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MINIREVIEW

Proposed Molecular and Cellular Mechanism for Aminoglycoside Ototoxicity

TIM HUTCHIN AND GINO CORTOPASSI*

Institute for Toxicology, Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, California 90033

INTRODUCTION

The ototoxic side effects of aminoglycoside antibiotics have been known for some time, but the mechanism by which such antibiotics cause cochlear hair cell death has been unclear. Genetic analysis of persons hypersensitive to aminoglycosides has recently shown that a particular mitochondrial mutation (1555^G; see below) can account for this rare trait (13, 21). From this it is inferred that aminoglycoside-induced hair cell death is the result of mitochondrial dysfunction. We review the data that support this notion and the implications for the identification of susceptible individuals.

AMINOGLYCOSIDE ANTIBIOTICS INTERFERE WITH PROTEIN SYNTHESIS

Aminoglycoside antibiotics exert their antibacterial effects at the level of the prokaryotic ribosome, inducing errors in protein synthesis (3, 8). The basis for the selective bactericidal effects of the aminoglycosides is presumably their preferential binding to the bacterial ribosome (1). Since mitochondrial ribosomes are structurally more similar to their prokaryotic ancestors than either ribosome is to the eukaryotic ribosome, aminoglycosides might be expected to interfere with mitochondrial protein synthesis, which could be the basis of their ototoxicity.

AMINOGLYCOSIDE HYPERSENSITIVITY IN HUMANS IS A MATERNALLY INHERITED TRAIT

Certain individuals display a hypersensitivity to aminoglycosides, suffering permanent loss of hearing after receiving normal, low doses of the antibiotic. This rare trait of hypersensitivity was found to be transmitted through the maternal lineage in 41 human pedigrees (10, 12).

A maternal mode of inheritance is the hallmark of mitochondrial genetic disease (for reviews, see references 25 and 26). One of the most common phenotypes among individuals with these diseases is sensorineural deafness (see, for example, references 7 and 18). Stria vascularis cells degenerate and die in persons with mitochondrial disease (17). These strial cells, which are dense with mitochondria, are essential for generating ion gradients in the inner ear. Together, these observations suggest that mitochondrial function is critical for inner ear function.

* Corresponding author. Mailing address: Department of Molecular Pharmacology and Toxicology, University of Southern California, 1985 Zonal Ave., Los Angeles, CA 90033. Phone: (213) 342-3293. Fax: (213) 224-7473. Electronic mail address: cortopas@zygote.hsc.usc.edu.

CORRELATION OF A MITOCHONDRIAL MUTATION WITH FAMILIAL HYPERSENSITIVITY TRAIT

Studies of the pedigrees of three Chinese (21) and two Japanese (13) families whose members displayed a maternally inherited hypersensitivity to aminoglycosides identified a rare transition mutation of A to G in the mitochondrial 12S rRNA gene at position 1555 (referred to as 1555^G). The same 1555^G mutation was not present in any of 414 control individuals (Table 1). Four of 78 individuals with sporadic cases of aminoglycoside-induced deafness also carried the 1555^G mutation, an occurrence which is statistically significantly different from that in the control population (13). Together, these data indicate a statistically significant correlation between the 1555^G mutation and aminoglycoside otosensitivity (13). Since all except one of the hypersensitive individuals received streptomycin (see footnote a of Table 1), it is possible that this trait might show some aminoglycoside specificity.

STERIC MODEL FOR FACILITATION OF AMINOGLYCOSIDE BINDING

If one compares the structures of prokaryotic and mitochondrial small rRNAs (Fig. 1), it can be seen from their secondary structures that position 1555 lies in a highly conserved region, the penultimate stem, which is involved in aminoglycoside binding to the bacterial ribosome (5, 11, 19). Mutations in this region alter the ribosome's susceptibility to aminoglycosides and have been shown to confer resistance to aminoglycosides in bacteria and the mitochondria of yeasts (6, 16).

A guanine at position 1555 would be expected to form a base pair with the cytosine at position 1494 on the opposite strand of the penultimate stem. Molecular dynamics simulation was used to see what effect this extra base-pairing might have on the structure of the ribosome. It was found that the additional pairing resulting from 1555^G shrank the volume of RNA at this site (13). This reduction in volume might leave more space for aminoglycosides to enter or bind to the mitochondrial ribosome, thus resulting in increased levels of binding of aminoglycoside to the ribosome. Further work will need to be done to provide direct evidence of enhanced binding of one or all aminoglycosides to such mitochondrial ribosomes.

MOLECULAR BASIS FOR AMINOGLYCOSIDE-INDUCED HAIR CELL DEATH

From the findings presented above, mitochondrial ribosomes are implicated as a likely target for aminoglycosides that induce hair cell death in individuals who carry the 1555^G mutation. The cause of hair cell death in normal individuals who receive large doses of aminoglycosides may be the same.

