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Survival of Auditory Hair Cells

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Abstract

The inability of mammals to regenerate auditory hair cells creates a pressing need to understand how to enhance hair cell survival following insult or injury. Hair cells are easily damaged by noise exposure, ototoxic medications, and as a consequence of aging processes, all of which lead to progressive and permanent hearing impairment as hair cells are lost. Significant effort has been invested in designing strategies to prevent this damage from occurring since permanent hearing loss has a profound impact on communication and quality of life for patients. In this mini-review, we discuss recent progress in using antioxidants, anti-inflammatories and apoptosis inhibitors to enhance hair cell survival. We conclude by clarifying the distinction between protection and rescue strategies and by highlighting important areas of future research.

Keywords

Hair cells; survival; antioxidants; anti-inflammatories; caspase inhibitors

Introduction

To survive is to continue to exist and function in spite of danger, severe suffering, or privation. For auditory hair cells, these challenges to survival can originate internally in the form of age-related processes or externally in the forms of medications or excessive stimulation by noise or sound. The mechanisms of damage from several of these sources are comprehensively reviewed elsewhere in this special issue. Irrespective of the source or mechanism of damage, any of these insults can injure hair cells to the extent that they are rendered non-functional or non-existent. The crux of this issue for humans and other mammalian vertebrates is that differentiated hair cells are post-mitotic and are not regenerated once they are lost, resulting in permanent, irreversible hearing loss (Ryan, 2003; Kelley, 2006; 2007). Our objective is to review promising interventions with the potential to improve survival of auditory hair cells, as well as to highlight future avenues of research.

Antioxidants

Antioxidants show promising enhancements of hair cell survival following drug-induced damage to the inner ear, called ototoxicity. Two clinically important classes of drugs are notable for inducing irreversible ototoxicity: aminoglycoside antibiotics (gentamicin, neomycin, kanamycin) and platinum-based antineoplastic chemotherapy drugs (cisplatin, carboplatin, oxaliplatin). Both aminoglycosides and cisplatin increase production of reactive oxygen species in the cochlea, and cisplatin also depletes cochlear levels of glutathione and antioxidant enzymes (Lautermann et al., 1995; Ravi et al., 1995; Clerici et al., 1996; Hirose et al., 1997; Rybak et al., 2000; Dehne et al., 2001; Ryan, 2003; Kelley, 2006; 2007; Ding et al., 2012; Schacht et al., 2012). Ultimately, this redox imbalance triggers cell death pathways, resulting in progressive and irreversible hearing loss (Nakagawa et al., 1998; Alam et al., 2000; Ylikoski et al., 2002; Watanabe et al., 2003; Schacht et al., 2012).

Antioxidants protect hair cells from damage with varying efficacy in model systems ranging from zebrafish to rodents. Zebrafish are a robust model for investigating ototoxicity and regenerative mechanisms. In this model, edaravone, a potent free radical scavenger, attenuates apoptosis and approximately doubled the number of surviving hair cells when administered concurrently with ototoxic levels of neomycin or cisplatin (Hong et al., 2013; Choi et al., 2014b). Similarly, trimetazidine, an anti-ischemic agent typically used to treat angina pectoris, is also known to reduce oxygen-derived free radicals and exhibited a three-fold attenuation of neomycin-induced hair cell loss in zebrafish (Chang et al., 2013). Evidence from mammalian models lacking hair cell regenerative capacity is equally compelling, both in the context of drug-induced ototoxicity and noise-induced hearing loss. Systemic administration of thiamine pyrophosphate, the active form of the metabolism and antioxidation cofactor thiamine, preserved cochlear antioxidant levels and activities, as well as the presence of hair cells in cisplatin-treated guinea pigs, though hair cell quantification was not part of the analysis (Kuduban et al., 2013). Antioxidant extracts from *Ginkgo biloba* leaves displayed a dose-dependent attenuation of cisplatin-induced hair cell death and threshold elevations in rat models (Choi et al., 2013). N-acetyl L-cysteine (NAC) replenishes reserves of the antioxidant glutathione and preserved ~80-88% of outer hair cells in whole organ cultures of neonatal mouse inner ears exposed to cisplatin (Tropitzsch et al., 2014). Recently, combination treatment with NAC and additional compounds that stabilize free radicals has been shown to improve outer and inner hair cell survival by 85% and 64%, respectively, and to attenuate hearing loss for up to 21 days after noise exposure in rodent models (Lu et al., 2014; Choi et al., 2014a). Combination treatment was effective when begun one to four hours after noise exposure.

