Mitochondrial dysfunction in hearing loss

Nathan Fischel-Ghodsian\textsuperscript{a}, Richard D. Kopke\textsuperscript{b,\*, c}, Xianxi Ge\textsuperscript{c}

\textsuperscript{a}Department of Pediatrics, Cedars-Sinai Medical Center, UCLA School of Medicine, Los Angeles, CA, USA
\textsuperscript{b}Hough Ear Institute, Oklahoma City, OK, USA
\textsuperscript{c}Department of Defense Spatial Orientation Center, Department of Otolaryngology, Naval Medical Center, San Diego, CA, USA

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Abstract

Mitochondrial pathology plays an important role in both inherited and acquired hearing loss. Inherited mitochondrial DNA mutations have been implicated in both syndromic and non-syndromic hearing loss, as well as in predisposition to aminoglycoside ototoxicity. Acquired mitochondrial dysfunction in the absence of mitochondrial DNA mutations has also been proposed as playing an important role in noise-induced and toxin-induced hearing loss. Presbycusis, the hearing loss associated with aging, may be caused by mitochondrial dysfunction resulting from the accumulation of acquired mitochondrial DNA mutations and other factors. The pathophysiological mechanisms and clinical implications of these findings are discussed.

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Keywords: Hearing loss; Presbycusis, ototoxicity; Noise induced hearing loss; Mitochondrial dysfunction

1. Introduction

Mitochondrial pathology plays an important role in both inherited and acquired hearing loss. Inherited mitochondrial DNA mutations have been implicated in both syndromic and non-syndromic sensorineural hearing loss (Jacobs, 1997; Fischel-Ghodsian, 1998, 1999). Acquired mitochondrial dysfunction in the absence of mitochondrial DNA mutations has also been proposed as playing an important role in noise-induced hearing loss (NIHL), and toxin-induced hearing loss (Kopke et al., 1999, 2000, 2002). The mechanisms of mitochondrial dysfunction are being studied, and in NIHL seem to involve glutamate toxicity, glutathione depletion, increased reactive oxygen species (ROS) and oxidative stress. Presbycusis, the hearing loss associated with aging, may lead to mitochondrial dysfunction through both the accumulation of mitochondrial DNA mutation, and other factors (Seidman et al., 2000). The purpose of this paper is to review some of the latest information linking inherited and acquired mitochondrial pathologies to a variety of etiologies of sensorineural deafness. The clinical relevance of these findings will be discussed, in particular with respect to preventive and therapeutic interventions.
2. Brief review of cochlear function

The function of the cochlea is to convert an acoustic waveform into an electrochemical stimulus transmitted to the central nervous system. The inner hair cell (IHC) function is primarily as a sensory receptor. Movement of the hair cell (HC) stereocilia opens transduction ion channels allowing entry of $\text{K}^+$ and $\text{Ca}^{2+}$, generating a transduction current. The transduction current then activates voltage sensitive calcium channels along the IHC lateral wall and base as well as $\text{Ca}^{2+}$-activated $\text{K}^+$ channels. The end result is release of the neurotransmitter glutamate at the HC base (Raphael and Altschuler, 2003). Glutamate binds to the afferent nerve terminals that surround the base of the HC, resulting in an action potential being propagated down the afferent nerve fibers. The role of the outer hair cell (OHC) is to provide the amplification of acoustic signal by elongation and contraction of the OHC that results from acoustically driven depolarization and hyperpolarization of the cell augmenting the displacement of the basilar membrane. The major functions of mitochondria in HCs are to provide adenosine triphosphate (ATP) formation by oxidative phosphorylation, modulation of intracellular calcium concentration, and the regulation of apoptotic cell death. The number of mitochondria is related to the metabolic activity of the cell. Mitochondrial density appears higher in the basal turn of the cochlea and in the infra-nuclear region of the HC, suggesting a higher metabolic activity in the basal turn, and a greater energy requirement in the region close to nerve endings. (Kopke et al., 2003) The stria vascularis contributes the metabolic energy necessary to maintain a positive endocochlear potential. Enzymes, specifically Na/K ATPase, utilize ATP generated by the mitochondria from cells of the stria and spiral ligament, to pump Na$^+$ and K$^+$ ions against their concentration gradients.

3. Inherited mitochondrial hearing loss

Inherited hearing loss can be due to both heteroplasmic and homoplasmic mitochondrial mutations. These data have recently been reviewed (Fischel-Ghodsian, 2002, 2003) and are summarized with the inclusion of the most recent data in Table 1.

3.1. Mitochondrial mutations and syndromic hearing loss

Systemic neuromuscular syndromes due to heteroplasmic mitochondrial DNA mutations, such as Kearns–Sayre syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), and mitochondrial encephalomyopathy with ragged red fibers, frequently have hearing loss as one of their

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Table 1
Mitochondrial mutations and hearing impairment

