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New developments in aminoglycoside therapy and ototoxicity

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Abstract

After almost seven decades in clinical use, aminoglycoside antibiotics still remain indispensable drugs for acute infections and specific indications such as tuberculosis or the containment of *pseudomonas* bacteria in patients with cystic fibrosis. The review will describe the pathology and pathophysiology of aminoglycoside-induced auditory and vestibular toxicity in humans and experimental animals and explore contemporary views of the mechanisms of cell death. It will also outline the current state of protective therapy and recent advances in the development of aminoglycoside derivatives with low toxicity profiles for antimicrobial treatment and for stop-codon suppression in the attenuation of genetic disorders.

Keywords

Aminoglycoside antibiotics; hair cells; reactive oxygen species; antioxidants; protective agents; stop-codon suppression

1. Introduction

In recent decades, the use of aminoglycosides has been relatively restrained, especially in industrialized countries. The decline in their once unbounded use was owed, in part, to the introduction of other broad-spectrum antibiotics such as cephalosporins, carbapenems and fluoroquinolones, but is also due to the incidence of serious side-effects associated with aminoglycoside therapy. However, their indispensable use in cystic fibrosis patients and the emergence of multi-drug-resistant bacteria in many types of infections, including urinary tract infections, tuberculosis and visceral leishmaniasis, have kept them in constant clinical use. In addition, they are very inexpensive to produce, which is of greatest importance to vulnerable and economically disadvantaged populations in developing countries where they all too often are the drugs of choice. These factors and their more recently recognized ability to attenuate the effects of certain genetic disorders have led to a renewed interest in these drugs.

Since the isolation of streptomycin in 1944, a variety of aminoglycosides have been introduced into antimicrobial chemotherapy (table 1). They are natural products from soil actinomycetes, a group of gram-positive bacteria, or their semisynthetic derivatives. All aminoglycosides share common structural elements: several six-membered cyclitol or

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hexose rings substituted with amino-and hydroxyl-groups and linked by glycosidic bonds. They are highly water-soluble and positively charged and insoluble in organic solvents.

2. Therapeutic characteristics

2.1 Therapeutic advantages of aminoglycosides

Aminoglycosides carry significant advantages over other classes of antibiotics. They are powerful broad-spectrum antibiotics; unlike penicillin, they exhibit great efficacy against aerobic gram-negative pathogens, including *Enterobacteriaceae* and *Pseudomonas* spp. Adding to their efficacy is their bactericidal potential, i.e. ability to kill bacteria, rather than being bacteriostatic as many other drugs. Synergistic bactericidal effects with other medications, especially beta lactams like ampicillin, make such combinations markedly efficacious. Aminoglycosides are also hypoallergenic, hence they are especially apt in emergency situations. However, they are poorly absorbed after oral dosing, necessitating systemic injections for most applications.

The antibacterial effect of aminoglycosides is afforded mainly by their binding to the 30S ribosomal subunit, leading to misreading of RNA (Kaul et al., 2006), disruption of protein synthesis, and, ultimately, accumulation of truncated and nonfunctional proteins leading to bacterial death. The RNA interactions are relatively selective for bacterial ribosomes because structural differences lower the drugs' affinity for eukaryotic ribosomes and allow for generally safe human use.

2.2 Current clinical use

In addition to frequently serving as primary antibiotics in developing countries, aminoglycosides are commonly used in industrialized nations against potentially life-threatening sepsis in infants, urinary tract infections with complications, intra-abdominal infections, and osteomyelitis; or as alternate drugs in cases of antibacterial resistance, for example in the world-wide prevalence of fluoroquinolone resistance (Radigan, et al., 2010). The United States Food and Drug Administration has approved nine aminoglycosides for clinical use: gentamicin, tobramycin, amikacin, streptomycin, neomycin, kanamycin, paromomycin, netilmicin and spectinomycin. Additional aminoglycosides may be available outside of the United States. Due to differences in structure and pharmacokinetics, the drugs exhibit somewhat different antimicrobial activities and therefore different clinical indications (table 1). Some of these applications deserve a more detailed consideration here.

Cystic fibrosis is the most common lethal autosomal recessive disorder in Caucasians with approximately 30,000 affected persons currently in the United States alone (Davies, et al., 2007). Mutation of the cystic fibrosis transmembrane conductance regulator gene leads to a dysfunctional protein expression which facilitates chronic pulmonary infections, mostly by *pseudomonas*. 85% of deaths from cystic fibrosis are due to pulmonary disease (Barto and Flume, 2010) but survival rates can be increased by regular inhalation or infusion of tobramycin or related aminoglycosides (Frederiksen et al., 1996).

Urinary tract infections are one of the most common bacterial complications of pregnancy carrying with it a risk of prematurity and fetal mortality if untreated (Schieve et al., 1994). Traditionally there was no need for treatment with aminoglycosides, but increasing resistance of *Escherichia coli* strains to previously effective drugs such as ampicillin has led to a growing use of gentamicin in pregnant women (Greer et al., 2008). As a consequence of treatment, however, the fetus is exposed to the drug because aminoglycosides cross the placental barrier. Conversely, the ability to cross the placental barrier is an advantage over other antibiotics in the case of chorioamnionitis, an intrauterine infection commonly treated with gentamicin (Lyell et al., 2010).