Interventions with dietary antioxidants show selective successes. Dietary intake of Coenzyme Q10 (CoQ10), a component of the electron transport chain that functions as an antioxidant, attenuated outer hair cell loss to 20% from 60% in untreated animals following noise-induced hearing loss (Fetoni et al., 2009). However, other antioxidant dietary supplements were less effective in protecting hair cells from noise-induced damage. Supplementation with a combination β -carotene, vitamins C and E, and magnesium prior to noise exposure protected against permanent threshold shifts and preserved morphologic integrity of subsets of hair cells; however, quantification of hair cell survival did not achieve

statistical significance (Le Prell et al., 2011). Dietary studies of resveratrol, an antioxidant of great interest in anti-inflammatory and aging research, reveal a similar pattern of selective effects. Dietary intake of low doses (0.1 mg/kg/day) of resveratrol preserved ultrastructural integrity of hair cells following cisplatin treatment, whereas higher doses (1 and 10 mg/kg/day) paradoxically worsened hair cells survival (Olgun et al., 2013). Improved hair cell survival and structure at low resveratrol doses did not translate to improvement or preservation of hearing function.

Anti-inflammatories

Cochlear inflammation is characterized by increased recruitment of immune cells to the cochlea and by swelling and dysfunction of the *stria vascularis*, a highly vascular structure along the lateral wall of the cochlear duct that produces and maintains the unique ion concentration and voltage of the endolymph. Dysfunction of the *stria vascularis* also compromises integrity of the blood-labyrinth barrier, increasing the exposure of hair cells to ototoxic medications in the bloodstream and highlighting the important role of inflammation in hair cell survival (Hirose et al., 2014). The anti-inflammatory effects of corticosteroids make them attractive candidates to improve hair cell survival. Due to the variety and severity of side effects associated with systemic corticosteroid treatment, interventions designed to protect the auditory system primarily use an intratympanic (IT) route of delivery. IT corticosteroid injections have a lengthy history of clinical use to treat patients with inflammatory inner ear diseases and sudden hearing loss; however, further clinical studies will be needed to provide evidence-based guidelines for optimum dosage (Alles et al., 2006; Li et al., 2014). In animal studies, IT dexamethasone protected guinea pigs and rats treated with a single dose of cisplatin from hearing loss in a frequency-dependent manner and preserved cochlear structures, especially when given 1 hour before or up to 48 hours after cisplatin (Murphy and Daniel, 2011; Topdag et al., 2012; Shafik et al., 2013). Interestingly, IT dexamethasone was not otoprotective when cisplatin was administered in multiple doses over five to ten days, suggesting that timing and dose of corticosteroid relative to the damaging agent are crucial factors (Hughes et al., 2014). Similarly, the route of administration is important since systemic dexamethasone failed to provide significant otoprotection against cisplatin-induced hair cell damage in guinea pigs, though it did have a protective effect on the *stria vascularis* (Waissbluth et al., 2013).

Apoptosis Inhibitors

Direct modulation of programmed cell death pathways, particularly those involving phosphoinositide 3-kinase (PI3-kinase) and caspase 3, promotes hair cell survival by targeting apoptosis as the final common pathway in the progression from injury to hair cell death. The PI3-kinase pathway promotes outer hair cell survival and opposes gentamicin-induced toxicity in rat organs of Corti (Chung et al., 2006). In rat organ of Corti explants, dexamethasone protects hair cells from Tnf- α induced apoptosis by activation of the PI3 kinase/Akt and NF κ B pathways and up-regulation of Bcl-2 and Bcl-xl, both of which inhibit signaling events that lead to activation of caspase 3 (Dinh et al., 2008; Haake et al., 2009). Indeed, Tnf- α is up-regulated and induces hair cell death after noise exposure (Fujioka et al., 2006; Huang et al., 2013). Suppression of inflammatory pathways involving Tnf- α is the putative mechanism underlying the preservation of a majority of hair cells, as well as

significant reduction in both age-related hearing loss through 17 months and noise-induced hearing loss two weeks after noise exposure, in C57BL/6J mice over-expressing Islet1 (Isl1), a LIM-homeodomain transcription factor (Huang et al., 2013).