<table>
<thead>
<tr>
<th>Hearing impairment</th>
<th>Mutations identified</th>
<th>Inherited</th>
<th>Acquired</th>
<th>Homoplasmy</th>
<th>Heteroplasmy</th>
</tr>
</thead>
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<tr>
<td><strong>Syndromic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syst. neuromuscular</td>
<td>Deletions, A3243G,…</td>
<td>Rare</td>
<td>Usually</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Diabetes + deafness</td>
<td>A3243G-tRNAleu(UUR)</td>
<td>Yes</td>
<td>Possible</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>Deletion/rearrangement</td>
<td>Yes</td>
<td>Not observed</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>AX296G-tRNAlys</td>
<td>Yes</td>
<td>Not known</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>T14709C in the tRNAglu</td>
<td>Yes</td>
<td>Not observed</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>PPK + deafness</td>
<td>A7445G-non-coding</td>
<td>Yes</td>
<td>Not observed</td>
<td>Yes</td>
<td>Minimal</td>
</tr>
<tr>
<td>Non-syndromic$^a$</td>
<td>A1555G-12S rRNA</td>
<td>Yes</td>
<td>Not observed</td>
<td>Yes</td>
<td>Minimal</td>
</tr>
<tr>
<td></td>
<td>A7445G-non-coding</td>
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<td>Not observed</td>
<td>Yes</td>
<td>Minimal</td>
</tr>
<tr>
<td></td>
<td>Cins7472-tRNAser(UCN)</td>
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<td>Nearly</td>
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<td></td>
<td>T7511C-rRNAser(UCN)</td>
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<td>Ototoxic</td>
<td>A1555G-12S rRNA</td>
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<td></td>
<td>C1494T-12S rRNA</td>
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<td>Not observed</td>
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<tr>
<td></td>
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<td>Multiplasmic</td>
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<tr>
<td>Presbycusis</td>
<td>Random</td>
<td>Not known</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

$^a$ A pathogenic role has been proposed, but not been established, for the T7510C and G7444A sequence changes (see text).
clinical signs (Schon et al., 1997; Chomyn, 1998; Sue et al., 1998).

In 1992 several families with diabetes mellitus and sensorineural hearing loss were described, and surprisingly were found to have inherited the heteroplasmic A3243G mutation in the gene for tRNAleu (UUR) the very same mutation associated with the systemic MELAS syndrome (Reardon et al., 1992; van den Ouweland et al., 1992). In none of these cases were other neurological symptoms present. One family had instead of the 3243 mutation a heteroplasmic large deletion/insertion event (Ballinger et al., 1992), and more recently the heteroplasmic point mutations T14709C in the tRNAglu gene and A8296G in the tRNAlys gene were also found to be associated with maternally inherited diabetes and deafness (Vialettes et al., 1997; Kameoka et al., 1998). This association between diabetes mellitus, hearing loss, and mitochondrial mutations has been confirmed in population studies of diabetic patients (Oka et al., 1993; Alcolado et al., 1994; Kadowaki et al., 1994; Katagiri et al., 1994; Sepehrnia et al., 1995; Newkirk et al., 1997; Rigoli et al., 1997; Guillausseau et al., 2001). Kadowaki et al. (1994), for example, found the 3243 mutation in 2–6% of diabetic patients in Japan, and in three out of five patients with diabetes and deafness. Twenty-seven of their 44 patients with diabetes and the 3243 mutation had hearing loss. The hearing loss is sensorineural and usually develops only after the onset of diabetes. In addition, diabetes mellitus, diabetes insipidus, optic atrophy and deafness have been well described as the Wolfram syndrome, a usually autosomal recessive condition (Cremers et al., 1977), but which may also occur as a consequence of mitochondrial deletions (Rotig et al., 1993; Bu and Rotter, 1993).

Some of the mutations described below in the non-syndromic section have been found associated occasionally with other symptoms. Most prominently, the A7445G in the tRNAser gene mutation was initially described as a non-syndromic deafness mutation, but was subsequently found to be also associated with the skin condition, palmoplantar keratoderma (PPK) in at least some of the cases (Reid et al., 1994a; Fischel-Ghodsian et al., 1995; Sevior et al., 1998). Another mutation in the tRNAser gene has also been found in one family to be associated with ataxia and myoclonus (Tiranti et al., 1995). The most common non-syndromic mutation, the A1555G mutation in the 12S rRNA gene has been described in one family with Parkinson, in another with a constellation of spinal and pigmentary disturbances, and in one case of a woman with a restrictive cardiomyopathy (Shoffner, 1999; Nye et al., 2000; Santorelli et al., 1999). It remains, however, not unlikely that these associations occurred by chance and are not causally related. Similarly, the homoplasmic mitochondrial sequence change A5568G in the tRNAtrp gene was proposed as pathogenic for a family with hearing loss and hypopigmentation, but the evidence for pathogenicity is speculative at this time (Hutchin et al., 2001).

