Ototoxicity-induced loss of hearing and inner hair cells is attenuated by HSP70 gene transfer

高田洋平

高次機能制御系耳鼻咽喉科・頭頸部外科学専攻第４学年

[1]Kansai Medical University

[2]Kresge Hearing Research Institute, University of Michigan

[3]National Institute on Deafness and Other Communication Disorders, NIH

Introduction:

The most common reason for sensorineural hearing loss is death of hair cells (HC) from diverse stressors. Heat shock proteins (HSPs) are molecular chaperones, participating in folding, targeting and degrading proteins in all cells. In addition, HSP expression is increased in response to various environmental stresses to protect cells from damage. One common HSP, HSP70, inhibits apoptosis caused by heat shock and other stresses. Previous studies showed that cultured mouse utricles exposed to HSP70 from conditioned media or adenovirus (Ad) mediated over-expression were protected from HC loss caused by aminoglycosides. Here we test whether Ad.*HSP70* gene transfer protects against a systemic ototoxic insult in the guinea pig cochlea, in vivo.

Material and methods:

Guinea pigs were deafened by administration of kanamycin (SC, 400 mg/kg) and furosemide (IV, 100 mg/kg) to induce a severe ototoxic lesion. The viral vector Ad.*HSP70*-mCherry (1.5μl) was injected into the scala media of the left (experimental) cochlea via basal turn cochleostomy 4 days before deafening. Control animals received an identical ototoxic insult after injection with Ad.mCherry. Hearing thresholds were measured by ABR before deafening and prior to sacrificing the animals, 14 days following deafening. Cochlear tissues were prepared for fluorescence microscopy as

whole mounts stained for Myosin VIIa (experimental ear) or Myosin VIIa and phalloidin (contralateral ear). HC were quantified in both ears in both groups.

Result:

Injection of Ad.*HSP70*-mCherry resulted in mCherry fluorescence in non-sensory cells of the organ of Corti. The ototoxic insult eliminated both outer HCs (OHC) and inner HCs (IHC) in the basal turns of both control (Ad.mCherry-injected) ears and contralateral (non-injected) ears. Ad.*HSP70*-mCherry-treated ears exhibited a less severe lesion with more IHC survival than Ad.mCherry-injected ears. OHC were not protected. ABR thresholds were significantly better in Ad.*HSP70*-mCherry treated ears than in control ears and contralateral ears. Because injection into the scala media involves a traumatic loss of some HCs, the degree of protection by HSP70 we observed may underestimate of its true protective effect.

Conclusions:

Our data show attenuation of severe ototoxic trauma by HSP70 over-expression in supporting cells after injecting Ad.*HSP70*-mCherry into the scala media of the guinea pig cochlea. Compared to controls, ABR thresholds were improved in treated ears and IHC were protected in the basal turn. HSP70 augmentation may represent a potential therapy against ototoxicity and other cochlear traumas. It is currently unknown whether HSP70 acts on the supporting cells and prevents elimination of damaged HC, or acts directly to protect the HC.

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