Ototoxicity

LP Rybak[1] and V Ramkumar[2]

[1]Department of Surgery, Southern Illinois University, School of Medicine, Springfield, Illinois, USA and [2]Department of Pharmacology, Southern Illinois University, School of Medicine, Springfield, Illinois, USA

This paper reviews intriguing recent findings on the mechanisms of drug induced hearing loss caused by two major classes of therapeutic agents: the aminoglycoside antibiotics and cisplatin. Both drug categories are nephrotoxic as well as ototoxic. Aminoglycosides and cisplatin target the outer hair cells in the basal turn of the cochlea to cause high frequency sensorineural hearing loss in a substantial percentage of patients treated with these drugs. Each group of agents appears to generate reactive oxygen species within the cochlea that trigger downstream mechanisms leading to cell death. Various protective agents including antioxidants show promise in protecting the inner ear from damage in experimental animals. The only successful double-blind, placebo controlled clinical trial using a protective agent to prevent ototoxicity was carried out in China. Aspirin or placebo was given in combination with gentamicin. A significant decrease in hearing loss was observed. Successful clinical implementation of protective agents will require a cautious approach, so that the therapeutic effect of the anti-infective agent or anti-neoplastic drug is not attenuated. This may require novel methods of administration of protective agents, such as injection within the middle ear. This would provide a maximal dose of protective agent without systemic interference with the desired effect of the ototoxic agent.

Kidney International (2007) **72,** 931–935; doi:10.1038/sj.ki.5002434; published online 25 July 2007

KEYWORDS: apoptosis; cell death; outer hair cells; cisplatin; aminoglycosides

Correspondence: *LP Rybak, Department of Surgery, Southern Illinois University, School of Medicine, 747 North Rutledge Street, PO Box 19653, Springfield, Illinois 62794-9653, USA. E-mail: lrybak@siumed.edu*

Received 24 April 2007; revised 29 May 2007; accepted 12 June 2007; published online 25 July 2007

Ototoxicity is the functional impairment and cellular degeneration of the tissues of the inner ear caused by therapeutic agents. The result of exposure to these agents is a loss of hearing and/or vestibular function. The most common ototoxic drugs in clinical use include: aminoglycoside antibiotics, platinum-based chemotherapeutic agents (cisplatin and carboplatin), loop diuretics, macrolide antibiotics, and antimalarials.[1] This mini review will be limited to the ototoxicity of aminoglycoside antibiotics and cisplatin, because both groups of drugs can cause the most severe and irreversible hearing loss and also cause severe nephrotoxicity.

AMINOGLYCOSIDE OTOTOXICITY

Background

Aminoglycoside antibiotics are used clinically for treating diseases, such as tuberculosis, and those produced by aerobic Gram-negative bacteria (such as bacterial endocarditis, urinary tract infections, pneumonia). However, the use of these agents has declined, especially in developed countries, because of their significant toxicities and the availability of better alternatives on the market. The incidence of hearing loss reported ranges from a few percent up to 33% and vestibular toxicity occurs in about 15% of patients administered aminoglycosides.[2] These drugs are still widely used in developing countries, because they are cost effective, are less regulated by prescription only sale, and have resulted in significantly higher incidences of drug toxicity.[2] The increased toxicities seen in these countries could be a result of increased use in multidrug-resistant tuberculosis requiring long-term therapy and other infections, over the counter availability and poor monitoring of auditory function following over the course of treatment.[2]

The first member of the aminoglycoside class, streptomycin, was isolated from *Streptomyces griseus* by Waksman and co-workers between 1939 and 1944. Other members of this group include natural products, such as neomycin, kanamycin, gentamicin, tobramycin, and semisynthetic products, such as netilmicin and amikacin. These agents are bactericidal and produce their therapeutic action by inhibiting bacterial protein synthesis. Aminoglycosides bind to the bacterial 30S ribosomal subunit and block initiation of protein synthesis, cause misreading of the mRNA, or facilitate premature termination of ongoing translation of mRNA template.