Visceral leishmaniasis is a frequently fatal parasitic disease affecting mostly the poor in South East Asia, parts of Africa, and some countries of South America. The disease is difficult to treat because of toxic side effects, high cost associated with some of the most effective drugs, and resistance developing in *Leishmania* so that fewer than half of treated patients are cured. Paromomycin has emerged as an efficacious and affordable alternative (Sundar et al., 2007).

Finally, streptomycin, kanamycin and amikacin are second-line anti-tuberculosis drugs as specified by the World Health Organization. Second-line drugs are employed after the first round of treatments has failed when, often, only resistant strains of the infection persist and proliferate. The incidence of tuberculosis has stabilized in recent years but an estimated one-third of the world's population remains infected, resulting in over nine million cases of currently active disease and approximately 1.3 million deaths per year (Haydel, 2010). An alarming trend is the emergence of multi-drug resistant (MDR) and XDR (extensively drug resistant) strains of tuberculosis, for which the second-line drugs are becoming increasingly important (Caminero et al., 2010).

3. Adverse effects

3.1 Types of adverse effects

Adverse effects of aminoglycosides prominently target the kidney (nephrotoxicity), vestibular and auditory organs (ototoxicity), and the neuromuscular junction. Nephrotoxicity and ototoxicity were recognized during the first clinical application of streptomycin in tuberculosis (Hinshaw and Feldman, 1945) and neuromuscular block was recognized later (Brazil and Corrado, 1957). The latter can be held infrequent by careful dosing and screening for potentiating factors like concomitant anesthetic administration, renal failure, or neuromuscular disease. Nevertheless, potential tetany still warrant attention (Thakur, 2008) particularly in some clinical circumstances, such as during perioperative periods when the co-treatment of aminoglycosides with neuromuscular blockers is possible (Paradelis et al., 1988; Dotan et al., 2003).

Nephrotoxicity and ototoxicity are the most persistent and pertinent serious side effects. The pathology of nephrotoxicity involves primarily the proximal tubules, a major site of drug accumulation. Acute tubular necrosis has been reported in 5–15% of patients after aminoglycoside therapy (Streetman, et al., 2001). Nephrotoxicity is reversible and can be clinically managed with hydration therapy so that patients generally recover normal renal function once treatment with aminoglycosides is discontinued (Heller, 1984). In contrast, ototoxicity may be initially overlooked as it might start after the end of drug treatment and develop only slowly thereafter.

Although aminoglycosides may have both nephrotoxic and ototoxic potential, there is no statistically significant relationship between the two side effects and the co-occurrence is 4.5% (de Jager and van Altena, 2002). Ototoxicity induced by aminoglycosides manifests as irreversible bilateral sensorineural hearing loss beginning at high frequencies (cochleotoxicity) or as any combination of vertigo, nausea, vomiting, nystagmus, and ataxia (vestibulotoxicity). The potential severity of auditory and vestibular deficits depends on the particular aminoglycoside used. Neomycin is considered the most highly toxic; followed by gentamicin, kanamycin, and tobramycin; while amikacin and netilmicin are considered the least toxic. Amikacin, neomycin and dihydrostreptomycin tend to be more cochleotoxic while gentamicin and streptomycin are more likely to target the vestibular sensory epithelium. Taking advantage of gentamicin's proclivity to cause vestibulotoxicity in humans, it is used to treat patients with debilitating unilateral Ménière's disease as an alternative to surgical ablation of the vestibular epithelium. Intratympanic injection of the

drug attempts to achieve significant vestibulotoxicity before the cochlear organ is affected, but cochleotoxicity often occurs (Kaplan, et al., 2000).

3.2 Detection and incidence of ototoxicity

Reported incidences of ototoxicity vary widely, depending upon subject groups, treatment parameters, assessment methods, and definitional criteria of hearing impairment. Guidelines for monitoring ototoxicity in aminoglycoside patients recommend threshold monitoring by pure-tone audiograms in both the conventional (8 kHz and below) and high frequency regions (8 kHz up to 16 or 20 kHz) at baseline, then once or twice per week during treatment and immediately following, and finally several months after drug discontinuation (American Academy of Audiology, 2009). Distortion-product otoacoustic emissions can be included as an adjunctive measure. Clinical diagnosis of vestibulotoxicity relies on reduced function in tests of the vestibular-ocular reflex or by vestibular autorotation or full-frequency rotary chair testing.

In the treatment of acute infections which may last for about 5–7 days, hearing loss induced by the most commonly used aminoglycosides may occur in about 20% of patients and balance may be affected in about 15% (Fee 1980; Moore et al. 1984; Lerner et al. 1986). However, using high-frequency testing procedures, a prospective study in 53 patients determined hearing loss in 47% of the ears studied (Fausti et al., 1992).

