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Research paper

Human audiometric thresholds do not predict specific cellular damage in the inner ear

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ABSTRACT

Introduction: As otology enters the field of gene therapy and human studies commence, the question arises whether audiograms – the current gold standard for the evaluation of hearing function – can consistently predict cellular damage within the human inner ear and thus should be used to define inclusion criteria for trials. Current assumptions rely on the analysis of small groups of human temporal bones *post mortem* or from psychophysical identification of cochlear "dead regions" *in vivo*, but a comprehensive study assessing the correlation between audiometric thresholds and cellular damage within the cochlea is lacking.

Methods: A total of 131 human temporal bones from 85 adult individuals (ages 19–92 years, median 69 years) with sensorineural hearing loss due to various etiologies were analyzed. Cytocochleograms – which quantify loss of hair cells, neurons, and strial atrophy along the length of the cochlea – were compared with subjects' latest available audiometric tests prior to death (time range 5 h–22 years, median 24 months). The Greenwood function and the equivalent rectangular bandwidth were used to infer, from cytocochleograms, cochlear locations corresponding to frequencies tested in clinical audiograms. Correlation between audiometric thresholds at clinically tested frequencies and cell type-specific damage in those frequency regions was examined by calculating Spearman's correlation coefficients.

Results: Similar audiometric profiles reflected widely different cellular damage in the cochlea. In our diverse group of patients, audiometric thresholds tended to be more influenced by hair cell loss than by neuronal loss or strial atrophy. Spearman's correlation coefficient across frequencies was at most 0.7 and often below 0.5, with 1.0 indicating perfect correlation.

Conclusions: Audiometric thresholds do not predict specific cellular damage in the human inner ear. Our study highlights the need for better non- or minimally-invasive tools, such as cochlear endoscopy, to establish cellular-level diagnosis and thereby guide therapy and monitor response to treatment.

1. Introduction

Hearing loss is the most common sensory deficit in humans and a major global health challenge. Three hundred and sixty million people are currently affected by moderate to profound hearing loss, and the increasing use of personal listening devices, mass attendance at sporting events and concerts, and the aging of a populous generation place 1.1 billion more at risk (Daniel, 2007; Wallhagen et al., 1997; World Health Organization). The underlying causes of hearing loss are diverse and include age, genetics, infection, trauma and exposure to noise or ototoxic drugs. While most of the

Abbreviations: ERB, Equivalent rectangular bandwidth; HL, Hearing level; PTA, Pure tone average; SNHL, Sensorineural hearing loss

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conductive forms of hearing loss – which affect transmission at the level of the middle ear – can be adequately treated with surgery or medications, there is still no cure for sensorineural hearing loss (SNHL), which reflects damage to the delicate mechanosensory structures of the inner ear (Geleoc and Holt, 2014). Therapeutic approaches to SNHL have rapidly evolved over the last few decades and now include astonishingly successful electronic devices, like cochlear and auditory brainstem implants. However, these devices provide a different and limited perception of sound and speech compared to that provided by "natural hearing" (Carlson et al., 2012).

To address limitations of the current rehabilitative approaches for SNHL, new strategies are being developed and include the administration of genes, small molecules and stem cells directly to the inner ear, with clinical trials currently ongoing and results yet to be published (Chien et al., 2015; ClinicalTrials.gov). For such approaches to be successful, it is of utmost importance to identify the extent of cochlear damage at the cellular level so to develop precise and personalized therapies.

Inner hair cells are the main sensory cells in the inner ear, and they perform mechanotransduction – the conversion of a mechanical stimulus into an electrical response, which is then processed by spiral ganglion neurons and the central nervous system. Outer hair cells are motile and they amplify the traveling wave in the cochlea to provide exquisite sensitivity of the hearing organ. Stria vascularis is a vascular structure in the cochlear lateral wall and generates the endocochlear potential that drives transduction current through hair cells.

The vast majority of human studies on hearing restoration rely on the well-established analysis of standardized audiograms, which reflect hearing thresholds in quiet as a function of frequency. Some studies include additional indirect metrics like word recognition scores, defined as the percentage of words a patient can correctly repeat after listening to a standardized word list in quiet; otoacoustic emissions, which are generated by outer hair cells and serve as a measure of those cells' integrity; and auditory brainstem responses, which are surface potentials consisting of several waves, the first of which reflects the summed activity of the cochlear nerve (Causey et al., 1984; Gelfand, 1997; Thornton and Raffin, 1978). However, although it is common in animal studies to verify the consequences of experimental procedures with histological data, very little is known about the predictive value of human audiometric thresholds to detect specific cellular damage in the inner ear. Capitalizing on the precious resources of the US Temporal Bone Registry, which has one of the world's largest collections of human post-mortem temporal bones, we sought to determine to what extent human audiograms predict changes in cochlear histopathology.

