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Does Otosclerosis Affect Dark and Transitional Cells in the Human Vestibular Labyrinth?

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Abstract

Hypothesis—The density of vestibular dark cells (DCs) and vestibular transitional cells (TCs) can be quantitatively decreased in human temporal bones with otosclerosis.

Background—Previous reports have shown that otosclerosis can lead to vestibular symptoms.

Methods—We examined 61 human temporal bone specimens from 52 deceased donors with otosclerosis group—with and without endosteal involvement (EI), and with and without endolymphatic hydrops (EH)—vs. 25 specimens from 18 age-matched controls. Using light microscopy, we evaluated the nonsensory epithelium of the lateral semicircular canal (LSC) and posterior semicircular canal (PSC) of the human vestibular labyrinth, focusing on the density of DCs and TCs.

Results—In both the LSC and the PSC, as compared with the control group, the mean density of DCs significantly decreased in the *EI (+) group*, in the *EI (+) and EH (+) subgroup*, and in the *EI (+) and EH (–) subgroup* ($P < 0.05$). In addition, we found a significant difference in the mean density of DCs between the *EI (+) group* and the *EI (–) group* in the LSC and in the PSC ($P < 0.05$). But we found no significant difference in the mean density of TCs in any of the otosclerosis groups or subgroups as compared with the control group ($P > 0.05$).

Conclusion—We found a decrease in the density of DCs associated with EI in human temporal bone specimens with otosclerosis, regardless of the presence of EH. This decrease might cause damage in ion and water transportation, leading to vestibular symptoms.

Keywords

Otosclerosis; dark cell; transitional cell; endosteal involvement; endolymphatic hydrops; human temporal bone; histopathology

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INTRODUCTION

Otosclerosis, a disease of the otic capsule and stapes, can lead to conductive and/or sensorineural hearing loss, dizziness, and tinnitus (1). Its well-established association with endolymphatic hydrops (EH) has been documented in numerous reports (2–4). Published studies have extensively examined the pathology and pathogenesis of the mechanisms of hearing loss in patients with otosclerosis, but few studies have investigated the mechanisms of vestibular symptoms in such patients (5, 6).

Vestibular dark cells (DCs) localized within the ampullae of the semicircular canals, utricle, and common crus between the melanocytes and endolymphatic surface play an important role: they help maintain the high-potassium and low-sodium content of the endolymph. They also help maintain homeostasis, volume regulation, osmolarity, calcium regulation, enzymatic activity, and glucose transportation (7–9). Studies have reported the degeneration of DCs in patients with Waardenburg's syndrome, Meniere's syndrome, and ototoxicity (10–11). In addition, vestibular transitional cells (TCs) can play a big role in maintaining labyrinthine fluid homeostasis because of their ability to transport ions (12).

Still, the exact pathogenesis of vestibular symptoms in patients with otosclerosis remains unknown. The maintenance of vestibular and auditory transduction relies on the balance between ion absorption and secretion by the special epithelial cells, such as DCs and TCs, bounding the endolymphatic spaces (13); therefore, we hypothesized that vestibular symptoms in patients with otosclerosis are associated with deterioration in those cells. A better understanding of the mechanisms of vestibular symptoms in patients with otosclerosis might help us create specific medical and surgical approaches to more effective treatment.

MATERIALS AND METHODS

Temporal bone specimens

For this study, we obtained all temporal bone specimens from our archived human temporal bone collection at the University of Minnesota. The temporal bones, from deceased donors, had previously been removed at autopsy and fixed in formalin solution. Each bone was decalcified, embedded in celloidin, and then serially sectioned in the horizontal plane at a thickness of 20 μ m. Every 10th section was stained with hematoxylin-eosin (H&E), and then mounted on a glass slide for light microscopic assessment. This study was approved by the institutional review board of the University of Minnesota (0206M26181).

We examined 61 human temporal bone specimens from 52 donors (31 male and 21 female) with otosclerosis. Their age range was 16 to 89 years; their mean age (standard deviation [SD]), 66.7 years (17.2). Using light microscopy, we classified the human temporal bone specimens into 2 groups depending on the presence of endosteal involvement (EI): the *EI (+) group* and the *EI (–) group*. In all specimens, we also checked for the presence of EH and thus created 4 subgroups: the *EI (+) and EH (+) subgroup*, the *EI (+) and EH (–) subgroup*, the *EI (–) and EH (+) subgroup*, and the *EI (–) and EH (–) subgroup* (Figure 1). We defined EI as an otosclerotic focus involving the endosteum of the otic capsule (Figure 2). We excluded specimens from donors with head and/or acoustic trauma, systemic autoimmune

disorders, labyrinthitis, active otitis media, neoplasms, and other otologic and systemic diseases that may affect the vestibular system.

