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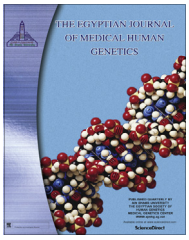

REVIEW

Aminoglycoside induced ototoxicity associated with mitochondrial DNA mutations

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Abstract Despite the risk of permanent ototoxic effects, aminoglycosides remain commonly utilized antibiotics worldwide due to low cost and efficiency in treating severe infections. Over the last two decades, mitochondrial mutations have been shown to enhance the likelihood of ototoxic injury. In particular the 1555A > G mutation in the mitochondrial gene *MTRNR1* has been strongly associated with the onset of aminoglycoside-induced deafness; though pinning down the exact mechanism of action has thus far been elusive. Clinically aminoglycoside-induced deafness has been characterized by variation in the degree of hearing loss, which has prompted an investigation into genetic modifiers. To date, several putative mutations have been categorized as contributing factors to the onset of deafness with no single variation being sufficient to bring about hearing loss. Meanwhile current methods to mitigate the risk of ototoxic injury are in various stages of development. Efforts to alter the molecular structure of aminoglycosides have shown a potential path to reducing ototoxicity while preserving antibacterial properties, but these drugs are not clinically available. On the other hand, application of preemptive audiometry provides the most readily available method to both monitor and reduce the extent of aminoglycoside-induced deafness.

Contents

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1. Introduction

Hereditary hearing loss is the most common sensory problem, affecting 1 and 1000 newborns [1]. While around half of congenital deafness is explained genetically, hearing loss has also been linked to environmental factors such as *in utero* contraction of cytomegalovirus and antibiotics [2]. Originally developed in the 1940's aminoglycosides (AG) have been effective in treating gram-negative bacterial infections [3]. Unfortunately though, systemic administration of AGs can lead to both nephrotoxicity and ototoxicity [4,5]. In populations where AGs were commonly prescribed familial inheritance of ototoxicity became evident. Matrilineal inheritance of aminoglycoside-induced deafness (AID) was a defining characteristic in several of these families, which suggested mitochondrial involvement [6].

Currently the genetic mutations most strongly associated with AID are 1555A > G and 1494C > T in the mitochondrial gene *MTRNR1*, which codes for the mitochondrial 12S ribosomal subunit [7,8]. These mitochondrial positions map to the corresponding 1409–1491 base pairing in bacteria, which are the AG binding sites on the 16S ribosome subunit [9]. Several additional variations in mitochondrial tRNA genes have been associated with AID and non-syndromic hearing loss [10–12], though biochemical analysis of these variations suggests many are not sufficient to generate the hearing loss phenotype. While the effect of systemic AG administration in individuals carrying a mitochondrial tRNA gene mutation is not well established, it is clear that genetic defects in the mitochondrial *MTRNR1* gene at positions 1555 and 1494 facilitate AG-induced deafness [13].

Modifiers in the nuclear genome may provide an explanation for familial cases of non-syndromic deafness involving the 1555A > G mitochondrial mutation and absence of AG treatment. The nuclear gene *TRMU* has been identified as one such modifier, as a missense mutation in *TRMU* was shown to compromise tRNA metabolism. Reduction of tRNA metabolism in combination with the reduction of accurate protein synthesis caused by the 1555A > G mutation may be sufficient to cause the hearing loss phenotype, though this is yet to be fully elucidated [14].

As AGs continue to be prescribed to treat common infections around the world, reducing or removing the potential for ototoxicity holds significant clinical value. While concomitant treatment of AGs and antioxidants yields moderate protective properties, recent research on structurally unique AGs shows promise in reducing ototoxicity [15–18]. Increasing the size of AGs prohibits entry to the cytosol of cochlear hair cells while maintaining its potent antibacterial properties. As the physical properties of the cationic channels of hair cells are further characterized, these data may lead to improvements in AG alteration, potentially removing the concern of ototoxicity.