Cystic fibrosis patients routinely receive repeated courses of aminoglycoside therapy in 3–4 month intervals as prophylaxis against pneumonia caused primarily by *Pseudomonas aeruginosa*. The incidence of adverse reactions should be expected to increase with the frequency and length of exposure and may approach 17% (Mulheran et al. 2001). When assessing hearing function using high-frequency audiometry and DPOAEs in children receiving intravenous aminoglycoside treatment, 21% had significant threshold shifts in the 8–20 kHz range and lower DPOAE amplitudes at 3.2–6.3 kHz (Al-Malky et al., 2011).

The incidence of ototoxicity in tuberculosis patients treated with aminoglycosides depends on the duration and dose. It may be as low as 3.2% during the initial phase of therapy using primary anti-tuberculosis drugs (Gülbay, et al., 2006), but prolonged treatment with aminoglycosides which might be necessary for 6–12 months will lead to hearing loss in all patients (Duggal and Sarkar, 2007).

Exposure of the fetus to aminoglycosides is a concern that might arise in acute treatment of pregnant women for genitourinary infections or in long-term treatments for tuberculosis. A “critical period” of fetal sensitivity to aminoglycosides has been established in several animal species and coincides with the developmental phase of the inner ear (Marot et al., 1980; Lenoir et al., 1983). Some clinical observations appear to agree with the inference that a similar sensitive period should exist in the human and that aminoglycosides might have an ototoxic action during intrauterine life (Rasmussen, 1969; Saunders et al., 2009). A recent survey, however, did not seem to support a generalization of these findings as no increased risk for audiologic impairment was seen in infants born to mothers exposed to gentamicin during pregnancy (Kirkwood et al., 2007). However, the treatments examined in the latter study occurred during 28 ± 6 weeks of pregnancy (i.e. past the critical period of inner ear development in human from the 18th to the 20th week of gestation), and treatment duration was relatively short at 2.7 ± 2.3 days.

3.3 Risk factors for susceptibility

There are a few known factors that increase a patient's susceptibility to aminoglycoside toxicity. One might draw from compelling results of animal experimentation that a patient's risk for toxicity increases with dose concentration, frequency of administration, and

treatment duration (Wu et al., 2001). In clinical reality, however, a significant association of hearing loss with those parameters is difficult to establish. A recent evidence-based systematic review analyzed 48 clinical studies and derived conclusions for gentamicin, tobramycin, and amikacin (ASLHA, 2010). There was no apparent correlation between the incidence of hearing loss and higher drug dosing for any of the three drugs examined. This finding does not contradict the animal studies. Rather, it points to the fact that clinical studies employ a much narrower range of drug dosages than animal studies with expectedly smaller effects on outcome. Individual risk factors, disease, and genetics that influence susceptibility in a patient population are likely to obscure a dose-response relationship.

Genetic predisposition is indeed a well-documented risk factor. A mitochondrial DNA A1555G point mutation in the 12S ribosomal RNA gene is associated with non-syndromic deafness and increased susceptibility to aminoglycoside-induced hearing loss (Prezant, et al., 1993). There are other known mutations that confer increased risk of aminoglycoside-induced hearing loss, and pre-treatment genetic screening can be used to exclude patients who are especially sensitive to ototoxic drugs. Despite the increased susceptibility to aminoglycoside-induced hearing loss in carriers of mitochondrial mutations, there is little or no vestibular involvement even though drugs like gentamicin are more vestibulotoxic than cochleotoxic in human (Prezant et al., 1993; Selimoglu et al., 2003).

4. Pathology

4.1 Pathological changes in human temporal bones

Physiological tests and temporal bone pathology have yielded a clear picture of clinical ototoxicity and provided a standard against which to compare results obtained in animal models. Effects on the balance organs have largely been studied in response to streptomycin, the most highly vestibulotoxic of the aminoglycosides. Typical for vestibular damage by streptomycin (and other aminoglycosides) is the loss of both types of hair cells, especially type I, in the cristae ampullares. Damage to sensory epithelia in utricle and sacculus can also be observed while loss of vestibular ganglia does not seem to be a primary effect of the drugs (Tsuji, et al., 2000).

The details of auditory pathology are also exemplified by a histopathological study of 21 patients with aminoglycoside ototoxicity. It demonstrated a progressive loss of outer hair cells from the base of the cochlea to the apex, the higher turns of the cochlea being spared until the more basal regions had sustained substantial losses. Damage to outer hair cells is followed by progressive loss of inner hair cells in more severe cases (Huizing and de Groot, 1987). This sequence appears to be a predominant pattern and the base-to-apex progression of hair cell loss correlates well with the initial hearing impairment at high frequencies. Degeneration of nerve fibers, spiral ganglion neurons, and supporting cells generally follow hair cell death in the same regions. However, degeneration of spiral ganglion neurons without loss of hair cells was seen in two cases, underscoring the potential complexity of human pathology. Both subjects had intact hearing prior to treatment and suffered hearing loss after treatment with gentamicin (Hinojosa and Lerner, 1987).