The age-matched control group had 25 temporal bone specimens from 18 individuals (11 male and 7 female) with no histopathologic findings of any otologic diseases. Their age range was 49 to 79 years; their mean age (SD), 65.5 years (9.9).

Density of DCs

For this study, we selected histologic sections in which the cristae of the lateral semicircular canal (LSC) and of the posterior semicircular canal (PSC) were cut close to perpendicular to its axis. We did not include superior semicircular canal, and utricle in our study because they did not have perpendicular sections to count DCs. Using light microscopy, we assessed the nuclei of the DCs. They can be distinguished by the location of their nuclei high and close to the endolymphatic space and by the melanin particles seen in the connective tissue just below them (Figure 3). We counted DCs in each perpendicularly sectioned cristae of the lateral and posterior semicircular canals whose viewable surface area was at least 0.002 mm². We expressed the number of DCs as the density (defined as the number of hair cells per 0.001 mm² of surface area). To determine the surface area, we multiplied the thickness of the section (20 µm) by the length of the sensory epithelium where we made the count (100 µm).

Density of TCs

For this study, we selected histologic sections in which the cristae of the LSC and of the PSC were cut close to perpendicular to its axis. We did not include superior semicircular canal, and utricle in our study because they did not have perpendicular sections to count TCs. We identified TCs by the location of their nuclei between vestibular hair cells and DCs (Figure 3). We counted TCs in each perpendicularly sectioned cristae of the lateral and posterior semicircular canal whose viewable surface area was at least 0.002 mm². We expressed the number of TCs as the density (defined as the number of hair cells per 0.001 mm² of surface area). To determine the surface area, we multiplied the thickness of the section (20 µm) by the length of the sensory epithelium where we made the count (100 µm).

Statistical analysis

To evaluate any relationship between otosclerosis and EH, we performed the chi-square test. To compare the density of DCs and TCs in all of our groups and subgroups, we used the Mann-Whitney U test. Significance was defined as $P < 0.05$.

RESULTS

Temporal bone specimens

The *EI (+) group* consisted of 38 temporal bone specimens from 32 donors; the *EI (-) group*, 23 specimens from 20 donors. Of the 32 donors in the *EI (+) group*, 6 (18%) had a history of dizziness; but of the 20 donors in the *EI (-) group*, only 1 (5%) had a history of dizziness. Of the total of 61 specimens in both of our otosclerosis groups combined—i.e., the *EI (+) group* and the *EI (-) group*—36 had EH and 25 did not. Of the 38 specimens in

the *EI (+) group*, 29 (76%) had EH. But of the 23 specimens in the *EI (-) group*, only 7 (30%) had EH ($\chi^2 = 12.470$, $P < 0.001$) (Figure 1).

Density of DCs

In the control group, the mean density (SD) of DCs was 7.54 (0.49) in the LSC and 7.02 (0.83) in the PSC. The *EI (+) and EH (+) subgroup* had the lowest density of DCs. As compared with the control group, the mean density (SD) of DCs significantly decreased in the *EI (+) group* [6.68 (0.82) in the LSC ($P < 0.001$) and 5.76 (1.05) in the PSC ($P < 0.001$)]; in the *EI (+) and EH (+) subgroup* [6.60 (0.82) in the LSC ($P < 0.001$) and 5.68 (0.82) in the PSC ($P < 0.001$)]; and in the *EI (+) and EH (-) subgroup* [6.94 (0.84) in the LSC ($P = 0.016$) and 6.00 (1.62) in the PSC ($P = 0.027$)] (Figure 4).

In addition, we found a significant difference in the mean density (SD) of DCs between the *EI (+) group* and the *EI (-) group* [LSC ($P = 0.011$); PSC ($P = 0.002$)]. In the *EI (-) group*, the mean density (SD) of DCs was 7.23 (0.64) in the LSC and 6.59 (0.54) in the PSC (Figure 4).

But we found no significant difference in the mean density (SD) of DCs between the *EI (-) group* and the control group; between the *EI (-) and EH (+) subgroup* and the control group; and between the *EI (-) and EH (-) subgroup* and the control group ($P > 0.05$) (Figure 4).

Density of TCs

In the control group, the mean density (SD) of TCs was 6.18 (0.78) in the LSC and 6.04 (0.59) in the PSC. We observed no significant difference between the control group and any other groups or subgroups ($P > 0.05$) (Figure 4).