2. Mechanism of aminoglycoside ototoxicity

Localization of aminoglycosides to hair cells begins by crossing the blood-endolymph barrier [19]. Once in the endolymph AGs transverse the apical membrane of hair cells via the mechanoelectrical transducer (MET) channel or other cationic channels [20–23]. AG entrance through the MET channel was initially proposed after the detection of AGs at the tip of the stereocilia prior to observing AG in the cytosol of hair cells [24]. Further investigation revealed the opening of the channel measures 1.25 nm in diameter which is sufficient for the passage of AGs [25]. Alharazneh et al. has also shown that AGs reduce the transduction current pointing to the involvement of the MET channel [21]. Moreover, various concomitant factors have been shown to potentiate AID. Noise induces the open state of the MET channel leading to an increased concentration of AGs in the hair cell cytosol [26]. Hirose et al. reports the combined administration of aminoglycosides with loop diuretics exacerbates ototoxic injury in mice when compared with controls and mice injected only with aminoglycosides [27]. Cochlear uptake of aminoglycosides is also increased by endotoxemia-induced inflammation [28]. The unidirectional MET channel compounds the effect of these additional potentiating factors, as AGs are unable to move back to the endolymph leading to higher concentration in the hair cell cytosol [23].

Once in hair cell cytosol AGs interact with mitochondrial 12S ribosomal subunit causing inaccurate translation of mitochondrial proteins. The downstream implication of faulty protein synthesis is eventual cell death either through caspase-mediated or non-caspase-mediated apoptosis [22]. Additionally, AG interaction with iron species has been shown to produce reactive oxygen species (ROS), which also leads to cell death through apoptosis [29]. Previous *in vitro* experiments have shown an alteration in antioxidant defense system in melanocytes induced by aminoglycosides, which may be an additional contributing factor in the onset of ototoxicity [30]. Rizzi et al. has also shown a clear progression of hair cell death originating at the basal turn and moving toward apical turn of the cochlea [31]. This progression moves in line with the onset of deafness, as high frequency loss is observed before the loss of lower frequencies.

3. Increased susceptibility to aminoglycoside ototoxicity due to mitochondrial mutations

The baseline incidence of cochleotoxic effects following the administration of systemic AGs has previously been reported between 2% and 25%, and has been known to fluctuate depending on the drug administered [31,32]. Amikacin, Gentamicin, and Tobramycin produce ototoxic effects at a rate of 5%, 8%, and 14% respectively [32]. The incidence of AID increases to 17–33% in cases involving genetic variations [22]. In particular the 1555A > G and 1494C > T mutations in the *MTRNR1* gene have been shown to facilitate the binding of AGs to the 12S ribosomal subunit [13].

Prezant et al. initially sequenced the mitochondrial genome in three families with maternally inherited AID revealing the 1555A > G variation in the mitochondrial *MTRNR1* gene [7]. Over the last two decades 1555A > G has been identified as

Table 1 1555A > G frequencies found during mitochondrial screening of hearing impaired people in various different populations.

Country	City or Province	1555A > G/Families Screened	Controls	AID cases in Cohort	Homoplasmy	Type of Deafness	Reference
European Cohort	–	18/9371 (0.19%) 1/520 population level	–	–	–	–	[50]
China	Xiamen	6/155 (3.87%)	0/–	4	Homoplasmic	NSHL	[51]
Finland	–	1/103 (0.97%)	–/–	–	–	SHL and NSHL	[52]
China	–	10/538 (1.85%)	0/256	10	Homoplasmic	NSHL	[53]
China	–	36/301 (12%)	–/–	–	–	Familial NSHL	[54]
China	Hubei	95/813 (11.68%)	0/126	78	Homoplasmic	HL	[55]
Taiwan	Zhongzheng	1/1017 (0.09%)	–/–	–	Homoplasmic	Newborn screening	[56]
Greece	–	2/513 (0.38%)	–/–	–	–	Various	[57]
Morocco	–	3/84 (3.57%)	0/100	0	Homoplasmic	NSHL	[58]
Turkey	–	3/168 (1.8%)	–	–	–	NSHL	[59]
China	–	5/284 (1.76%)	0/200	5	–	NSHL	[60]
Poland	–	1/250 (0.4%)	–/–	–	–	Unknown	[61]
Belgium	–	2/466 (0.42%)	0/400	1	Homoplasmic	NSHL	[62]
Korea	–	2/227 (0.88%)	0/217	–	Heteroplasmic/ Homoplasmic	NSHL	[63]
Italy	–	9/167 (5.38%)	–/–	9	Homoplasmic	NSHL	[64]
China	Gansu	44/514 (8.56%)	0/117	23	–	NSHL	[65]
United States	–	7/41 (17%)	–	–	–	NSHL	[66]
Taiwan	–	10/315 (3.17%)	–/120	2	Homoplasmic	NSHL	[67]
China	–	8/60 (13.33%)	0/354	60	Homoplasmic	AID	[68]
Spain	–	69/443 (15.57%)	–/100	24	–	NSHL	[69]
Tunisia	–	1/100 (1%)	0/100	0	–	NSHL	[70]
Brazil	–	5/204 (2.45%)	0/300	1	–	Various NSHL	[71]
Spain	–	42/209 (20%)	0/200	–	–	NSHL	[72]
United Kingdom	–	2/80 (2.5%)	–/–	–	–	NSHL	[73]
United States	Cincinnati	1/164 (0.6%)	–/226	0	Homoplasmic	NSHL	[74]
Spain	–	105/648 (16.2%)	–/–	2	Various	NSHL	[75]
Indonesia	Sulawesi	4/75 (5.33%)	0/100	–	Homoplasmic	NSHL	[76]
Denmark	–	2/85 (2.4%)	–	–	–	NSHL	[77]
Finland	–	2/117 (1.7%)	–	–	–	NSHL	[78]
Germany	–	1/139 (0.7%)	0/160	–	–	NSHL	[79]
Poland	–	3/125 (2.4%)	0/89	–	–	NSHL	[79]
Mongolia	–	37/480 (7.7%)	0/389	–	–	NSHL	[80]
Poland	–	9/250 (3.6%)	0/250	7	Homoplasmic	NSHL/AID	[81]