2. Materials and methods

2.1. Temporal bone preparation and study

The archival collection of human temporal bones from the US Temporal Bone Registry at Massachusetts Eye and Ear Infirmary was inspected to identify the specimens that had been quantified using cytocochleograms, which are graphic representations of structural integrity of sensory hair cells, cochlear neurons and stria vascularis along the cochlear length (see Fig. A1) (Schuknecht, 1968). A total of 131 temporal bones from 85 hearing-impaired adult patients (age range 19–92 years, median 69 years; see Fig. A.2A) were analyzed. The most common diagnosis was presbycusis, i.e. age-related hearing loss (22 ears), followed by a combination of presbycusis and acoustic trauma (11 ears), kanamycin ototoxicity (7 ears), sudden SNHL (6 ears), "isolated" SNHL (6 ears), otosclerosis (5 ears) and several rare diseases and syndromes (see Table A.1). Written informed consent had been obtained prior to death and the study was carried out according to The Code of Ethics of the World Medical Association (Declaration of Helsinki). The current study was approved by the institutional review board. As previously described by Schuknecht, bones were removed after death (post-mortem time range 2–63 h, median 10 h, no information for 4 subjects; see Fig. A.2B) and fixed in 10% neutral buffered formalin or Heidenhain Susa solution, decalcified in ethylenediaminetetraacetic acid, embedded in celloidin, serially sectioned at a thickness of 20 μm in the horizontal (axial) plane, and every tenth section was stained with hematoxylin and eosin (Schuknecht, 1968). The specimens on glass slides were examined using light microscopy. Cytocochleograms were created to quantify fractional loss of hair cells and cochlear neurons as well as strial atrophy along the cochlear spiral after standardizing cochlear length to 32 mm for all ears (Schuknecht, 1993). Up to 320 different data points per cochlea (one every 100 μm) were collected. The resulting cytocochleograms were sorted from most to least extensive damage for every cell type to generate waterfall plots (Figs. 1–5, see below). Earlier cytocochleograms combined inner and outer hair cells into "hair cells," while more recent cytocochleograms classify inner and outer hair cells separately.

2.2. Hearing tests

Standardized pure tone audiometric thresholds, identified at 250, 500, 1000, 2000, 4000 and 8000 Hz, were determined as a part of the routine clinical examination by increasing sound level in 5 dB increments from 0 dB to a maximum of 100 dB. The current study focused on the latest available audiograms recorded for each patient prior to death, which ranged from 5 h to 22 years, with a median of 24 months (see Fig. A.2D). Three individuals did not undergo a standardized hearing test, but were diagnosed with profound deafness during clinical examination. For one person, the date of audiologic testing was not specified. Pure tone average (PTA) was noted, defined as the average dB hearing level of the two frequencies with the lowest thresholds in the frequency range from 500 to 2000 Hz as this provides the closest agreement with speech reception thresholds (Fletcher, 1953). PTA and damage at the corresponding position in the cytocochleogram were compared according to tonotopic arrangement along the length of the cochlea. Each audiogram was compressed into a linear heat map, with the color reflecting dB of hearing loss across increasing frequencies; an increasing gray scale was used for increasing dB of hearing loss from 0 dB (white) to 100 dB or no response (black) (see Fig. A.1B). Word recognition scores were available and analyzed for 70 temporal bones from 44 patients (see Fig. A.2C).

2.3. Correlation of cellular damage and audiograms

The frequency, f, corresponding to a specific location along the cytocochleograms, x, was calculated according to a modified Greenwood function: $f = 165.4(10^{2.1x} - 0.88)$ (Greenwood, 1961a,b). The cochlear region responding to a certain frequency was determined using the equivalent rectangular bandwidth (ERB), a measurement defined in psychoacoustic studies to represent an approximation of the bandwidths of the filters in human hearing (Glasberg and Moore, 1990; Moore and Glasberg, 1983). In humans, the ERB reflects a constant distance of 0.9 mm on the basilar membrane. Consequently, we calculated the average damage for each location corresponding to a specific frequency, +/− 0.45 mm (Moore, 1986). For word recognition scores, we combined all frequency-specific results and weighted them based on the octave-band Speech Intelligibility Index described by the American