DISCUSSION

Vestibular symptoms, including unsteadiness, dizziness, or vertigo, are common, affecting up to 30% of patients with otosclerosis (14, 15). Yet the associated pathogenesis is still unknown. It has been speculated that otosclerotic foci are associated with EH and cause vestibular symptoms via end-organ or vestibular nerve degeneration, and/or via the changing biochemistry of the labyrinthine fluid (16–19). According to Liston et al., otosclerotic involvement of the vestibular wall may lead to EH (3). In our study, in temporal bone specimens from deceased donors with otosclerosis, we found a strong relationship between EI and EH.

DCs, one of the main cells responsible for endolymph production, are functionally similar to stria marginal cells in the cochlea (20). DCs play a crucial role in maintaining the high-potassium and low-sodium content of the endolymph (7). The survival and functions of vestibular hair cells depend on the fine balance among electrolytes, water, and the electrical potential of inner ear compartments (13, 21). In a previous study, we showed a significant decrease in vestibular hair cells in patients with otosclerosis and endosteal involvement (22). We have speculated that any changes in the density of DCs play a role in the imbalance of homeostasis, leading to losses of vestibular hair cells.

The spreading of toxic metabolites (released by otosclerotic bones) into the inner ear fluids may lead to sensorineural hearing loss via vascular and neuroepithelial injury, or via alteration of electrolytes (23). Previous studies have found hydrolytic enzymes produced by otosclerotic foci in the perilymph and have concluded that they contribute to the damaging of cochlear elements (24–26). These enzymes enter into the inner ear fluids either by splashing or by flowing through canaliculi, and they can change the results of electronystagmography (ENG) (26). Furthermore, inflammatory cytokines produced by otosclerotic foci can cause some disturbances in labyrinthine fluid and of inner ear homeostasis (27). Moreover, Cowan et al. showed that mice lacking the EphB2 receptor tyrosine kinase expression in their DCs displayed circling behaviors associated with vestibular dysfunction (28). In our study, the loss of DCs that we observed in the temporal bone specimens with EI was significant in both the LSC and the PSC. This injury might be due to the inflammatory and/or enzymatic activities of otosclerosis that showed endosteal involvement.

TCs have enzymatic activity (Na^+/K^+ ATPase and Na^+/H^+ exchanger) and ion conductance (Na^+ and Cl^-) (12, 29–31). Those findings suggest that TCs play a role in inner ear fluid homeostasis. In our current study, we did not observe any changes in TCs under light microscopy, perhaps because they might be more resistant to the harmful effects of pathologic processes in otosclerosis. Still, some ultrastructural changes may well have occurred that might not be visible in those cells under light microscopy.

In conclusion, in this study, we clearly showed a decrease in the density of DCs—in the cristae of both the LSC and the PSC—in human temporal bone specimens from deceased donors who had otosclerosis with EI, regardless of the presence of EH. This pathologic change may be associated with vestibular symptoms secondary to altered homeostasis in the ion-fluid transport mechanism.

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Figure 1.

Groups and subgroups of temporal bone specimens with otosclerosis. EI = endosteal involvement; EH = endolymphatic hydrops; n = number of temporal bone specimens; minus sign (-) = absent; plus sign (+) = present

Figure 2. Photomicrograph of the right temporal bone specimen from a 75-year-old deceased woman with otosclerosis (staining with H&E). Arrows = otosclerotic foci involving the cochlear and vestibular endosteum; C = cochlea; F = facial nerve; O = otosclerosis.

Figure 3. Photomicrograph of the right temporal bone specimen from the patient in Figure 2 (staining with H&E). (A) Magnified view of boxed area in Figure 2. (B) Magnified view of boxed area in (A). Arrowheads = transitional cells; thick arrows = melanin particles; thin arrows = dark cells.

Figure 4.

(A) Mean density ($/10^{-3} \text{ mm}^2$) of dark cells and transitional cells in the *EI (+)* group, in the *EI (-)* group, and in the control group. (B) Mean density ($/10^{-3} \text{ mm}^2$) of dark cells and transitional cells in the *EI (+)* and *EH (+)* subgroup, in the *EI (+)* and *EH (-)* subgroup, and in the control group. (C) Mean density ($/10^{-3} \text{ mm}^2$) of dark cells and transitional cells in the *EI (-)* and *EH (+)* subgroup, in the *EI (-)* and *EH (-)* subgroup, and in the control group. DC = dark cell; EI = endosteal involvement; EH = endolymphatic hydrops; error bars = standard deviation; LSC = lateral semicircular canal; minus sign (-) = absent; plus sign (+) = present; PSC = posterior semicircular canal; TC = transitional cell. * $P < 0.05$."