### 3.2. Mitochondrial mutations and non-syndromic hearing loss

The first mutation associated with non-syndromic deafness was identified in an Arab–Israeli pedigree, when the striking pattern of transmission only through mothers was noted (Jaber et al., 1992; Prezant et al., 1993). Most of the deaf family members had onset of severe to profound sensorineural hearing loss during infancy, but a minority of family members had onset during childhood or even adulthood (Braverman et al., 1996). The homoplasmic A1555G mutation in the mitochondrial 12S ribosomal RNA gene was identified as the pathogenic mutation (Prezant et al., 1993). Initially, in all additional pedigrees and individual patients with the same A1555G mutation, the hearing loss occurred only after aminoglycoside exposure (Hutchin et al., 1993; Fischel-Ghodsian et al., 1993, 1997; Matthijs et al., 1996; Pandya et al., 1997; Gardner et al., 1997). Predisposing mutations for aminoglycoside ototoxicity will be discussed more generally in Section 4.3. below. However, subsequently a significant number of pedigrees were described in Spain, with family members who went deaf with and without aminoglycosides (El-Schahawi et al., 1997; Estivill et al., 1998). The age of onset of hearing loss in the Spanish families was rarely congenital, which is different from the Arab–Israeli pedigree. In particular, the study by Estivill et al. is remarkable for two reasons, both of which indicate a higher than previously expected frequency of this mutation. First, it describes 19 families with the A1555G mutation out of a total of 70 families with sensorineural hearing loss collected. Even if the selection of families led to a bias toward
families with multiple affected individuals, and even when only the individuals without aminoglycoside exposure are considered, this represents an unexpectedly high frequency of familial sensorineural hearing loss due to the A1555G mutation. Second, the fact that the mutation was identified on different haplotypes, a finding supported by the study of Torroni et al. (1999) indicates that this mutation exists in other populations as well, and may not be rare. Similarly, in Mongolia the mutation appears to be common, although it is not clear to what extent this is a selection bias due to aminoglycoside exposure (Pandya et al., 2001). However, unpublished results from screening of hearing-impaired populations in other parts of the world seem so far to indicate a very low frequency of the A1555G mutation. In the United States, newborn screening of 1173 anonymized random dried blood spot cards revealed a single case of the A1555G mutation (Tang et al., 2002). Despite that, families with the A1555G and non-syndromic non-ototoxic hearing loss have been described in different places of the world (Usami et al., 1997; Casano et al., 1998; Bu et al., 2000; Feldmann et al., 2001; Mingroni-Netto et al., 2001).

Another close to homoplasmic inherited mutation leading to hearing loss is the A7445G mutation. It was first described in a family from Scotland, and confirmed and established in two unrelated pedigrees from New Zealand and Japan (Reid et al., 1994a; Fischel-Ghodsian et al., 1995; Sevior et al., 1998). In the New Zealand and Japanese pedigrees, a mild form of the skin condition palmoplantar keratoderma also segregates in the maternal line (Sevior et al., 1998). Interestingly, the penetrance of this mutation for hearing loss in the Scottish pedigree is quite low, while in the New Zealand and Japanese pedigrees is very high. Thus, in similarity to the Arab–Israeli pedigree, the mitochondrial mutation by itself does not appear to be sufficient to cause hearing loss, but requires additional genetic or environmental factors, which seem to be rare in the Scottish pedigree and common in the New Zealand and Japanese pedigrees. The difference in penetrance in this situation appears to be due to a difference in mitochondrial haplotype. In the New Zealand pedigree, complete sequencing of the mitochondrial DNA revealed three additional sequence changes in complex I protein genes, two of which have been also labeled as secondary Leber’s hereditary optic neuroretinopathy mutations (Fischel-Ghodsian et al., 1995). Since these or similar sequence changes are not present in the Scottish pedigree (Reid et al., 1994b), mitochondrial haplotype appears to account for the differences in penetrance in this case.

A third mitochondrial mutation, a cytosine insertion at position 7472 in the tRNAser (UCN) gene, was identified in one large Dutch family (Verhoeven et al., 1999). The same mutation had been previously described in a Sicilian family with hearing loss, some of the family members having also other neurological symptoms, such as ataxia and myoclonus (Tiranti et al., 1995). In the Dutch family, the hearing loss is sensorineural progressive with onset in early adulthood. Most of the individuals over 30 years of age were deaf, indicating that the penetrance in this family is high. The mutation is heteroplasmic, although most individuals have over 90% of abnormal mitochondrial chromosomes in the tissues examined. More recently, a large African American pedigree with maternal inheritance and non-syndromic hearing loss has been identified (Friedman et al., 1999), and shown to have a close to homoplasmic T7511C mutation in the tRNAser (UCN) gene (Sue et al., 1999). The same mutation was subsequently found in a Japanese and two French families (Ishikawa et al., 2001; Feldmann et al., 2001). Also, a British family with a T7510C mutation, and non-syndromic deafness was described, although the mitochondrial chromosome was not fully evaluated (Hutchin et al., 2000). Lastly, the G7444A substitution has been described in deaf individuals with and without the A1555G mutation, but its pathogenicity has not been established (Pandya et al., 2001).

3.3. Pathophysiology of hearing loss due to mitochondrial DNA mutations

Mitochondrial DNA is essential for normal ATP production in nearly every cell of the body, and thus it is not unexpected that hearing loss occurs in systemic neuromuscular disorders due to mitochondrial DNA mutations. Similarly, in syndromic hearing loss with heteroplasmic mitochondrial DNA mutations, it is possible to speculate that the distribution of abnormal mitochondrial DNA molecules is responsible for the phenotype. For the homoplasmic mitochondrial DNA mutations associated with non-syndromic hearing
loss, at a first glance, it is possible to speculate that mitochondrial mutations interfere with energy production, that the cochlea is highly dependent on sufficient energy production, and that insufficient energy production leads to degeneration of cochlear cells. However, the cochlea is not the most energy-dependent organ in the body, and in the systemic neuromuscular disorders, the extraocular muscles appear to be the most energy-sensitive cells, and hearing loss is certainly not the most prominent clinical sign. Thus, in order to understand the pathophysiological pathways leading from the mitochondrial mutations to hearing loss, two major biological questions need to be answered: Why does the same mutation cause severe hearing loss in some family members but not in others (phenotypic expression), and why is the ear the only organ affected (tissue specificity)?

