The Role of TNFα in Aging Processes of Mouse Inner Ear Cells

Cody D. Spence[1,2#]; Bo Ding[1,2]; XiaoXia Zhu[1,2,3]; Mark A. Bauer[2,3]; Robert D. Frisina[1,2,3*]

[1]Medical Engineering Dept., University of South Florida Morsani College of Medicine, [2]Global Center for Hearing and Speech Research, [3]University of South Florida College of Engineering

Introduction: Age-related hearing loss (ARHL) is a highly prevalent chronic communication disorder of the elderly, yet its mechanisms are not clear and current treatments are mostly inadequate. This condition is frequently treated with hearing aids, which have low rates of compliance due to factors such as discomfort and high cost. Development of a drug that prevents or treats ARHL could help alleviate some of the shortcomings of current treatments and significantly improve quality of life. To find a potential target for ARHL drug development, we examined cochlear inflammatory mechanisms of ARHL.

Materials and Methods: Young adult (2-4 months, n=6) and old (30-32 months, n=4) CBA/CaJ mice were used. Hearing loss was quantified with auditory physiology measurements, recorded in a soundproof chamber with anesthetized mice. The Auditory Brainstem Response (ABR) audiogram and Distortion Product Otoacoustic Emissions (DPOAE) were measured with a TDT Biosig system (Alachua, FL). Complementary *in vitro* studies with SVK-1 cells from the mouse stria vascularis and HEI-OC1 cells from the mouse organ of Corti were used for gene expression measurements. Here, H_2O_2 was used in varying doses (for 24 hours) to simulate oxidative stress associated with aging. RT qPCR was used to measure gene expression of TNFα, an inflammatory cytokine, normalized to GAPDH. TNFα expression was compared to control (no treatment). 1-way ANOVA, 2-way ANOVA with multiple comparisons, and unequal variance t-tests were performed using *GraphPad Prism 8.1.2* (La Jolla, CA).

Results and Discussion: DPOAE amplitudes decreased and ABR and DPOAE thresholds increased in older mice compared to young adults ($p<0.0001$), indicating ARHL had occurred. HEI-OC1 cells had peak TNFα expression at 120 µM H_2O_2 ($p=0.0003$). SVK-1 cells had peak TNFα expression at 80 µM ($p<0.0001$) with a higher fold change over control than in the HEI-OC1 cells ($p=0.0095$). This may be due to the more vascularized nature of the stria compared to the organ of Corti. No unspecified bands were seen in gel electrophoresis of PCR products, suggesting that we detected the specific TNF-α gene signal in cochlear cell lines. These results show inflammatory-induced changes of the inflammatory cytokine TNFα for mouse inner ear cells. If similar changes of TNFα and other inflammatory gene expression biomarkers are shown for future *in vivo* aging studies, then it could provide a basis for the use of anti-inflammatory agents in the prevention or treatment of ARHL.

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Email: cdspence@health.usf.edu