the most frequent mutation associated with AID (Table 1). This base-pair change maps to a conserved position within the A-site of the 12S small ribosomal subunit, which is the homologous decoding region AGs bind to on the bacterial 16S ribosomal subunit [9]. The aberrant C–G base-pairing alters the secondary structure of the mitochondrial 12S small rRNA causing it to take on more of the bacterial 16S small rRNA characteristic [9]. Moreover, these structural changes have been shown to result in a higher affinity for AGs in *E.coli* carrying the corresponding mutation at the 1491 position when compared with controls [33].

The 1494C > T mutation has been shown to cause both non-syndromic hearing loss and AID, but the occurrence of this mutation has thus far been limited to the Chinese and Spanish populations and is characterized by phenotypic variation [8,34]. Nonetheless, the 1494C > T change occurs at the same conserved base pairing as 1555A > G in the mitochondrial 12S ribosomal subunit. Lymphoblastoid cell lines derived from a patient who carried this particular mutation showed reduced oxygen consumption and growth when exposed to AGs [8].

Outside of the *MTRNR1* gene, several reports show mutations occurring in mitochondrial tRNA genes [35]. While a strong causative link between mutations in mitochondria tRNA genes and hearing loss has yet to be established due to inconsistency in mutation segregation and penetrance, previously conducted *in vitro* experiments show reduced metabolism of mitochondrial tRNA in cell lines carrying certain mutations. Lymphoblastoid cell lines derived from patients with 7445A > C, 7511T > C or 7505T > C have shown a clear reduction in steady-state tRNA [36–38]. Given the multifactorial nature of mitochondrial disease, concomitant occurrence

of several genetic defects such as these tRNA mutations and the more common 1555A > G mutation may produce sufficient stress to result in the observed hearing loss phenotype.

Identification of additional mitochondrial variations that increase sensitivity to AGs holds clinical value, as it will allow for the development of effective screening panels. A recent comprehensive meta-analysis found a significant association of 10 previously reported variations not including 1555A > G and hearing loss [39]. These include 839A > G, 961delTinsC (2_7) (Significant only in European subpopulation), 988G > A, 1009C > T, 1095T > C, 1107T > C, 1382A > C, 1494C > T, 1557A > C and 15927G > A. When considering AG exposure only five of these variations were significantly associated with AID: 839A > G, 1095T > C, 1107T > C, 1494C > T, 1555A > G.

4. Genetic modifiers

Variation in the degree of hearing loss in patients with 1555A > G or 1494T > C points to the involvement of additional environmental factors or genetic modifiers [9]. Previous reports have proposed a > 50% decrease in mitochondria protein synthesis will decrease cellular respiration and growth [40]. The 1555A > G and 1494C > T mutations have been shown to reduce protein synthesis by ~34% and ~40% in cybrid cell lines; a model that controls for nuclear modifiers by combining the nuclear material from a wild-type cell with the mitochondria isolated from patients carrying a mutation [41,42]. Both 1555A > G and 1494C > T mutations alone are not sufficient to meet the 50% reduction threshold, and therefore subsequent respiration and growth stress are unlikely to generate the hearing loss phenotype [41]. While administration of AGs in a patient with 1555A > G or 1494C > T mutation leads to hearing loss, the combination of nuclear variation and either 1555A > G or 1494C > T may yet prove to be pathogenic.