Study of the mitochondrial mutations leading to hearing loss has led to three possible precipitating factors modulating phenotypic expression, and it is likely that a combination of them also play a significant role in the phenotypic expression of acquired mitochondrial disorders. The first such factor involves environmental agents, and aminoglycosides are the prime example as a triggering event in the case of the 1555 mutation. It is not unlikely that other, as yet unrecognized environmental factors, could play similar, but perhaps less dramatic, roles. Diet and drugs affecting oxygen radical formation and breakdown come to mind. The second factor involves the mitochondrial haplotype, and, as noted above, the 7445 mutation provides a dramatic example of that effect. The third factor involves nuclear genes. The Arab–Israeli pedigree and some of the Spanish and Italian pedigrees are good examples of the role of nuclear genes. For example, the entire Arab–Israeli family lives in the similar environmental surroundings of a small Arab village in Israel, and all maternal relatives share the same mitochondrial haplotype. Biochemical differences between lymphoblastoid cell lines of hearing and deaf family members with the identical mitochondrial chromosomes provide direct support for the role of nuclear factors (Guan et al., 1996). An extensive genome wide search has led to the conclusion that this nuclear effect is unlikely to be due to the effect of a single nuclear locus but involves a number of modifier genes (Bykhovskaya et al., 1998, 2000). The chromosomal location of one of these modifier genes has been identified, and linkage disequilibrium has been obtained in families from varied ethnic backgrounds (Bykhovskaya et al., 2000, 2001).

Additional nuclear-encoded putative modifier genes have been identified using a candidate gene approach (Bykhovskaya et al., 2003). Thus, the model that emerges for explaining penetrance is a threshold model, where a combination of environmental, mitochondrial and nuclear factors can push a cell over a threshold, with dramatic clinical differences on either side of this threshold.

The second major biological question relates to tissue specificity: If a homoplasmic mutation affects oxidative phosphorylation (the only known function of the human mitochondrial chromosome and an essential process in every nucleated cell of the human body), it is unclear how the clinical defect remains confined to the cochlea, rather than affecting every tissue. We propose that cochlea-specific isoforms or splice-variants involved in mitochondrial RNA processing or translation interact abnormally with the mutated rRNA, tRNA, or polycistronic mRNA, and lead to qualitative or quantitative changes in the protein products. Different processing of mitochondrial RNA and protein, leading to tissue specific defects or functions have been described. Several examples of tissue specificity in oxidative phosphorylation and of tissue specific secondary functions of mitochondrial RNAs exist. Tissue-specific subunits for oxidative phosphorylation have been described (Arnaudo et al., 1992). Even more relevant is the case report of a 22-year-old patient who died from respiratory failure due to a mitochondrial myopathy. It was shown that the causative mutation in the mitochondrial trNAleu (UUR) gene causes a RNA processing defect in skeletal muscle but not in the patient’s fibroblasts (Bindoff et al., 1993), raising the possibility of a skeletal muscle specific mitochondrial RNA processing gene. Examples of secondary function include the mitochondrial large ribosomal RNA gene in Drosophila melanogaster, which in addition to being involved in mitochondrial translation, can also be processed in a few cells for export into the cytoplasm where it induces pole cell formation in embryos, a key event in the determination of the germ line (Kobayashi et al., 1993). Similarly, in the mouse
the ND1 protein can be processed in two different ways, part of it being presented on the cell membrane with a minor histocompatibility protein (Wang et al., 1991).

It can be hoped that the identification of the nuclear modifier genes through genetic positional cloning or candidate gene testing will shed light on the pathophysiological pathways leading from the mitochondrial mutation to hearing impairment, and provide targets for prevention and therapy. In addition, the recent identification of the first mouse model of a naturally occurring pathogenic mitochondrial DNA mutation provides a ready experimental model to dissect the complex genetic factors and interactions (Johnson et al., 2001). In that model, a mitochondrial DNA mutation in the tRNA-Arg gene worsens hearing impairment when combined with the nuclear-encoded Ahl gene locus on mouse chromosome 10, which has been described as a major gene for age-related hearing loss in mice (Johnson et al., 2000, 2001). It is possible that the pathways to hearing loss are similar in mice and humans, and thus the human homologues of all nuclear genes and/or pathways identified in mice will become candidates for testing in humans.

4. Acquired mitochondrial hearing loss

4.1. Noise-induced hearing loss

4.1.1. Mechanisms for mitochondrial injury secondary to noise

4.1.1.1. Glutamate excitotoxicity. In a manner analogous to CNS excitotoxicity, acoustic overexposure may initiate mitochondrial damage as the result of glutamate excitotoxicity (Vercesi et al., 1997; Castilho et al., 1998, 1999). Glutamate excitotoxicity is triggered primarily by massive Ca^{2+} influx arising from over stimulation of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors. Mitochondrial damage results from calcium overload in the mitochondria (Pereira and Oliveira, 2000). Calcium overload disrupts mitochondrial cristae and internal membranes (Chandrasekaran et al., 2002). Mitochondria are primary targets for excitotoxicity evidenced by confocal imaging of intracellular Ca^{2+} and mitochondrial membrane potential (Schinder et al., 1996). Glutamate excitotoxicity in neurons involves essentially two components. The first component marked by acute neuronal swelling depends on the uptake of extracellular Na^{+} and Cl^{-} by the cell that causes plasma membrane depolarization and Ca^{2+} channel opening. This triggers the second component, which is marked by delayed neuronal degeneration. The massive influx of extracellular Ca^{2+}, together with any Ca^{2+} release triggered from intracellular stores, increases cytosolic-free Ca^{2+} and initiates a cascade-like effect leading to cell death. Excitotoxicity, induced by an excessive release of glutamate by the IHC, causes afferent nerve ending swelling with a disruption of the postsynaptic structures. After repetitive noise stimulation, excitotoxicity may develop metabolic events triggered by the entry of Ca^{2+}, which leads to neuronal death in the spiral ganglion (Puel, 1995; Pujol et al., 1995).