Caspase 3 activation is a critical signaling event during loss of hair cells, and inhibitors of caspase activation have also shown potential in promoting hair cell survival. Minocycline, a broad-spectrum tetracycline antibiotic, attenuates gentamicin-induced hair cell loss from 70-95% of hair cells being absent to 20-40% by inhibiting p38 MAP kinase phosphorylation and caspase 3 activation (Corbacella et al., 2004; Wei et al., 2005). Intriguingly, modulation of histone modifications also reduces caspase-3 activation following aminoglycoside exposure. Delivery of small molecular inhibitors of lysine-specific demethylase 1 (LSD-1) to the inner ear prior to neomycin treatment prevented demethylation of H3K4me2 in hair cell nuclei and reduced caspase 3 activation, doubling the number of surviving hair cells and maintaining hearing thresholds in mice (Corbacella et al., 2004; Wei et al., 2005).

Future Perspectives

Extensive research has focused on discovering or developing agents that will either protect or rescue hair cells from damage, thus increasing hair cell survival and preserving hearing function. Protection primarily focuses on preventing hair cells from sustaining damage, whereas rescue implies the ability to recover or reverse damage that has already been sustained. Though the terms are often used interchangeably even within the reviewed body of literature, the distinction between protection and rescue is important, particularly in the absence of regenerative capabilities. Understandably, this inability to regenerate lost or damaged hair cells has fostered an emphasis on prevention. With the exception of three studies (Topdag et al., 2012; Lu et al., 2014; Choi et al., 2014a), the work highlighted in this review falls into the realm of preventative strategies because interventions were either given prior to or concurrently with the induction of damage. The findings yielded from this type of experimental design have great clinical value since ototoxicity can be anticipated in many circumstances. For example, a patient may require treatment with an ototoxic medication or experience occupational or recreational noise-exposure. In both situations, ototoxicity is not unexpected, and treatment to protect hair cells could be given prophylactically.

The promise and value of preventative strategies do not diminish the need for future studies to focus on rescue, or therapeutic, interventions for situations in which damage could not be anticipated or prevented and may have already occurred to some extent. Evidence that antioxidant combination therapy and IT dexamethasone may be able to rescue damage caused by noise exposure or cisplatin therapy, respectively, when administered after damage has occurred is intriguing (Topdag et al., 2012; Lu et al., 2014; Choi et al., 2014a). However, these interventions were begun between one and forty-eight hours after noise or cisplatin exposure with follow-up measurements spanning from two to twenty-one days after the final intervention. Thus, whether the improvement of hair cell survival in these studies primarily reflects a true rescue of damage sustained during that first interval or a prevention of further damage sustained during the subsequent interval to follow-up assessment remains unclear. The salient questions that remain to be answered by rescue studies are: (1) how much and what type of damage can hair cells sustain and still survive?; (2) is there a time

frame after the initial damage during which intervention can increase hair cell survival?; (3) to what extent can hair cells be rescued, and does restoration of function always follow quantitative survival?; and (4) are 'rescued' hair cells as resilient as undamaged counterparts in the face of subsequent insults?

Collectively, the findings discussed in this review provide compelling evidence that antioxidants, anti-inflammatories and apoptosis inhibitors can effectively attenuate hair cell death following a variety of insults (Figure 1). However, the efficacy of these agents was dependent upon numerous factors, including dosage regimen, route of administration, and timing of intervention relative to induction of damage. Future experiments will need to focus on determining the optimal conditions for each agent before asking subsequent questions about the clinical feasibility and usefulness of each approach in patient populations.

