17β-estradiol Blocks Stress-Induced Apoptosis through Autophagy Enhancement for Cochlear Hair Cells

#Bo Ding¹,³, Xiaoxia Zhu²,³, Mckenzie Watson³, *D. Frisina¹,²,³

1. Department of Communication Sciences and Disorders, College of Behavioral & Community Sciences, University of South Florida, Tampa, FL-33620
2. Department of Medical Engineering, College of Engineering, University of South Florida, Tampa, FL-33620
3. Global Center of Hearing and Speech Research, University of South Florida, Tampa, FL-33612

Introduction: Oxidative stress is a dominant factor in aging. The finding that estradiol therapy can slow down age-related hearing loss (ARHL) in females suggests it can prevent cochlear aging processes. Autophagy, a highly conserved cellular mechanism, and plays a critical role in the pathology of a number of neurodegenerative age-linked diseases. Whether or not estradiol can prevent cochlear age-related oxidative stress via autophagy pathways is not clear. To investigate this, we used the HEI-OC1 cochlear hair cell line and CBA/CaJ mice to gain novel insights into the mechanisms of inner ear aging disorders and possible roles of sex hormones such as estrogen.

Methods: HEI-OC1 cells were used as an in vitro model and CBA/CaJ mice as the in vivo model. Treatments, such as estradiol, and hydrogen peroxide (H₂O₂) were used for in vitro experiments; and chloroquine and arsenic trioxide in vivo. In addition, CBA/CaJ mice were divided into two groups: young adult at 3-months old and old age at 30-months.

Results: We observed that iNOS and TNF-α increased in the old cochlea compared with young adults. In addition, we detected autophagy marker increases (LC3II and p62) in the aged cochlea in vivo. These changes indicate the presence of oxidative stress in the aged cochlea and are similar to the results of our in vitro experiments where HEI-OC1 cells were treated with H₂O₂. 17β-estradiol treatment blocked the effects of H₂O₂ on changes of iNOS, TNF-α, LC3II and p62 in vitro. In terms of cellular and molecular mechanisms underlying therapeutic effects, additional experiments revealed that HEI-OC1 cell survival improves with 17β-estradiol therapy, as H₂O₂ related intrinsic and extrinsic apoptotic pathways are inhibited. The hallmarks of mTOR activation, the phosphorylation of p70S6K at threonine 389 and 4E-BP at serine 65, were also evaluated. S6K1 and 4E-BP1 showed an increase of their phosphorylation with H₂O₂ treatment; similar to the results for in vivo cochlear samples (increased phosphorylation of S6K and 4E-BP for young vs old cochlea). Interestingly, 17β-estradiol blocked this phosphorylation increase for HEI-OC1 cells. Ligation-mediated polymerase chain reactions (LMPCR), to amplify the nucleosomal ladder, showed that increases in apoptosis occurred for H₂O₂ treated HEI-OC1 cells, indicating elevated levels of cleaved genomic DNA, but 17β-estradiol treatment inhibited this H₂O₂-induced DNA fragmentation.

Conclusion: Estrogen increases autophagy flux through mTOR modulation and blocks H₂O₂-induced autophagy changes and cell death, which may contribute to a therapeutic strategy for treatment of ARHL.

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Poster only, email: ding1@usf.edu, phone No: 001-585-6830170