4.1.1.2. Oxidative stress. Oxidative stress associated with glutamate excitotoxicity, mitochondrial respiration and other events that increase ROS, also causes mitochondrial damage. In early stages of injury, oxidative stress may affect the mitochondria and cause auditory functional impairment prior to HC death. Early elevation of cochlear ROS was observed following noise exposure (Ohlemiller et al., 1999). Dilated mitochondria were observed in the afferent nerve endings after auditory overstimulation (Omata and Schatzle, 1984). Initially, ROS production could reduce mitochondrial respiration making mitochondria vulnerable targets of ROS (Lenaz et al., 1998; Poderoso et al., 2000). The increasing ROS production is expected to deplete cellular antioxidant defenses, leading to a general enhancement of oxidative stress and radical-mediated injury throughout the cell (Ciani et al., 1996).

4.1.1.3. Glutathione depletion. Glutathione (GSH) in mitochondrion is an important antioxidant defense. A small fraction of the total cellular pool of GSH is sequestered in mitochondria by the action of a carrier that transports GSH from cytosol to the mitochondrial matrix. Depletion of mitochondrial GSH renders the cell more susceptible to oxidative stress (Fernandez-Checa et al., 1998). Buthionine
sulfoximine (BSO) was used to block GSH synthesis. The GSH-depressed ears showed more vulnerability to noise (Henderson et al., 1999). GSH was diminished in HCs post noise exposure (Kopke et al., 1999).

4.1.2. Indirect evidence for a prominent role of mitochondrial injury in NIHL

Indirect evidence published in the literature indicates that the mitochondria play a prominent role in NIHL. Hyde and Rubel (1995) evaluated the role of mitochondrial biogenesis in HC survival after injury by inhibiting mitochondrial protein synthesis with chloramphenicol. Addition of chloramphenicol to noise exposure significantly increased HC loss by 80% demonstrating that mitochondrial biogenesis is involved in cellular responses to injury. Kopke et al. (2002) studied the use of acetyl-L-carnitine, carbamathione and d-methionine as protective agents in a chinchilla model of NIHL. Acetyl-L-carnitine (ALCAR) was chosen because of its capacity to enhance mitochondrial bioenergetics and repair in the face of oxidative stress. ALCAR serves as a precursor for acetyl-CoA, a mitochondrial energy substrate, and restores a key mitochondrial lipid, cardiolipin, in oxidatively injured cells, further restoring mitochondrial integrity. Permanent threshold shifts and HC loss were significantly reduced in animals pre-treated with these metabolites (Figs. 1 and 2). These data suggest that oxidative stress, glutamate excitotoxicity, impaired mitochondrial function, and GSH depletion, play a role in cochlear injury induced by noise. Similar results were noted when pre-treated with N-acetylcysteine (NAC) to bolster cochlear antioxidant defenses (Kopke et al., 2000).

HC mitochondria are damaged after noise exposure. Kopke et al. (2003) reported localized destruction of mitochondrial cristae in cochlear HCs 2 h after noise exposure. Three weeks post-noise exposure, cristae exhibited shortening, blurring and dissolution. Mitochondria showed extreme swelling and vacuolization. The external and internal membranes were destroyed, and numerous mitochondria were ruptured. Mitochondrial damage was very limited in the apical turn, apparent in the middle turn and severe in the basal turn. These authors also reported that HC mitochondrial injury was reduced with ALCAR treatment (Kopke et al., 2004). Compared to saline treated, noise-exposed animals where most of the mitochondria were damaged in the area of noise injury to the cochlea, ALCAR-treated animals demonstrated normal appearing mitochondria (Fig. 3). The volume density of normal-appearing mitochondria decreased both in IHC and OHC after noise exposure in saline-treated animals ($P<0.01$) but was preserved with ALCAR treatment ($P<0.01$) three weeks post noise exposure (Fig. 4).