4.2 Pathology in animal models

Ototoxicity has been studied in many species including primates, but primarily in guinea pigs and, to a lesser extent, rats and chinchillas. Mice were not routinely used until the last decade because adult animals appeared resistant to typical doses of aminoglycosides. However, the recent establishment of an adult mouse model of gentamicin-induced hearing loss (Wu, et al., 2001) brought with it the availability of a vast array of molecular and genetic data and tools. In addition, the organotypic culture of the early postnatal murine cochlea (fig. 1) is a convenient means for drug screening and studying molecular

mechanisms in a controlled environment (Richardson et al., 1987; Chen et al., 2009). Zebra fish are yet another recent model for the examination of ototoxic mechanisms in which genetic and pharmacologic modulators of toxicity have been explored (Ou, et al., 2010).

Attesting to their validity, mammalian models replicate the pattern of aminoglycoside damage observed in humans. Loss of hair cells in the organ of Corti progresses from the base to the apex of the cochlea, and from the outer hair cells to the inner hair cells (fig. 2). Consequently, the resulting hearing loss, mostly assessed via the auditory brain stem response in animals, begins at high frequencies and proceeds to lower frequencies (Causse, et al., 1949; Stebbins et al., 1969). At high concentrations of aminoglycosides, supporting cells are damaged as well, and degeneration of the auditory nerve may be seen. The stria vascularis and spiral ligament initially remain structurally and functionally intact but damage to these tissues may eventually occur. In fact, Hawkins suggested that aminoglycosides could cause injury to cochlear secretory structures, thereby disrupting homeostasis and contributing to hair cell damage (Hawkins, 1973). While auditory dysfunction mediated by such non-receptor cell damage is possible, the overwhelming evidence points to a primary drug action on the sensory cells. Also reflecting human pathology in the vestibular organ, type I hair cells are more sensitive in animal models than type II hair cells, and hair cells of both types are more susceptible than supporting cells (Wersall, et al., 1969).

5. Mechanisms of ototoxicity

5.1 Uptake of aminoglycosides

Because aminoglycosides are selectively toxic to the kidney and the inner ear, research has long focused on the mechanism of drug transport into these two organs. Of particular interest in this context was the question whether an exclusive or preferential drug uptake into hair cells might explain their sensitivity to insult. Notwithstanding the prerequisite of tissue entry for toxicity, uptake studies have not resolved this issue. In fact, aminoglycosides penetrate almost all cell types of the cochlea (De Groot, et al., 1990; Imamura and Adams, 2003) including those not damaged by the drugs. Aside from the hair cells, the areas with highest affinity for and longest retention of aminoglycosides are the mesenchymal cells under the basilar membrane, the interdental cells of the spiral ligament, and type III fibrocytes. Taken together, the results suggest no direct relationship between cellular uptake and cell death.

The molecular mechanism of uptake into inner ear structures has not yet been resolved. The glycoprotein megalin has been suggested as an aminoglycoside transporter in proximal tubules of the kidney (Moestrup, et al., 1995), but its distribution in the inner ear does not match the pattern of aminoglycoside uptake and ototoxicity (Mizuta et al., 1999). Observations that lysosomes appear in the subcuticular portion of hair cells soon after treatment of guinea pigs with kanamycin prompted the suggestion of an endocytotic uptake at the apex of the hair cell (Darrouzet and Guilhaume, 1974). This suggestion was supported by observations that aminoglycosides initially appeared at the apical end of hair cells both in mammals (De Groot, et al., 1990; Hiel, et al., 1992) and birds (Hashino et al., 1997). Myosin VII-A also seems to regulate aminoglycoside uptake (Richardson et al., 1997), as explants from the inner ear of mutant mice lacking myosin VII-A do not take up the drugs. Other potential transport mechanisms include a polyamine-like transport (Williams et al., 1987) and vesicular receptor-mediated endocytosis at the base of hair cells (Lim, 1986). Entry through ion channels such as the mechanoelectrical transducer channel (Marcotti et al., 2005) or aminoglycoside-permissive TRP channels (Myrdal and Steyger, 2005) are alternate or additional options. From the sum of the evidence, it seems reasonable to conclude that more than one mechanism of uptake for aminoglycosides operates in the inner ear.

5.2 Formation of ROS

Aminoglycoside-induced generation of reactive oxygen species (ROS) is presumed to be a principal mechanism underlying sensory cell death. Under normal physiological conditions, a variety of enzymatic processes produce ROS as primary metabolic products and physiological mediators (such as nitric oxide), or as byproducts (such as superoxide by mitochondrial “leakage”). Any detrimental effects of ROS are contained by the cellular antioxidant systems that maintain a redox balance. However, an overproduction of ROS challenges this balance and might yield highly reactive oxidative species such as hydrogen radicals or peroxynitrite with injurious consequences to cell constituents from DNA to lipids and proteins, ultimately leading to cell death.