Clinical and experimental aspects of aminoglycoside ototoxicity

The clinical benefits of these agents are counterbalanced by significant toxicities, affecting the cochlea, vestibular apparatus, and the kidney. Individual aminoglycosides differ in their ability to produce cochlear versus vestibular toxicity. Cochlear damage is generally observed with the use of amikacin, kanamycin, and neomycin, whereas the use of streptomycin and gentamicin is associated with vestibular toxicity. Tobramycin is equally effective at producing both ototoxicity and vestibular toxicity. The aminoglycosides rapidly enter the cochlea after systemic administration, but the distribution within inner ear tissues does not correlate with their preferential toxicity to particular cells in the cochlea and vestibular system.[3] Drug accumulation within the inner ear does not seem to occur. The inner ear concentration of aminoglycosides does not exceed that of the plasma.[4] However, it is interesting to note that the aminoglycosides persist in the inner ear tissues for 6 months or longer after administration.[5] This finding may explain the enhanced susceptibility of patients to the ototoxicity of aminoglycosides when they have a history of previous aminoglycoside therapy.

Prolonged exposure of the cochlear cells to aminoglycosides is apparently linked to the killing of outer hair cells in the organ of Corti and type I sensory hair cells in the vestibular organ, leading to permanent hair cell loss and vestibular damage. Damage to the hair cells progresses from the base of the cochlea (an area for high frequency sound detection) to the apex (an area for low frequency sound detection).[2] This is followed by retrograde damage to the auditory nerve. The degree of hair cell damage and hearing loss is directly proportional to the dose of the drug to which the hair cells are exposed. Repeated exposure to aminoglycosides leads to an additive damage to hair cells and other structures and subsequently to deafness. Damage is more significant in the elderly who may have fewer hair cells at the beginning of treatment or lower endogenous protective mechanisms or in other individuals with compromised auditory function. In addition, damage may be potentiated by the concurrent administration of diuretics, such as ethacrynic acid and furosemide, which produce reversible hearing loss by themselves. Individuals with renal insufficiency are more susceptible to aminoglycoside ototoxicity, because of reduced renal excretion that can result in higher serum levels and prolonged half-life. This could lead to increased exposure of the inner ear to toxic concentrations of aminoglycosides resulting in more severe hearing loss.

Mutations in the mitochondrial 12S ribosomal rRNA renders patients highly susceptible to aminoglycoside ototoxicity. The first described mutation was an A1555G mutation in the 12S rRNA. Mutations in the mitochondrial 12S ribosomal RNA in humans make this mammalian RNA more similar to the bacterial ribosomal RNA, the primary target of the bactericidal activity of aminoglycosides.[6] This mutation has been associated with spontaneous, as well as aminoglycoside-induced hearing loss. Persons with this mutation may incur hearing loss after a single dose of aminoglycoside. It is interesting to note that the vestibular system is not affected by aminoglycosides in patients with this mutation. In China, where this mutation appears to occur in 5–6% of sporadic patients, approximately one-third of patients with aminoglycoside ototoxicity appear to have the A1555G mutation.[6]

Mechanisms of aminoglycoside ototoxicity

Several reports have concluded that the generation of reactive oxygen species (ROS) is linked to ototoxicity.[2] The generation of ROS involves the formation of an aminoglycoside-iron complex, which catalyzes their production from unsaturated fatty acids.[7] ROS are believed to promote apoptotic and necrotic cell death[2] (Figure 1a). A critical role