There are a number of other potentially lucrative lines of enquiry that would be applicable to both preventative and therapeutic approaches. The first is the evaluation of combination therapies for synergistic effects since damage stimulates multiple imbalances, such as the induction of both inflammation and oxidative stress. Another avenue is the modulation of supporting cell function to promote auditory hair cell survival, particularly since supporting cells actively participate in the process of hair cell death and scar formation. Finally, the National Institutes of Health (NIH) has recognized that men and women respond differently to medications, and the absence of gender as a variable in the reviewed body of literature might impact the clinical efficacy of any proposed interventions.

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References

- Alam SA, Ikeda K, Oshima T, Suzuki M, Kawase T, Kikuchi T, Takasaka T. Cisplatin-induced apoptotic cell death in Mongolian gerbil cochlea. *Hearing Research*. 2000; 141:28–38. [PubMed: 10713493]
- Alles MJRC, Gaag der MA, Stokroos RJ. Intratympanic steroid therapy for inner ear diseases, a review of the literature. *Eur Arch Otorhinolaryngol*. 2006; 263:791–797. [PubMed: 16724210]
- Chang J, Im GJ, Chae SW, Lee SH, Kwon S-Y, Jung HH, Chung A-Y, Park H-C, Choi J. Protective Role of Trimetazidine Against Neomycin-induced Hair Cell Damage in Zebrafish. *Clin Exp Otorhinolaryngol*. 2013; 6:219–225. [PubMed: 24353861]
- Choi C-H, Du X, Floyd RA, Kopke RD. Therapeutic effects of orally administrated antioxidant drugs on acute noise-induced hearing loss. *Free Radic Res*. 2014a; 48:264–272. [PubMed: 24182331]
- Choi J, Chang J, Jun HJ, Im GJ, Chae SW, Lee SH, Kwon S-Y, Jung HH, Chung A-Y, Park H-C. Protective role of edaravone against neomycin-induced ototoxicity in zebrafish. *J Appl Toxicol*. 2014b; 34:554–561. [PubMed: 24795994]
- Choi SJ, Kim SW, Lee JB, Lim HJ, Kim YJ, Tian C, So HS, Park R, Choung YH. Ginkgo biloba extracts protect auditory hair cells from cisplatin-induced ototoxicity by inhibiting perturbation of gap junctional intercellular communication. *Neuroscience*. 2013; 244:49–61. [PubMed: 23583760]
- Chung W-H, Pak K, Lin B, Webster N, Ryan AF. A PI3K pathway mediates hair cell survival and opposes gentamicin toxicity in neonatal rat organ of Corti. *J Assoc Res Otolaryngol*. 2006; 7:373–382. [PubMed: 17053865]