ROS and their reaction products (fig. 3a, b) arise after aminoglycoside treatment in hair cells or in organ of Corti tissue explants (Clerici et al., 1996; Sha and Schacht, 1999a; Hirose et al., 1997) as well as in the inner ear *in vivo* after systemic drug injections (Jiang et al., 2005). Initially balancing the detrimental actions of ROS are intrinsic antioxidants (Chen, et al., 2008) and protective signaling pathways such as those linked to NF- κ B (Jiang et al., 2005), ERK (Battaglia et al., 2003) or PI 3-kinase (Chung et al., 2006). Such pathways may up-regulate genes for antioxidant enzymes or slow the rate of cell death signaling until an excess of ROS overwhelms the redox balance and skews cell metabolism towards the activation of apoptosis or necrosis. A compelling piece of evidence that ROS formation is causally linked to hair cell death is the demonstration that antioxidants can protect against aminoglycoside-induced loss of hair cells and auditory function (see section 6). A recent study confirmed that the accumulation of highly reactive ROS (hROS) is an important initial step in gentamicin-induced hair cell damage and that the differential sensitivity of hair cells in the organ of Corti is closely related to differences in hROS accumulation (Choung, 2009). This notion is in accordance with the fact that hair cells are more susceptible to ROS damage than supporting cells and basal hair cells more so than apical hair cells (Sha et al., 2001). Differential ROS production coupled with differential sensitivity may explain – at least in part – the base-to-apex gradient in ototoxic damage.

Despite the clear link between ROS formation and cell death as a consequence of treatment with aminoglycosides, the sources of ROS remain uncertain. Aminoglycosides can accelerate the non-enzymatic formation of ROS via redox-active iron complexes (Priuska and Schacht, 1995) and also invoke enzymatic reactions in the cell. Metabolic contributions may include ROS generation after mitochondrial damage or the activation of the NADPH oxidase complex via the Rac/Rho pathway, a primary source of superoxide radicals (Jiang et al., 2006a).

5.3 Involvement of phosphoinositide lipids

Leitner et al. (2011) attributed aminoglycoside ototoxicity at least partly to the inhibition of potassium channels induced by depletion of phosphoinositides. This suggestion was supported by the fact that dysfunction or genetic ablation of KCNQ4 also results in hair cell degeneration and subsequent hearing loss. This recent evidence is interesting in the context of the long-standing suggestions of an involvement of these lipids in ototoxicity based on observations that aminoglycosides strongly bind phosphoinositides (Schacht, 1979) and that the binding affinity correlates with ototoxicity (Lodhi et al., 1980). Furthermore, it is well established that the drugs alter phosphoinositide metabolism (Orsulakova et al., 1976) and deplete phosphatidylinositol trisphosphate in hair cells of mice after *in-vivo* treatment with kanamycin leading to epigenetic changes in the cells (Jiang et al., 2006b).

5.4 Epigenetic changes and cell death pathways

Epigenetic changes are alterations of gene expression without changes to the DNA sequence accomplished by, for instance, the phosphorylation, sumoylation, acetylation, or other covalent modification of histone core proteins. Aberrations of histone-mediated nuclear processing can lead to transcriptional dysfunction or gene silencing and have been increasingly linked to disease pathogenesis (see Mohtat and Susztak, 2010 for a review). Phosphoinositides form complexes with and decrease the acetylation of histones in the nuclei of outer hair cells following the administration of aminoglycosides to mice *in vivo* (Jiang et al., 2006b) or in organotypic cultures (fig. 3c, d). The diminished acetylation of histone core proteins is caused by increased protein levels of histone deacetylases, while histone acetyl transferase levels remain unchanged (Chen et al., 2009). The fact that inhibitors of histone deacetylases rescue both histone acetylation and hair cells indicates a close link between epigenetic alterations of gene expression and ototoxicity.

It is tempting to speculate that such epigenetic changes in gene expression contribute to the inhibition of homeostatic pathways or the emergence of cell death pathways in hair cells, but such a link remains to be established. Cell death pathways of ototoxicity are complex and include necrosis as well as caspase-dependent and caspase-independent apoptosis (Jiang et al., 2006c). Specific observations, partly based on studies of inhibition of delay of cell death, have also implicated involvement of c-jun N-terminal kinases (JNK) and caspase cascades (Ylikoski et al, 2002; Eshraghi et al., 2007), as well as the nuclear translocation of endonuclease G (Endo G) and activation of μ -calpain (Jiang et al., 2006c). In concert with the activation of cell death pathways, aminoglycosides inhibit cell survival pathways such as PI 3-kinase/Akt signaling (Jiang et al., 2006b).

6. Attenuation and prevention of aminoglycoside ototoxicity

6.1 Prevention of hearing loss in animal models

Along with investigations of the mechanisms behind toxicity, therapeutic approaches for prevention of hearing loss have long been explored. Currently, most attempts at protection target ROS, the presumed up-stream cause of toxicity, or one of the metabolic branches that lead from ROS insult to cell death.