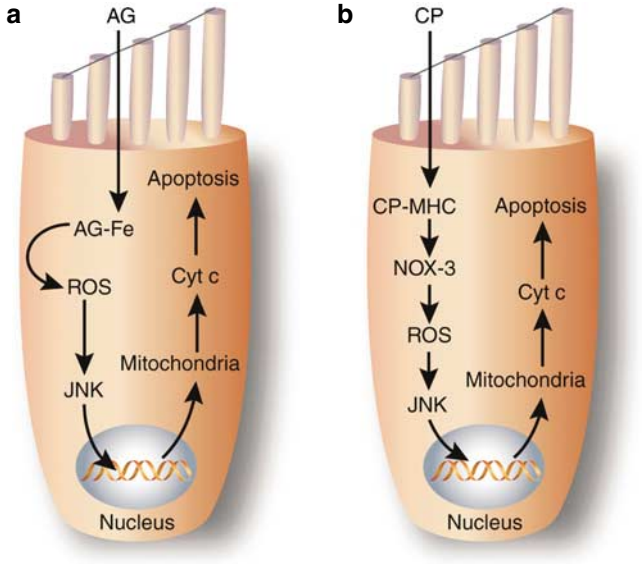

Figure 1 | Mechanisms of aminoglycoside and cisplatin-induced outer hair cell death. (**a**) Aminoglycoside (AG) entry into outer hair cell results in cell death by either caspase-dependent or caspase-independent mechanisms. The steps that appear to be involved include: (1) aminoglycoside entry into outer hair cell through the mechano-electrical transducer channels; (2) formation of an AG-iron complex can react with electron donors, such as arachidonic acid (AA) to form ROS, like superoxide, hydroxyl radical, and hydrogen peroxide; (3) ROS can then active JNK, which can then (4) translocate to the nucleus to activate genes in the cell death pathway; (5) these genes can then translocate to the mitochondria, causing (6) the release of cytochrome *c* (cyt *c*), which can trigger (7) apoptosis via caspases. Cell death may also result from caspase-independent mechanisms. (**b**) Cisplatin (CP) entry into outer hair cell results in cell death, which appears to be primarily caspase-dependent. The steps that may be involved include: (1) CP entry into the outer hair cell through mechanotransducer channels; (2) CP within cells can be aquated to form the monohydrate complex (MHC), which is more highly reactive; (3) CP and/or MHC can activate NOX-3, resulting in ROS production; (4) ROS may, in turn, activate JNK; (5) these molecules can translocate to the cell nucleus to activate genes involved in the cell death pathway; (6) these genes can then translocate to the mitochondria, causing (7) the release of cyt *c*, which can trigger (8) apoptosis via caspase-dependent mechanisms.

of ROS in aminoglycoside ototoxicity is supported by the observation that animals overexpressing the superoxide scavenging enzyme, superoxide dismutase, demonstrate less aminoglycoside-induced ototoxicity compared with the wild-type controls.[8] One signaling pathway activated by aminoglycosides via ROS is the c-Jun N-terminal kinase (JNK) pathway and contributes to cell apoptosis.[9] Inhibition of JNK protected the cochlea from neomycin-mediated ototoxicity,[10–12] suggesting that this pathway is critical in inducing hair cell death. One of the downstream targets of the JNK is the transcription factor, activating protein-1. Gentamicin treatment of cochlear explants results in increased activating protein-1 activity in the outer hair cells. The main component of the activating protein-1 complexes was found to be the c-Fos protein, which was found only in gentamicin-treated explants and not in controls.[13]

A recent study has also indicated a role of the transcription factor, nuclear factor-κB in mediating cytoprotection against kanamycin-induced ototoxicity.[14] These investigators demonstrated that the death of mouse outer hair cells following kanamycin administration was associated with a loss of p65 and p50 labeling in the nucleus, whereas immunolabeling of these two subunits of nuclear factor-κB was observed in the surviving inner hair cells. Mice given protective agents in combination with kanamycin were found to have preservation or outer hair cells in the basal turn. These cells exhibited a greater expression of nuclear factor-κB than controls.

Otoprotective approaches to preventing aminoglycoside toxicity

Aminoglycosides can react with iron to generate ROS. Protection against ototoxicity may be achieved by reducing the availability of iron using chelators, such as deferoxamine and dihydroxybenzoate. This leads to a dramatic reduction of aminoglycoside ototoxicity. Importantly, these iron chelators do not interfere with the therapeutic efficacy of the aminoglycosides.[2] Another approach to protect the cochlea is to administer antioxidants. Antioxidants, which show protection against aminoglycoside ototoxicity in experimental animals, include lipoic acid, *d*-methionine, salicylates, and dihydroxybenzoate (for review see Lesniak *et al.*[7]).