Noise injury is associated with release of cytochrome c and activation of caspases, and induction of programmed cell death (PCD) leading to the loss of OHC. Hu et al. (2002) reported involvement of the apoptotic pathway in the progression of OHC death in the chinchilla cochlea following noise exposure. OHC apoptosis developed asymmetrically toward the apical and basal parts of the cochleae following the noise exposure. Two days after the noise exposure, there was still active OHC pathology with condensed and fragmented nuclei in the basal part of the cochleae. Caspase-3 activation, an intracellular marker for apoptosis, showed a spatial agreement with the apoptotic nuclei (Fig. 5). Nicotera et al. (2003) further reported that intense noise exposure causes OHC death primarily through apoptosis. The authors investigated the apoptotic signal pathways after noise exposure by examining the activity of each of three caspases, including caspase-3, -8, or -9 with carboxyfluorescein-labeled fluoromethyl ketone (FMK)-peptide inhibitors. The cochleae were further examined for cytochrome $c$ release from mitochondria by immunohistology and for DNA degradation by the TUNEL method. Noise exposure triggered the activation of caspase-8 and -9 leading to activation of caspase-3. Caspase activation occurred only in the apoptotic OHCs and not in the necrotic OHCs (Fig. 6). These results indicate that multiple signaling pathways leading to caspase-3 activation take place simultaneously in the apoptotic OHCs. Noise exposure also caused the release of cytochrome $c$ from mitochondria. In contrast to activation of caspases, the release of cytochrome $c$ took place in both apoptotic and necrotic OHCs (Fig. 7). Moreover, the release of cytochrome $c$ in a subpopulation of OHCs took place early in the cell death process, prior to any outward signs of necrosis or apoptosis.
Fig. 1. (Right) (A) Auditory threshold shifts for saline-treated and ALCAR-treated animals. Mean threshold shifts plotted for treatment groups (saline-noise and ALCAR-noise treatment), time [1 h (or 0 week), or 1, 2, 3 weeks post noise] by test frequency for 2, 4, 6 and 8 kHz. Noise exposure was six continuous hours of 105 dB SPL octave band noise centered at 4 kHz. Initial threshold shifts (week 0) were from 65 to 97 dB for both treatment groups over the test frequencies. There was an overall treatment effect for the ALCAR-noise group compared with the saline-noise group ($P < 0.001$) for all test frequencies beginning at week one. Error bars are ± SEM. Sample ($n$) size is 12 ears (six animals) for this and B and C data. (B) Outer hair cell cytocochleogram data. Depicted are means ± SE (SE, shaded area) cytocochleograms for outer hair cells (OHCs) of noise-exposed animals pre-treated with either ALCAR (solid line) or saline (dotted line). The $Y$-axis is mean percent missing OHCs. The lower $X$-axis represents percent distance from the cochlear apex, and the upper $X$-axis is the associated frequency range of the cochleae in kHz. Very little OHC loss occurred in the ALCAR-protected cochleae (less than 10%), whereas there is substantial OHC loss ($P < 0.001$) in the saline-noise-exposed group (about 60–70% for the 4–10 kHz region). (C) Inner hair cell cytocochleogram data. Illustrated are the mean inner hair cell (IHC) cytocochleograms with missing IHC percentages on the $Y$-axis as a function of the measured percent distance from the cochlear apex (axis are the same as in B). ALCAR treatment (solid line) afforded significant protection ($P < 0.01$) of IHCs (3% or less loss verses 10–30% in the saline-treated animals). (Left) Postulated cell death pathway from mitochondrial injury: cytochrome $c$ release to activation of caspases leading to apoptosis of the sensory cells. An injury (noise) generates oxidative stress (ROS and free radicals), which can activate a stress kinase cell death pathway (c-JNK and c-Jun) triggering BIM and damaging both the inner and outer mitochondrial membranes. Membrane damage (pores) allows cytochrome $c$ release into the cytoplasm to interact with APAF-1 converting inactive caspase-9 to an activated form. These in turn activate different downstream effector caspases to degrade membrane lipids, proteins and nuclear DNA resulting in apoptotic cell death. Bcl-2 and BclxL act to inhibit pore formation (anti-apoptotic) while BIM (pro-apoptotic) acts to inhibit the mitochondrial membrane stabilizing action of Bcl-2 and BclxL. Bax acts directly (pro-apoptotic) to form pores in the mitochondrial membranes. (Kopke et al., 2002, used with permission).
4.2. Cisplatin ototoxicity

As with noise, the cancer chemotherapy agent, cisplatin, may induce oxidative stress. Cisplatin, a relatively strong ototoxin, will damage HCs (particularly OHCs), the stria vascularis (Tsukasaki et al., 2000), and auditory neurons (Zheng and Gao, 1996), making cisplatin-induced deafness the dose-limiting side effect of this otherwise useful drug (Blakley et al., 2002). In vitro studies have indicated that exposure of cochlear neuroepithelium to cisplatin leads to the production of ROS and depletion of HC GSH followed by HC death and that these processes can be prevented by a variety of antioxidant compounds (Kopke et al., 1997; Feghali et al., 2001). Theories regarding the genesis of this cisplatin-induced oxidative stress include DNA damage induced by cisplatin (Saito et al., 1997), interference with the glutathione antioxidant defense system (Rybak et al., 1995), or increases in lipid peroxidation (Teranishi et al., 2001). The consequences of this oxidative stress can include HC loss and permanent deafness.

Devarajan et al. (2002) examined the mechanisms of auditory HC death induced by cisplatin in an immortalized OHC line from mice. They noted involvement of both cell death receptor and mitochondrial-based cell death apoptotic mechanisms. Cisplatin induced truncation of Bid and upregulation of p53.
coincident with the activation of caspase-8. Furthermore, cisplatin induced mitochondrial membrane permeability transition and loss of cytochrome c from the mitochondria into the cytosol along with activation of Bax. Caspase-8 activation peaked at 6-h post cisplatin exposure, and this was followed later by caspase-9 activation. The result of these processes was dose- and duration-dependent apoptosis of the HCs in culture. These findings suggest an induction of apoptosis caused by cisplatin involving mitochondrial and possibly cell death receptor pathways. Wang et al. (2003a) have recently made pertinent and interesting observations about the mechanisms of cisplatin-induced cochlear injury in vivo. Using a locally applied thiol protective agent, they were able to show the prevention of cisplatin-induced mitochondrial damage, cytochrome c release, DNA fragmentation and apoptotic or necrotic HC death. Besides preventing the morphologic and biochemical changes caused by cisplatin, they were also able to preserve hearing function.