Consistent with the hypothesis of ROS involvement in ototoxicity, co-administration of antioxidants affords protection against aminoglycoside-induced hair cell damage *in vitro* and *in vivo*. There is a remarkable consistency in the reported literature in the ability of a variety of antioxidants to protect against various aminoglycoside antibiotics, attesting to the robustness of the approach. Compounds successfully applied include iron chelators such as desferoxamine and dihydroxybenzoate (Song et al., 1996; 1998) as well as antioxidants such as lipoic acid, α -tocopherol, ebselen, D-methionine, salicylates, and traditional medicines (see Rybak and Whitworth, 2005 for a list). Antioxidants were effective against various aminoglycosides including gentamicin, neomycin, kanamycin, streptomycin, amikacin and tobramycin. The routes of administration of antidotes likewise can vary without apparent detriment to the efficacy: systemic or intracochlear; or via the round window; or the introduction of antioxidant enzymes to the inner ear via viral vectors (Kawamoto et al., 2004).

Protection from ototoxicity by targeting presumed downstream signaling pathways has also been demonstrated. For example, blocking the jun kinase, caspase or calpain pathways with specific inhibitors has rescued hair cells from aminoglycoside ototoxicity (Pirvola et al., 2000; Wang et al., 2003). Pretreatment with an adenovector expressing bcl-2, an endogenous anti-apoptotic protein, also offered morphologic preservation of hair cells and protection of hearing (Pfannenstiel, et al., 2009), as did over-expression of Hsp70, a stress

response protein (Taleb et al., 2009). Likewise, in agreement with epigenetic routes of hair cell death, trichostatin and butyrate, inhibitors of histone deacetylation, both attenuated gentamicin toxicity (Chen et al., 2009). Trophic factors, primarily glial cell line-derived neurotrophic factor, also afforded protection (Yagi et al., 1999), although their mode of action remains speculative.

The challenge from these successful animal studies is to delineate the most appropriate drugs for human use. Despite the success of a compound in an animal study, there are many considerations that affect the viability of extending a treatment to human medicine, such as additional side effects of the protective agent, efficacy, and drug-drug interactions. An ion chelator such as dihydroxybenzoic acid, for example, although highly efficient in animals is unlikely to be used in seriously ill infectious disease patients. Desferoxamine, despite being a clinical drug against iron overload in thalassemia has potential neuro- and ototoxicity of its own (Ryals et al., 1997). A concern with blockers of a cell death pathway is that they might only introduce a delay in the process as other cell death pathways would be activated. This concern is particularly pertinent for aminoglycoside-induced hair cell death which may proceed via several caspase-dependent and caspase-independent pathways (Jiang, et al., 2006c). More promising might be drugs that are already medically used or in clinical trials in different contexts. These include D-methionine (Sha et al., 2000) which has been studied as a dietary supplement (Kies et al., 1975) and inhibitors of histone deacetylases several of which are currently being evaluated as antineoplastic agents (Balakin et al., 2007). Furthermore, experimental drugs that require delivery to the cochlea or the round window will have limited, if any, clinical potential. Finally, a single solution to the problem of aminoglycoside ototoxicity might not be possible, because the indications for aminoglycoside therapy vary widely from short-term use in acute infections to repeated applications in cystic fibrosis and long-term treatment in tuberculosis. Antioxidants, for example, that can expected to be tolerated well in the short term might create unanticipated problems in long-term treatment by interfering with ROS as physiological cell signaling molecules.

6.2 Prevention of hearing loss in clinical trials

Based on laboratory studies of salicylate prevention of ototoxicity (Sha and Schacht, 1999b) aspirin (acetyl salicylate) was the first protectant successfully tested against gentamicin-induced hearing loss in a randomized double-blind clinical trial with placebo-controls (Sha, et al., 2006). While 13% of placebo-group patients met the criteria for hearing loss, only 3% of patients receiving salicylate suffered hearing loss, indicating a 75% reduction of risk. The salicylate treatment did not influence gentamicin serum levels or interfere with its antibacterial effect. With the aspirin dose used, no increased incidence of tinnitus was reported, although gastric complications expectedly occurred in 3% of patients. The beneficial effect of aspirin was subsequently confirmed in another clinical trial (Behnoud et al., 2009). The presumed underlying mechanism of protection may be multifaceted. Not only is salicylate a weak iron chelator and free radical scavenger, it may also influence expression of genes related to cell survival and death. Salicylate can induce protective heat shock proteins (Ramesh et al., 2004) and an attenuation of cisplatin nephrotoxicity by salicylate was linked to an inhibition of tumor necrosis factor α (Ishihara, et al., 2003).

Other antioxidants must also be considered for clinical application because the use of aspirin carries a risk of gastrointestinal disorders, is contraindicated in individuals at risk of bleeding disorders and in children, including the bulk of the population with cystic fibrosis. Co-administration of N-acetylcysteine significantly reduced the incidence of hearing loss in a small group of patients receiving hemodialysis and gentamicin for bacteremia (Feldman et al., 2007). In contrast, the antioxidant vitamin E did not confer a statistically significant protection against gentamicin ototoxicity (Kharkheli et al., 2007). These clinical studies

demonstrate that animal experimentation on aminoglycoside-induced hearing loss provides a good, but not yet perfect, basis for translation to the clinic.