Another drug of this class is aspirin or sodium salicylate. A recent double-blind trial in China has demonstrated that a 14 day administration of aspirin attenuates gentamicin ototoxicity.[2] The incidence of hearing loss in patients administered gentamicin and placebo was 13%, whereas in patients administered gentamicin and aspirin showed a 3% incidence of hearing loss was observed. The dose of aspirin used caused gastrointestinal complications in 3/92 patients. Gastric bleeding was confirmed in each case by endoscopy and these patients were removed from the study. These complications may have been reduced or eliminated if enteric-coated aspirin had been used or if the patients were treated with proton pump inhibitors.[2] Salicylates are both iron chelators and free radical scavengers. These actions may partially explain the protective effect of aspirin in this clinical trial.

CISPLATIN OTOTOXICITY

Background

Cisplatin is a widely used chemotherapeutic agent for the treatment of various malignancies, including testicular, ovarian, bladder, cervical, head and neck, and non-small cell lung cancers. The mechanism of antitumor action of cisplatin involves uptake by the cancer cell; aquation within the tumor cell, which makes the drug more reactive for cellular targets; then, the platinum atom of cisplatin forms covalent bonds with DNA at the N7 positions of purine bases to form intrastrand and interstrand crosslinks. A number of downstream signaling pathways can then be activated to cause DNA-damage. These can include MAPK/JNK/ERK pathways. Cell death in tumor cells is primarily through apoptosis, although *in vitro* tumor cells may undergo necrosis when exposed to high concentrations of cisplatin.[15]

The use of this agent is limited by nephrotoxicity, neurotoxicity, and ototoxicity. Cisplatin ototoxicity is manifested by sensorineural hearing loss, which can be severe to profound after high-dose chemotherapy.[16] Cisplatin-related hearing loss is usually bilateral and appears first at high frequencies. Progression to lower frequencies may occur with continued therapy.

Some audiometric studies have reported elevated hearing thresholds in 75–100% of patients.[16] There is substantial variability in susceptibility to the ototoxic effects of cisplatin. Risk factors include: rapid intravenous bolus injections; high cumulative doses; pre-existing hearing loss; renal insufficiency; anemia; hypoalbuminemia; and prior cranial irradiation. Cisplatin ototoxicity appears to be related to age of the patient. Both elderly and pediatric patients are reportedly more sensitive to cisplatin ototoxicity. Li *et al.*[17] demonstrated that pediatric patients under 5 years of age are most susceptible. They developed a logistic regression model that predicted that about 40% of children fewer than 5 years of age would develop a moderate to severe hearing loss after a cumulative dose of 400 mg/m^2 as opposed to a 5% risk in children between 15 and 20 years of age.[17]

There may be genetic factors that make patients more sensitive to cisplatin ototoxicity. A recent study of survivors of testicular cancer who received cisplatin chemotherapy showed differences in functional polymorphisms in glutathione-*S*-transferase. The presence of both alleles of ^{105}Val-GSTP1 appeared to offer protection against hearing loss from cisplatin. The risk of having a poor hearing result was more than four times higher in patients with ^{105}Ile/105Ile-GSTP1 or 105Val/105Ile-GSTP1. Those genotypes associated with poor hearing after cisplatin therapy could indicate a limited amount of glutathione available for detoxification of cisplatin.[18]

Mechanisms of cisplatin ototoxicity

Cisplatin has been shown to target three areas in the cochlea: the hair cells in the basal turn of organ of Corti, the spiral ganglion cells and the lateral wall tissues (spiral ligament and stria vascularis). Outer hair cells, cells in the stria vascularis,