- Clerici WJ, Hensley K, DiMartino DL, Butterfield DA. Direct detection of ototoxicant-induced reactive oxygen species generation in cochlear explants. *Hearing Research*. 1996; 98:116–124. [PubMed: 8880186]
- Corbacella E, Lanzoni I, Ding D, Previati M, Salvi R. Minocycline attenuates gentamicin induced hair cell loss in neonatal cochlear cultures. *Hearing Research*. 2004; 197:11–18. [PubMed: 15504599]
- Dehne N, Lautermann J, Petrat F, Rauen U, de Groot H. Cisplatin ototoxicity: involvement of iron and enhanced formation of superoxide anion radicals. *Toxicol Appl Pharmacol*. 2001; 174:27–34. [PubMed: 11437646]
- Ding D, Allman BL, Salvi R. Review: ototoxic characteristics of platinum antitumor drugs. *Anat Rec (Hoboken)*. 2012; 295:1851–1867. [PubMed: 23044998]
- Dinh CT, Haake S, Chen S, Hoang K, Nong E, Eshraghi AA, Balkany TJ, Van De Water TR. Dexamethasone protects organ of corti explants against tumor necrosis factor- α -induced loss of auditory hair cells and alters the expression levels of apoptosis-related genes. *Neuroscience*. 2008; 157:405–413. [PubMed: 18838114]
- Fetoni AR, Piacentini R, Fiorita A, Paludetti G, Troiani D. Water-soluble Coenzyme Q10 formulation (Q-ter) promotes outer hair cell survival in a guinea pig model of noise induced hearing loss (NIHL). *Brain Research*. 2009; 1257:108–116. [PubMed: 19133240]
- Fujioka M, Kanzaki S, Okano HJ, Masuda M, Ogawa K, Okano H. Proinflammatory cytokines expression in noise-induced damaged cochlea. *J Neurosci Res*. 2006; 83:575–583. [PubMed: 16429448]
- Haake SM, Dinh CT, Chen S, Eshraghi AA, Van De Water TR. Dexamethasone protects auditory hair cells against TNF α -initiated apoptosis via activation of PI3K/Akt and NF κ B signaling. *Hearing Research*. 2009; 255:22–32. [PubMed: 19442713]
- Hirose K, Hockenbery DM, Rubel EW. Reactive oxygen species in chick hair cells after gentamicin exposure in vitro. *Hearing Research*. 1997; 104:1–14. [PubMed: 9119753]
- Hirose K, Li S-Z, Ohlemiller KK, Ransohoff RM. Systemic Lipopolysaccharide Induces Cochlear Inflammation and Exacerbates the Synergistic Ototoxicity of Kanamycin and Furosemide. *JARO*. 2014; 15:555–570. [PubMed: 24845404]
- Hong SJ, Im GJ, Chang J, Chae SW, Lee SH, Kwon S-Y, Jung HH, Chung A-Y, Park H-C, Choi J. Protective effects of edaravone against cisplatin-induced hair cell damage in zebrafish. *Int J Pediatr Otorhinolaryngol*. 2013; 77:1025–1031. [PubMed: 23628221]
- Huang M, Kantardzhieva A, Scheffer D, Liberman MC, Chen Z-Y. Hair cell overexpression of Islet1 reduces age-related and noise-induced hearing loss. *Journal of Neuroscience*. 2013; 33:15086–15094. [PubMed: 24048839]
- Hughes AL, Hussain N, Pafford R, Parham K. Dexamethasone otoprotection in a multidose cisplatin ototoxicity mouse model. *Otolaryngol Head Neck Surg*. 2014; 150:115–120. [PubMed: 24233060]
- Kelley MW. Regulation of cell fate in the sensory epithelia of the inner ear. *Nat Rev Neurosci*. 2006; 7:837–849. [PubMed: 17053809]
- Kelley MW. Cellular commitment and differentiation in the organ of Corti. *Int J Dev Biol*. 2007; 51:571–583. [PubMed: 17891718]
- Kuduban O, Kucur C, Sener E, Suleyman H, Akcay F. The Role of Thiamine Pyrophosphate in Prevention of Cisplatin Ototoxicity in an Animal Model. *The Scientific World Journal*. 2013; 2013:1–5.
- Lautermann J, McLaren J, Schacht J. Glutathione protection against gentamicin ototoxicity depends on nutritional status. *Hearing Research*. 1995; 86:15–24. [PubMed: 8567412]
- Le Prell CG, Gagnon PM, Bennett DC, Ohlemiller KK. Nutrient-enhanced diet reduces noise-induced damage to the inner ear and hearing loss. *Transl Res*. 2011; 158:38–53. [PubMed: 21708355]
- Li H, Feng G, Wang H, Feng Y. Intratympanic Steroid Therapy as a Salvage Treatment for Sudden Sensorineural Hearing Loss After Failure of Conventional Therapy: A Meta-analysis of Randomized, Controlled Trials. *Clin Ther*. 2014
- Lu J, Li W, Du X, Ewert DL, West MB, Stewart C, Floyd RA, Kopke RD. Antioxidants reduce cellular and functional changes induced by intense noise in the inner ear and cochlear nucleus. *JARO*. 2014; 15:353–372. [PubMed: 24497307]