4.3. Aminoglycoside ototoxicity

Aminoglycoside antibiotics are well known ototoxins. Three mitochondrial DNA mutations confer an increased susceptibility to aminoglycoside antibiotics. These mutations have recently been reviewed (Fischel-Ghodsian, 2004), and include the A1555G, the ΔT961Cn, and the C1494T mutations in the 12S rRNA gene. While these known predisposing mutations account for a significant proportion of cases, most cases are probably sporadic and related to receiving a generally toxic dose of the drug. As with cochlear damage due to noise and cisplatin,

![Fig. 3. Saline-treated noise exposed animals demonstrated that almost all of the mitochondria were damaged in the area of noise injury to the cochlea. ALCAR-treated animals, in contrast, demonstrated normal appearing mitochondria. (A) Normal mitochondria (no noise exposure), (B) damaged mitochondria (noise exposure, saline control), (C) normal appearing mitochondria (noise exposure, ALCAR-treated).](image)

Fig. 4. Mitochondrial volume density of normal-appearing mitochondria decreased both in IHC (A) and OHC (B) after noise exposure in saline treated animals ($P < 0.01$) and were preserved with ALCAR treatment compared to noise saline ($P < 0.01$) 3 weeks post noise exposure.
permanent hearing loss related to the destruction of initially OHCs, and then IHCs, by aminoglycosides, is associated with the induction of oxidative stress. A gentamicin–iron complex is felt to be responsible for the generation of ROS in cellular systems (Priuska and Schacht, 1995; Sha and Schacht, 1999a,b) and a gentamicin-induced increase of ROS in cochlear explants has been measured (Clerici et al., 1996). In addition, variation in antioxidant levels including GSH can modulate the cochlear damage caused by aminoglycosides (Lautermann et al., 1995). Toxic levels of ROS can in turn lead to HC death through apoptosis (Nakagawa et al., 1998; Forge and Fradis, 1985) or necrosis, and mitochondria appear to play an important role in these processes.

An early indication that mitochondria play a role in gentamicin-induced HC injury was the report that inhibition of mitochondrial homeostatic mechanisms accentuated gentamicin-induced auditory HC death (Hyde and Rubel, 1995). Gentamicin was shown to cause cytochrome c release and apoptosis-inducing factor by inducing mitochondrial membrane permeability transition in liver mitochondria (Mather and Rottenberg, 2001). Cytochrome c is released from vestibular HC mitochondria after gentamicin exposure in vivo (Nakagawa and Yamane, 1999). All of these data are consistent with earlier observations that gentamicin can decrease inner ear mitochondrial respiration (Sato et al., 1969, Ann. Otol. Rhinol) and cause degeneration of mitochondria in cochlear HCs of guinea pigs (Wersall et al., 1973).

Dehne et al. (2002) reported that lower concentrations of gentamicin induced HC death through a process of apoptosis. Preceding the cell death
there was a consistent nearly universal loss of mitochondrial membrane potential noted in the HCs. Cyclosporin A was able to protect the tissue from gentamicin toxicity implicating involvement of the mitochondrial permeability pore in the pathologic process. Dehne et al. suggested that in their in vitro model, the major apoptotic pathway involved the ROS-mediated induction of mitochondrial membrane permeability transition. However, others have described receptor-mediated processes leading to the activation of the Jun kinase pathway of apoptosis (Pirvola et al., 2000). The sum of the data accumulated by these reports confirms the notion that mitochondrial injury caused by gentamicin-induced oxidative stress plays a prominent role in aminoglycoside ototoxicity.

4.4. Solvents, asphyxiants, and acrylonitrile

A variety of workplace chemicals are potentially ototoxic if exposures are above certain levels. These include solvents such as trichloroethylene, xylene, styrene, hexane, carbon disulfide, toluene, as well as carbon monoxide, and a variety of heavy metals (Rybak, 1992). It has been estimated by NIOSH that some 9.8 million workers are regularly exposed to such organic solvents (NIOSH, USDHHS, PHS, CDC, 1987). Of concern is the ability of many of these toxins to potentiate the ototoxicity of noise. Sub-toxic doses of toxins and noise when experienced together may cause hearing loss that would not be seen with either agent alone, or the hearing loss associated with a noise exposure may be worse than expected if there is a concomitant exposure even to ‘safe’ levels of toxin. For example, in animal studies, Fechter et al. (2000, 2002) have shown that the asphyxiants carbon monoxide (CO) and hydrogen cyanide (HCN) can potentiate NIHL. Model-based calculations extrapolated from their data suggested that the potentiation of noise damage could be seen with current permissible levels of these two asphyxiants (Fechter et al., 2000, 2002). Additionally, toluene has been reported to enhance cochlear noise injury in rat (Johnson et al., 1988, 1990; Lataye and Campo, 1997) and human (Morata et al., 1993). Another solvent, styrene, has also been reported to potentiate NIHL (Lataye et al., 2000; Campo et al., 2001).