and spiral ligament each have been shown to undergo apoptosis and platinated DNA immunoreactivity has been localized to the nuclei of outer hair cells, and cells in the stria vascularis and spiral ligament (reviewed by Rybak *et al.*[16]). ROS mimic the effects of cisplatin on outer hair cells *in vitro*, and cisplatin reacts with cochlear tissue explants to generate ROS. This can lead to calcium influx within cochlear cells resulting in apoptosis. A unique isoform of nicotinamide adenine dinucleotide phosphate oxidase, NOX 3 has been demonstrated in the rat cochlea and is upregulated following ototoxic doses of cisplatin.[19] This can lead to large increases in superoxide production, which can lead to the formation of hydrogen peroxide. The latter molecule can be catalyzed by iron to form the highly reactive hydroxyl radical, which interacts with membrane polyunsaturated fatty acids for the highly toxic aldehyde, 4-hydroxynonenal. Superoxide can also react with nitric oxide to form peroxynitrite, which reacts with proteins to form nitrotyrosine. Cisplatin-treated animals were found to have immunoreactivity for 4-hydroxynonenal and peroxynitrite in the cochlea. Antioxidant defenses in cochlear tissues can be depleted by cisplatin, allowing ROS to increase. Excessive ROS generated by cisplatin could overwhelm the antioxidant defense mechanisms within the cochlea, activating the apoptotic pathway causing cell death in outer hair cells[16] (Figure 1b).

Otoprotective approaches to the prevention of cisplatin ototoxicity

Antioxidants have been used to reduce cisplatin ototoxicity in animal experiments, presumably by scavenging ROS. These have included D- or L-methionine, *N*-acetyl-cysteine, sodium thiosulfate, lipoic acid, ginkgo biloba extract, aminoguanidine, alpha-tocopherol, ebselen combined with allopurinol, and salicylates. A potential problem with the administration of antioxidants is a reduction in antitumor efficacy of cisplatin.[16] Sodium thiosulfate and *N*-acetylcysteine are able to bind covalently to platinum, producing an inactive complex.[16] This can be obviated by intratympanic administration of the protective agent, so that its action against cisplatin will be confined to the cochlea. D-methionine readily traverses the round window membrane and has been shown to prevent cisplatin ototoxicity in animals when applied to the round window membrane before cisplatin.[16]

Other experimental approaches have included the application of inhibitors of the cell death pathway, for example the p53 inhibitor, pifithrin-alpha, caspase inhibitors, and gene therapy with adeno-associated virus-mediated delivery of the X-linked inhibitor of apoptosis protein.[16,20] Clinical trials with amifostine have shown that this drug is not effective in reducing cisplatin-induced hearing loss (cf 16). No clinical trials have yet been published to show efficacy of cisplatin protection.

CONCLUSION

Both aminoglycosides and cisplatin cause a high frequency hearing loss that is associated with loss of outer hair cells in the basal turn of the cochlea. It is interesting to note that these drugs are also nephrotoxic. Both classes of ototoxic agents can cause the formation of ROS within the cochlea. The ROS formed can lead to cellular damage and apoptosis, resulting in hearing loss. The possibility exists that protective agents could ameliorate or prevent hearing loss from these drugs. Experimental studies in animals have shown that a variety of antioxidants can attenuate the ototoxicity of either aminoglycosides or cisplatin. To date few clinical trials have investigated the safety and efficacy of administering protective agents to prevent ototoxicity. Future clinical trials should examine the appropriate route and timing of administration of protective agents to determine the optimum method for preservation of hearing in patients receiving these drugs. Such routes could include injection of a protective agent into the middle ear (intratympanic injection) to obtain a high local concentration of the protective molecule, while sparing the therapeutic effect of these drugs.

DISCLOSURE

The authors have been performing research under a contract with Quark Biotech Inc., Fremont CA to test putative protective agents against cisplatin ototoxicity. None of these agents is mentioned in this mini review.

ACKNOWLEDGMENTS

Dr Rybak and Dr Ramkumar are supported by NIH (NIDCD) Grant no. R01 DC 02396.