TABLE 1. Association of the 1555^G polymorphism with aminoglycoside-induced deafness[a]

Group	Occurrences in individuals surveyed[b]	Percent
AG-deaf pedigrees	5/5	100
AG-deaf sporadic	4/78	5
AG-resistant	0/34	0
Asian hearing controls	0/274	0
Total hearing controls	0/414	0

[a] Data are from references 13 and 21. The individuals of the three Chinese pedigrees (AG-deaf pedigrees) received normal therapeutic doses of streptomycin. Individuals with sporadic cases of aminocycloside-induced deafness (AG-deaf sporadic) received various aminoglycoside antibiotics for periods of 5 to 60 days, three of the four patients with 1555^G received streptomycin, and the other patient received kanamycin. Aminoglycoside-resistant persons (AG-resistant) are those who received aminoglycosides for between 17 days and 3 months with no loss of hearing (further details are given in reference 13).

[b] Data are number of occurrences of aminoglycoside-induced deafness/total number of individuals.

The aminoglycoside streptomycin is known to work by inducing the mistranslation of mRNAs at the ribosome, which has an effect similar to that of missense mutations in the DNA (14,15).

Perhaps the more interesting question is by what mechanism could the mistranslation of mitochondrial mRNAs kill cells? One reasonable possibility is that aminoglycoside treatment leads to a deficiency of mitochondrial complex I. Genes encoding proteins of mitochondrial complex I make up 56% of the protein-coding region of the mitochondrial DNA (mtDNA) (2). Thus, mistranslation is more likely to affect complex I than any other mitochondrial protein. It has been shown by others in vitro that drugs which induce complex I deficiency are lethal to cells and induce the production of mitochondrial superoxide (4, 9, 22–24). We have recently found that the lethality of a complex I inhibitor is suppressed by the mitochondrial superoxide dismutase (MnSOD) (27). Thus, one possible result of aminoglycoside treatment is the mistranslation of complex I-encoding genes, leading to excess mitochondrial superoxide production and oxidative damage to mitochondria, which may trigger cell death (Fig. 2). A prediction of this model is that animals which overexpress MnSOD should be resistant to aminoglycoside-induced hair cell death.

CLINICAL IMPLICATIONS OF 1555^G MUTATION

Of relevance to the clinician is that hypersensitive individuals and families could be identified prior to aminoglycoside administration. This would be most practical in instances in which one individual of a family has experienced aminoglycoside-induced deafness. This individual could be tested for the 1555^G mutation, and if positive, maternal relatives could then be informed beforehand that they are at risk for aminoglycoside ototoxicity. Since all except one of the patients with 1555^G received streptomycin, it is possible that certain aminoglycoside antibiotics do not show enhanced binding to the mutated ribosome and thus might be used safely.

The only pedigrees studied so far have been of Asian origin. The authors would be very interested to hear of families in any other racial groups with sensitivity to aminoglycosides so that we may assess the frequency of the 1555^G mutation in those groups. Since the 1555^G mutation may be only mildly deleterious in the context of aminoglycosides, one would not have expected it to have been eliminated from the evolving human population by natural selection. Furthermore, since mtDNA

