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

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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 (H_2O_2) 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 H_2O_2. 17β-estradiol treatment blocked the effects of H_2O_2 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 H_2O_2 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 H_2O_2 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 H_2O_2 treated HEI-OC1 cells, indicating elevated levels of cleaved genomic DNA, but 17β-estradiol treatment inhibited this H_2O_2-induced DNA fragmentation.

Conclusion: Estrogen increases autophagy flux through mTOR modulation and blocks H_2O_2-induced autophagy changes and cell death, which may contribute to a therapeutic strategy for treatment of ARHL.

Work Supported by NIH grant P01 AG009524 from the Nat. Inst. on Aging.

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