REFERENCES

1. Arslan E, Orzan E, Santarelli R. Global problem of drug-induced hearing loss. *Ann N Y Acad Sci* 1999; **884**: 1–14.
2. Chen Y, Huang W-G, Zha D-J *et al.* Aspirin attenuates gentamicin ototoxicity from the laboratory to the clinic. *Hear Res* 2007; **226**: 178–182.
3. Dulon D, Aran JM, Zajic G *et al.* Comparative pharmacokinetics of gentamicin, netilmicin and amikacin in the cochlea and vestibule of the guinea pig. *Antimicrob Agents Chemother* 1986; **30**: 96–100.
4. Henley CM, Schacht J. Pharmacokinetics of aminoglycoside antibiotics in blood, inner ear fluids and tissues and their relationship to ototoxicity. *Audiology* 1988; **27**: 137–146.
5. Dulon D, Hiel H, Aurousseau C *et al.* Pharmacokinetics of gentamicin in the sensory hair cells of the organ of Corti: rapid uptake and long term persistence. *Comptes Rendus de L'Academie des Sciences—Serie III, Sciences de la Vie* 1993; **316**: 682–687.
6. Fischel-Ghodsian N. Genetic factors in aminoglycoside toxicity. *Pharmacogenomics* 2005; **6**: 27–36.
7. Lesniak W, Pecoraro VL, Schacht J. Ternary complexes of gentamicin with iron and lipid catalyze formation of reactive oxygen species. *Chem Res Toxicol* 2005; **19**: 357–364.
8. Sha S-H, Zajic G, Epstein CJ *et al.* Overexpression of copper/zinc-superoxide dismutase protects from kanamycin-induced hearing loss. *Audiol Neurotol* 2001; **6**: 117–123.
9. Davis RJ. Signal transduction by the JNK group of MAP kinases. *Cell* 2000; **103**: 239–252.
10. Pirvola U, Xing-Qun L, Virkkala J *et al.* Rescue of hearing, auditory hair cells, and neurons by CEP-1347/KT7515, an inhibitor of c-Jun N-terminal kinase activation. *J Neurosci* 2000; **20**: 43–50.
11. Wang J, Van De Water TR, Bonny C *et al.* A peptide inhibitor of c-Jun N-terminal kinase protects against both aminoglycoside and acoustic trauma-induced auditory hair cell death and hearing loss. *J Neurosci* 2003; **23**: 8596–8607.
12. Eshraghi AA, Wang J, Adil E. Blocking c-Jun-N-terminal kinase signaling can prevent hearing loss induced by both electrode insertion trauma and neomycin ototoxicity. *Hear Res* 2007; **226**: 168–177.

13. Albinger-Hegyi A, Hegyi I, Nagy I *et al.* Alteration of activator protein 1 DNA binding activity in gentamicin-induced hair cell degeneration. *Neuroscience* 2006; **137**: 971–980.
14. Jiang H, Sha S-H, Schacht J. NF-κB pathway protects cochlear hair cells from aminoglycoside-induced ototoxicity. *J Neurosci Res* 2005; **79**: 644–651.
15. Wang D, Lippard SJ. Cellular processing of platinum anticancer drugs. *Nat Rev Drug Discov* 2005; **4**: 307–320.
16. Rybak LP, Whitworth CA, Mukherjea D *et al.* Mechanisms of cisplatin-induced ototoxicity and prevention. *Hear Res* 2007; **226**: 157–167.
17. Li Y, Womer RB, Silber JH. Predicting cisplatin ototoxicity in children: the influence of age and the cumulative dose. *Eur J Cancer* 2004; **40**: 2445–2451.
18. Oldenburg J, Kraggerud SM, Cvancarova M *et al.* Cisplatin-induced long-term hearing impairment is associated with specific glutathione-*S*-transferase genotypes in testicular cancer survivors. *J Clin Oncol* 2007; **25**: 708–714.
19. Mukherjea D, Whitworth CA, Nandish S *et al.* Expression of the kidney injury molecule (KIM) 1 in the rat cochlea and induction by cisplatin. *Neuroscience* 2006; **139**: 733–740.
20. Chan DK, Lieberman DM, Musatov S *et al.* Protection against cisplatin-induced ototoxicity by adeno-associated virus-mediated delivery of the X-linked inhibitor of apoptosis protein is not dependent on caspase inhibition. *Otol Neurotol* 2007; **28**: 417–425.