- Murphy D, Daniel SJ. Intratympanic dexamethasone to prevent cisplatin ototoxicity: a guinea pig model. *Otolaryngol Head Neck Surg.* 2011; 145:452–457. [PubMed: 21521888]
- Nakagawa T, Yamane H, Takayama M, Sunami K, Nakai Y. Apoptosis of guinea pig cochlear hair cells following chronic aminoglycoside treatment. *Eur Arch Otorhinolaryngol.* 1998; 255:127–131. [PubMed: 9561858]
- Olgun Y, Kirkim G, Kolatan E, Kıray M, Bağrıyanık A, Olgun A, Kızmazoglu DC, Ellidokuz H, Serbetcioglu B, Altun Z, Aktas S, Yılmaz O, Güneri EA. Friend or foe? Effect of oral resveratrol on cisplatin ototoxicity. *The Laryngoscope.* 2013; 124:760–766. [PubMed: 23900991]
- Ravi R, Somani SM, Rybak LP. Mechanism of cisplatin ototoxicity: antioxidant system. *Pharmacol Toxicol.* 1995; 76:386–394. [PubMed: 7479581]
- Ryan AF. The cell cycle and the development and regeneration of hair cells. *Curr Top Dev Biol.* 2003; 57:449–466. [PubMed: 14674489]
- Rybak LP, Husain K, Morris C, Whitworth C, Somani S. Effect of protective agents against cisplatin ototoxicity. *Am J Otol.* 2000; 21:513–520. [PubMed: 10912697]
- Schacht J, Talaska AE, Rybak LP. Cisplatin and aminoglycoside antibiotics: hearing loss and its prevention. *Anat Rec (Hoboken).* 2012; 295:1837–1850. [PubMed: 23045231]
- Shafik AG, Elkabarity RH, Thabet MT, Soliman NB, Kalleny NK. Effect of intratympanic dexamethasone administration on cisplatin-induced ototoxicity in adult guinea pigs. *Auris Nasus Larynx.* 2013; 40:51–60. [PubMed: 22884636]
- Topdag M, Iseri M, Gelenli E, Yardimoglu M, Yazir Y, Ulubil SA, Topdag DO, Ustundag E. Effect of intratympanic dexamethasone, memantine and piracetam on cellular apoptosis due to cisplatin ototoxicity. *J Laryngol Otol.* 2012; 126:1091–1096. [PubMed: 22947376]
- Tropitzsch A, Arnold H, Bassiouni M, Müller A, Eckhard A, Müller M, Löwenheim H. Assessing cisplatin-induced ototoxicity and otoprotection in whole organ culture of the mouse inner ear in simulated microgravity. *Toxicology Letters.* 2014; 227:203–212. [PubMed: 24709139]
- Waissbluth S, Salehi P, He X, Daniel SJ. Systemic dexamethasone for the prevention of cisplatin-induced ototoxicity. *Eur Arch Otorhinolaryngol.* 2013; 270:1597–1605. [PubMed: 22907029]
- Watanabe K-I, Inai S, Jinnouchi K, Baba S, Yagi T. Expression of caspase-activated deoxyribonuclease (CAD) and caspase 3 (CPP32) in the cochlea of cisplatin (CDDP)-treated guinea pigs. *Auris Nasus Larynx.* 2003; 30:219–225. [PubMed: 12927282]
- Wei X, Zhao L, Liu J, Dodel RC, Farlow MR, Du Y. Minocycline prevents gentamicin-induced ototoxicity by inhibiting p38 MAP kinase phosphorylation and caspase 3 activation. *Neuroscience.* 2005; 131:513–521. [PubMed: 15708492]
- Ylikoski J, Xing-Qun L, Virkkala J, Pirvola U. Blockade of c-Jun N-terminal kinase pathway attenuates gentamicin-induced cochlear and vestibular hair cell death. *Hearing Research.* 2002; 163:71–81. [PubMed: 11788201]

Figure 1. Model depicting the induction of auditory hair cell (HC) damage by drugs (aminoglycoside antibiotics and platinum-based anti-neoplastic agents), aging processes, and excessive stimulation by noise or sound

HC damage results in hearing impairment with ultimate progression to HC death and permanent hearing loss. Dashed arrows and inhibition symbols depict the steps at which preventative and rescue interventions act to enhance hair cell survival. The italicized parentheses beneath each intervention indicate the damaging agents against which each intervention demonstrates efficacy. IT indicates the intratympanic route of delivery.