Guan et al. identified *TRMU* as a modulating gene in several families carrying the 1555A > G mutation [14]. *TRMU* codes for the protein 5-methylaminomethyl-2-thiouridylate-methyltransferase, which plays a role in the modification of tRNAs. A missense mutation at position c.28G > T leads to the amino acid change p.A10S, which is a highly conserved region of the protein. This mutation co-segregated with hearing loss in one branch of a large Arab-Israeli family and six Spanish families who also have 1555A > G. The c.28G > T mutation occurs with high frequency in both Chinese and Spanish populations, which indicated that the mutation is unlikely to cause hearing loss alone. Further functional analysis of the c.28G > T mutation revealed loss of enzymatic activity, and subsequent reduction in tRNA metabolism. Therefore, combination of reduced tRNA metabolism and the loss of functionality at A-site of the small 12S rRNA resulted in a protein synthesis rate less than 50%, which suggests that high energy-consuming cochlear hair cells may be damaged. However, complete co-segregation of the *TRMU* variation and the 1555A > G in patients with hearing loss was absent from this study.

GJB2 is the most common cause of congenital profound hearing loss in many populations [43]. *GJB2* codes for the protein connexin 26 a gap junction protein, which regulates ion concentration in the endolymph and subsequently allows for normal hearing [43]. Reduced production of ATP caused by the 1555A > G mutation has been suggestively linked with cellular inability to efficiently turnover the gap-junction protein coded for by *GJB2* [44]. However, a previous report investigating the two common *GJB2* mutations c.35delG and c.235delC in association with 1555A > G revealed no strong correlation between genotype and phenotype [45].

Additionally, comparison of 10 Chinese haplogroups containing the 1555A > G mutation has also revealed a significant difference in penetrance when certain haplogroups were compared to the average of the collective [46]. This provides additional evidence that the mitochondrial genetic environment plays a role in the eventual phenotypic presentation.

A limited number of studies have characterized variations in the nuclear genome that co-segregate with the 1555A > G mutation, and no study has utilized next-generation sequencing to elucidate the involvement of nuclear background to date. Given that both 1555A > G and 1494C > T do not reduce the mitochondria protein synthesis below the 50% threshold to compromise cellular respiration, uncovering a potential modification role nuclear genes play provides an interesting topic for future study. In particular screening the nuclear genome in future studies may provide mechanistic insights for rare cases in which patients carrying a homoplasmic 1555A > G mutation and has been repeatedly treated with aminoglycosides, but experiences no deafness [47].

5. Current methods of reducing ototoxicity

Increased concentration of ROS within hair cells following systemic administration of AGs has led to investigating antioxidants as means to mitigate ototoxicity. While studies have shown reduction of ototoxicity *in vitro*, complete protection from ototoxic injury has not been achieved in humans [22]. Although other mitigating agents such as Aspirin have been successful in reducing the ototoxicity, serious side-effects including higher incidence of gastrointestinal bleeding offset the benefits [48]. Huth et al. has directly altered the size of AGs, leveraging the 1.25 nm pore size of the MET channel [15]. Increasing the size the AG molecule precludes entrance to the hair cell cytosol, while preserving the intended antibacterial properties. The clear advantage to this approach is administration of a single high-efficacy compound, as opposed to supplementing a previously derived AG with antioxidants.

Proactive utilization of both audiometry and genetic screening prior to AG administration will allow for early detection and quicker clinical response. As the basal turn of the cochlea is more susceptible to ototoxic injury, early and regular screening of high-frequency deafness will allow physicians to stop treatment and exercise other treatment options [49]. Prior to administering AGs family history of deafness should be evaluated. If a matrilineal inheritance pattern is uncovered the mitochondrial genome should be screened for the presence of mutations associated with AID. Finally, newborn screening for the DNA variants associated with AID, would determine individuals at risk before they get exposed to AGs.

6. Conclusion

As aminoglycosides continue to be utilized for the treatment of gram-negative bacterial infections, consideration of preexisting genetic defects may prove to be clinically valuable. To date,

hypersensitivity to AGs is facilitated by the 1555A > G and 1494C > T mitochondrial mutations. While several additional mitochondrial variations may eventually prove to play an integral role in the onset of hearing loss, the underlying biochemical basis has yet to be fully elucidated. Nonetheless, the current characterization of the MET channel has already led to research on altering the structure of AGs, which may provide a method to alleviate concern for potential ototoxicity. Meanwhile, clinicians should continue to consider the increased susceptibility that comes from genetic defect, as the combination of AGs and genetic defect could result in sufficient mitochondrial stress to cause hearing loss.

Conflict of interest

None.

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