As suggested by Fechter (1995) additive effects of combined noise and toxin damage may stem from the possibility that the noise and toxin may each damage a different part of the auditory pathway (i.e. hexane damages the central auditory pathway while...
noise damages the cochlea). Noise may enhance the toxicity of some chemicals by increasing cochlear blood flow thus enhancing the exposure of the toxin to the cochlear tissue, or noise damage might render the cochlea unable to exclude toxins that could have been excluded from the cochlea without injury, or the enhanced activity of the cells undergoing acoustic stimulation might make them more susceptible to a toxin (Fechter, 1995). In addition, the fact that some of these toxins potentiate the cochlear damage associated with noise exposure suggests the possibility that noise and the toxins share common injury mechanisms. In fact, evidence is accumulating that a common theme for the toxin noise interaction is oxidative stress in some cases (Fechter et al., 1997, 2003; Rao et al., 2001). Fechter et al. (1997) reported that the free radical scavengers PBN or allopurinol prevented hearing loss caused by carbon monoxide
suggesting that free radical generation plays an important role in CO cochlear injury. Additional work has demonstrated that another free radical scavenger, POBN, was protective against the combined toxicity of noise and CO (Rao et al., 2001). These authors also found evidence for the formation of free radical spin adduct using electron paramagnetic resonance spectroscopy. Acrylonitrile (ACN), a common potential manufacturing toxin, potentiated NIHL in Long–Evans rats and was found to lower cochlear GSH levels during the time course of the noise exposure (Fechter et al., 2003).

As stated previously in this review, oxidative stress, the generation of free radicals and the depletion of GSH, can all injure mitochondria enhancing further ROS production, depleting cellular energy, and eventuating in cell death. However, many of these toxins may also primarily injure mitochondria. It may then be possible that toxins potentiate cochlear noise damage because they both injure this important organelle. For example, a metabolite of ACN is hydrogen cyanide HCN. HCN can interfere with electron flow through the respiratory chain in mitochondria by inhibiting cytochrome c oxidase (Fechter et al., 2002, 2003; Jiang et al., 1998). It has also been postulated that solvents like toluene may cause brain toxicity by damaging mitochondrial membranes and interfering in that way with oxidative phosphorylation, in addition to altering mitochondrial membrane permeability directly (Garbe and Yukawa, 2001). Thus it can be seen that there is accumulating evidence that a number of industrial ototoxicants damage the ear or potentiate NIHL through oxidative stress mechanisms. More work needs to be done, but it appears that cochlear mitochondria are a plausible target of injury by these toxins primarily or secondarily through oxidative stress caused by the toxins.

4.5. Presbycusis

Oxidative damage has been implicated to be a major factor in the decline in physiologic function that occurs during the aging process. Decreased activity of electron transport chain complexes and increased release of ROS from the mitochondria with age suggest that alterations in mitochondrial function occur with age as a consequence of increased oxidative damage (Van Remmen and Richardson, 2001; Staecker et al., 2001). Alterations in mitochondrial turnover with age could also contribute to an increase in the number of dysfunctional mitochondria. Mutations and deletions within the mtDNA occur with increasing frequency in age and presbycusis (Fischel-Ghodsian et al., 1997; Seidman et al., 2000). When enough mtDNA damage accrues, the cell becomes bioenergetically deficient. This mechanism is the basis of the mitochondrial clock theory of aging, also known as the membrane hypothesis of aging (Seidman et al., 2000).

It is proposed that treatment with antioxidants or dietary restriction can attenuate age-related hearing loss. Nutritional compounds have been identified that enhance mitochondrial function and reverse several age-related processes. Seidman (2000) provide evidence that long-term treatment with compounds such as vitamin E, vitamin C, melatonin and lazaroid that block or scavenge reactive oxygen metabolites attenuate age-related hearing loss and reduce the impact of associated deleterious changes at the molecular level. The antioxidant-treated subjects had improved auditory sensitivities, and a trend toward fewer mtDNA deletions. Compounds that upregulate mitochondrial biogenesis in an aging model may improve hearing and reduce some of the effects of aging. Seidman et al. (2000) describe the effects of two mitochondrial metabolites, alpha-lipoic acid and ALCAR, on the prevention of age-related hearing loss. This effect may be related to the mitochondrial metabolite’s ability to protect from and repair age-induced cochlear mtDNA damage. Further study is warranted.

5. Therapeutic implications

(a) Prevention of aminoglycoside ototoxicity. The ototoxicity associated with the inherited mitochondrial mutations is preventable through a combination of taking family histories and molecular screening. In the future, the elucidation of the genetic factors predisposing to aminoglycoside ototoxicity will result in the development of non-toxic aminoglycoside analogs or in treatment strategies that prevent irreversible cochlear damage.

(b) Antioxidant approach. Oxidative damage has been implicated to be a major factor in the aging,
Decrease glutamate excitotoxicity. Glutamate excitotoxicity can be reduced by antagonizing the action of cochlear-methyl-D-aspartate (NMDA) receptors using carbamathione or other agents such as memantine and MK801 (Bienkowski et al., 2000).

e) Enhance mitochondrial biogenesis. NIHL may be caused by a defect in mitochondrial bioenergetics and biogenesis. Therefore, sensorineural hearing loss may be reduced by acetyl-L carnitine (ALCAR), an endogenous mitochondrial membrane compound that helps maintain mitochondrial bioenergetics and biogenesis in the face of oxidative stress.

(f) Cell death inhibition. Noise exposure is known to result in oxidative stress, which contributes to both the apoptotic and necrotic HC death. Blocking cell death pathway activation may be a useful strategy to reduce hearing loss (Pirvola et al., 2000; Hu et al., 2002; Wang et al., 2003b; Salvi et al., 1998).

6. Summary and conclusions

An increasing body of evidence supports that mitochondrial dysfunction plays an important role in both inherited and acquired hearing loss. Mitochondrial dysfunction is enhanced by glutamate ototoxicity, increasing ROS, oxidative stress and glutathione depletion. Preventive and therapeutic strategies to prevent or reduce hearing loss due to mitochondrial dysfunction are feasible and are currently being studied in humans.

7. Disclaimer

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, Department of Defense, or the United States Government.

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