7. Emerging developments

7.1 Novel non-ototoxic aminoglycosides

With prolonged and frequent clinical use of antibiotics, the bacterial pathogens develop rapid and extensive resistance. One tactic to mitigate this problem in clinical practice is the administration of a combination of multiple drugs across different classes of antibiotics. Another approach is the synthesis of novel aminoglycoside derivatives with the goal of creating potent antibiotics that are effective against resistant strains of bacteria and have minimal side effects, including minimal ototoxicity.

The mechanism of bacterial resistance to aminoglycosides primarily involves two actions: 1) a reduction in the drug concentration in bacterial cells via changes in influx and efflux transport, notably by multidrug-resistant transporters (Sobel, et al., 2003); and 2) enzymatic modification of aminoglycosides to reduce the binding affinity between the drugs and the bacterial ribosome (Magnet and Blanchard, 2005). The second action is considered a primary mechanism for the loss of aminoglycoside effectiveness and includes covalent modifications by phosphotransferases (APHs), acetyltransferases, and adenyltransferases.

To combat this resistance mechanism, semisynthetic aminoglycosides have been introduced over the years, such as amikacin, debekacin, and netilmicin that lack some of the structural elements required by the modifying enzymes. This approach is still viable; for example, a derivative with an extra sugar ring on the C5"-OH position of neomycin B is no longer susceptible to APH (3') while maintaining comparable or improved antibacterial activity against native and resistant strains (Hainrichson et al., 2005). A new class of 3',4'-methylidene protected aminoglycosides has also exhibited improved resistance combined with reduced toxicity (Pokrovskaya, 2010). Another example of a novel antibiotic is ACHN-490, the first "neoglycoside" to begin clinical development, which has the favorable bactericidal and synergistic properties of aminoglycosides and shows activity against resistant bacteria (Armstrong and Miller, 2010).

There is also emerging interest in the development of hybrid antibiotics. The purpose of hybrid drugs is to impede the success of drug resistance by combining multiple antibiotic properties in a single compound, similar to the administration of a combination of different classes of antibiotics (Bremner et al., 2007). Based on the knowledge that cationic amphiphiles such as cationic lipids, cationic peptides, and chlorhexidine have been used for decades with little or no susceptibility to resistance, amphiphilic aminoglyco-conjugates were introduced which show promise for treatment of drug-resistance bacteria (Bera, et al., 2010). Further investigation of the ototoxicity of these aminoglycosides derivatives is needed.

7.2 'Designer aminoglycosides' to treat genetic diseases

In the last few years, the use of aminoglycosides to target to genetic diseases has been gaining momentum. More than 1800 human genetic disorders, including cystic fibrosis, Duchenne muscular dystrophy (DMD), ataxia telangiectasia, Hurler syndrome, and hemophilia A, are caused by mutations introducing a premature stop codon into the DNA sequence (Kaufman, 1999). The result is a premature abortion of translation, yielding truncated and ineffective proteins. Although the anti-bactericidal mechanism of aminoglycosides of misdirecting protein synthesis is relatively prokaryote-specific, aminoglycosides do bind to eukaryotic RNA, albeit with less affinity and hence less resulting mistranslation (Böttger, 2001; Eustice and Wilhelm, 1984). Burke and Mogg

(1985) suggested that aminoglycosides might promote a “misreading” of disease-causing stop codons and that protein synthesis might continue and produce at least a small amount of functional protein. Several in-vitro and clinical studies have indeed shown a sensitivity of premature stop codons to aminoglycosides resulting in a limited synthesis of functional proteins and attenuating symptoms of several disorders (Clancy et al., 2001; Sleat et al., 2001; Wilschanski et al., 2003; Howard et al., 2004; Malik et al., 2010). As an example, DMD is the most common and most lethal X-linked genetic disease, and 1 in 3500 live male births are affected. Roughly 13–15% of DMD cases are caused by mutations introducing stop codons and aminoglycosides were successful in restoring dystrophin protein function in mouse mutants of the disease (Barton-Davis, et al., 1999). Subsequent clinical trials confirmed significantly increased dystrophin levels in patients (Wagner et al., 2001; Malik et al., 2010).

As the efficiency of read-through is directly related to the dosing and the frequency of aminoglycoside treatment, the therapy has to be balanced against the toxic side effects of the drugs. As a result, subtoxic dosages that avoid ototoxicity but might not be optimal for treatment need to be employed. This predicament has led to the quest for synthetic drugs that combine high read-through efficiency with reduced toxicity. One line of pursuit is the use of non-aminoglycosides with read-through activity but less intrinsic toxicity (Kerem et al., 2008). The other line is the development of ‘designer aminoglycosides’ for efficient stop codon suppression and minimal ototoxicity. Initial results of this approach are promising (Nudelman et al., 2009).