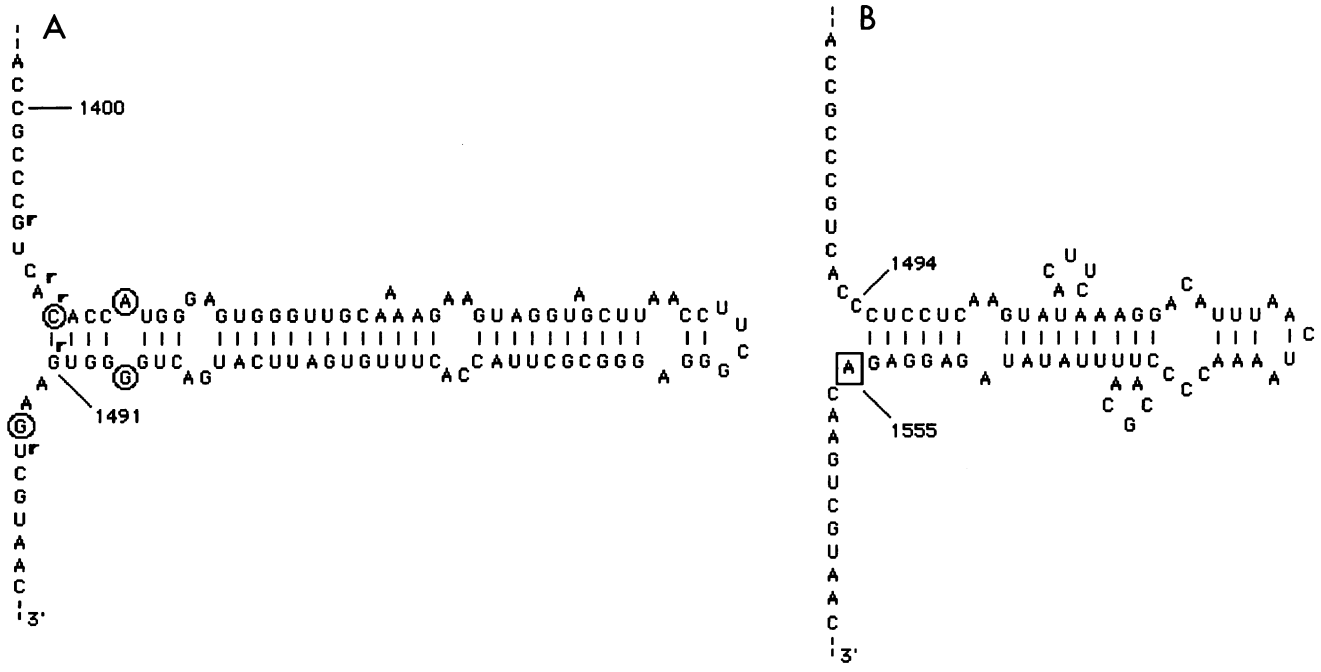

FIG. 1. Localization of 1555^G mutation to aminoglycoside-binding sites in small rRNA. The secondary structures of the 3′ ends of the *Escherichia coli* 16S rRNA (A) and human mitochondrial 12S rRNA (B) are shown. Bases protected by aminoglycosides are circled, bases conferring aminoglycoside resistance as a result of either mutation or methylation are marked **r**. Position 1555 on the mitochondrial 12S rRNA (boxed) is shown here as it exists in the wild type, i.e., an A residue, which when mutated to a G residue would be expected to pair to the C residue at position 1494. (Reprinted from *Nucleic Acids Research* [13] by permission of Oxford University Press).

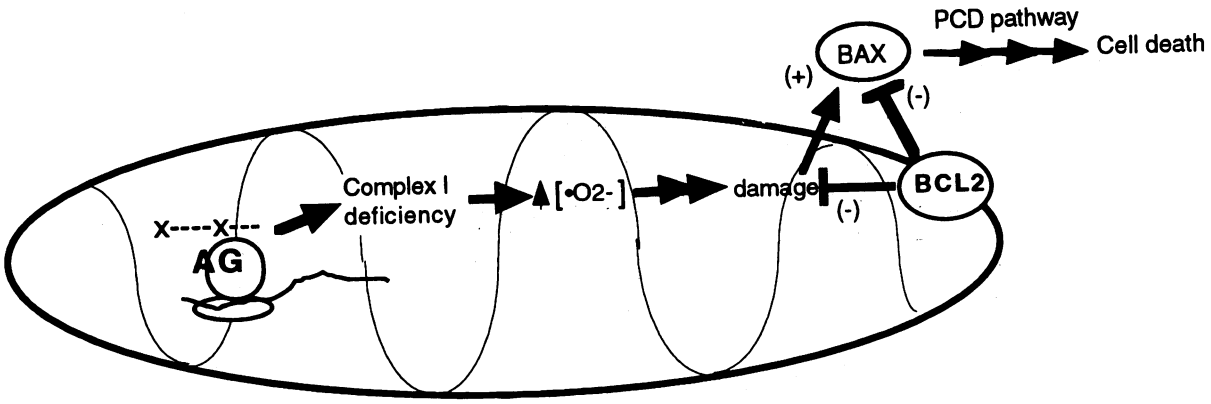

FIG. 2. Hypothetical model of mitochondrial superoxide-induced hair cell death. x----x---, complex I transcript misread by mitochondrial ribosomes in the presence of aminoglycoside (AG); $\cdot O_2^-$, superoxide anion; BCL2, B-cell lymphoma/leukemia locus-2 protein; BAX, B-cell accelerated cell death protein (20); PCD, programmed cell death.

evolves clonally, once the 1555^G mutation occurs in a particular mtDNA background, the mutation will stay linked to that background. Our recent studies suggest that persons with particular mtDNA genetic backgrounds may be at a greater risk of carrying 1555^G than others. We have found that the 1555^G mutation is seven times more frequent in one mtDNA type than another. Because this type is rare in Caucasians it might explain the relative scarcity of such aminoglycoside-hypersensitive persons in the Caucasian population.

CONCLUSIONS

A role for fully functional mitochondria in the inner ear is suggested from the high incidence of sensorineural hearing loss in individuals with mitochondrial genetic disease. Persons with a particular mtDNA mutation, 1555^G, are hypersensitive to aminoglycosides. A mechanism of aminoglycoside-induced hair cell death in which an interference with mitochondrial protein synthesis is central has been inferred. The molecular and cellular model for aminoglycoside-induced ototoxicity presented above provides several testable predictions of the relationship between mitochondrial dysfunction and deafness.

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