etiology in a given patient, enabling personalized, targeted therapy recommendations.

REFERENCES
Otopathology Mini-Travel Fellowship Program

The NIDCD National Temporal Bone Registry’s mini-travel fellowships provide funds for research technicians and young investigators to visit a temporal bone laboratory for a brief educational visit, lasting approximately one week. The emphasis is on the training of research assistants, technicians, and junior faculty.

These fellowships are available to:

- U.S. hospital departments who aspire to start a new temporal bone laboratory
- Inactive U.S. temporal bone laboratories who wish to reactivate their collections
- Active U.S. temporal bone laboratories who wish to learn new research techniques

Up to two fellowship awards will be made each year ($1,000 per fellowship). The funds may be used to defray travel and lodging expenses. Applications will be decided on merit.

Interested applicants should submit the following:

- An outline of the educational or training aspect of the proposed fellowship (1–2 pages)
- Applicant’s curriculum vitae
- Letter of support from temporal bone laboratory director or department chairman
- Letter from the host temporal bone laboratory, indicating willingness to receive the traveling fellow

Applications should be submitted to:

Michael J. McKenna, MD
NIDCD Temporal Bone Registry
Massachusetts Eye and Ear
243 Charles Street, Boston, MA 02114
michael_mckenna@meei.harvard.edu

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