8. Conclusion

By all accounts, aminoglycosides, antibiotics with a rich history, are experiencing a renaissance. Never having been completely abandoned in the clinic thanks to their highly desirable antibacterial spectrum, they increasingly fill emerging needs. For one, mounting bacterial resistance to other mainstay drugs require aminoglycosides for successful chemotherapy. Ironically, the utility of aminoglycosides against resistant bacteria stems in part from their relatively restrained use during the last decades lowering the development of global resistance to them. Another facet is the structural diversity within the class of aminoglycosides that precludes cross-resistance. For example, bacteria can become immune to neomycin through an aminoglycoside-O-phosphotransferase (APH(3’)-II) which inactivates neomycin-related aminoglycosides by a phosphorylation of the 3’-hydroxyl residue on aminohexose I. This resistance mechanism would also render the bacterium immune to kanamycin and paromomycin but still leave it susceptible to gentamicin or other aminoglycosides.

These applications and the novel uses, such as in stop codon suppression, still carry the risk of ototoxic side effects. First clinical trials have clearly shown the utility of antioxidant therapy as a preventive measure, but more research is needed. Enhanced protection might be achieved by using combination treatment, in particular, combinations that target potentially separate mechanisms of drug action such as complementary therapies to modulate oxidative stress, epigenetic changes, and apoptotic pathways. In this context, a more detailed understanding of initial events in the cascade of drug-induced pathways of cell death and protection might guide further designs of preventive therapies. Standardization of animal models might facilitate comparisons of studies between different laboratories and accelerate translation to the clinic.

Protective therapies indeed appear within reach but, as much as they are currently needed, will remain an imperfect solution. The development of non-ototoxic derivatives with similar antibacterial spectra or stop codon suppression seems a more prudent, albeit longer-term,

approach. This approach must include evaluations of ototoxicity in order to prevent the major irreversible side effect of any promising new drugs. Only by following stringent testing criteria will translational medicine fill a need for those who can benefit from the next generation of aminoglycoside antibiotics.

- > We first analyze the current use of aminoglycoside antibiotics
- > We detail the physiological and molecular pathology of hearing loss
- > We evaluate the current status of protective therapy
- > The outlook for novel antibiotics and novel therapeutic applications is discussed

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Fig. 1. Organ of Corti explant from a post-natal day 3 mouse. The preparation was stained for F-actin with rhodamine phalloidin (red fluorescence), outlining a bright outer rim of hair cells. Scale bar = 2 mm.

Fig. 2.

Gentamicin-induced hair cell loss. Organotypic cultures of the organ of Corti of the mouse were stained with myosin VIIa (red fluorescence) outlining hair cells. Scale bar = 20 μ m.

a: The basal part of an explant, control without drug treatment.

b to d: Progressive loss of OHC from base to the apex following a 24-h incubation with 0.2 mM gentamicin. b, basal part of explant, severe loss of OHC; c, middle section of the same explant with deranged and partly lost hair cells; d, hair cells in the apical portion remain intact.

Fig. 3.

Biochemical reactions preceding gentamicin-induced hair cell loss in mouse organotypic cultures of the organ of Corti. All examples are from comparable basal regions of explants. Scale bar = 20 μ m.

a and b. Control explant (a) and explant after gentamicin treatment (b) stained for myosin VIIa to outline hair cells (red) and for nitrotyrosine, a marker for ROS action (green). Blue fluorescence is a marker for nuclei. Nitrotyrosine appears in outer hair cells after exposure to the drug.

c and d: Control explant (c) and explant after gentamicin treatment (d) stained for myosin VIIa to outline hair cells (red) and for histone acetylation (H3 AcK9, green). Blue fluorescence is a marker for nuclei. Acetylated histone H3 is found in outer and inner hair cells and (more strongly) in supporting cells. After drug treatment, histone acetylation has almost completely disappeared from the cells.

Table 1

Therapeutic uses of aminoglycosides.

Aminoglycoside	Specific Therapy (FDA-approved and off-label indications)	Prophylaxis
Streptomycin *	Given IV or IM for tuberculosis, brucellosis, tularemia, and pneumonic, septicemic, and bubonic plagues caused by <i>Yersinia pestis</i> , among others	
Neomycin	Topical application for skin infections from minor wounds, orally for as an adjunct for hepatic encephalopathy (questionable efficacy) or hypercholesterolemia	Topical application to skin grafts, also taken orally for bowel surgery
Kanamycin *	Intraperitoneal application for post-operative peritonitis, IV or IM for tuberculosis, gonorrhea, orally for necrotizing enterocolitis in fetus or newborn, or hepatic encephalopathy	Taken orally for suppression of intestinal flora, often for bowel surgery preparation
Paromomycin	Taken orally for intestinal amebic infections, tapeworms, giardiasis, leishmaniasis, trichomoniasis, and hepatic encephalopathy	
Gentamicin	Administered IV or IM for meningitis, pneumonia, <i>Pseudomonas</i> infections, septicemia, <i>E. coli</i> infections, <i>Staphylococcus</i> infections, listeria, tularemia, brucellosis, endocarditis, respiratory tract infections, urinary tract infections, bone infections, cystic fibrosis, diverticulitis, neutropenia, and sepsis and necrotizing enterocolitis in newborns, among others. Oral or intraperitoneal treatment for peritonitis, topical treatment for burns and skin infections, ophthalmic drops for eye infections, intratympanic injection for Meniere's disease	Applied for urological, abdominal, and cardiac surgeries, as well as vascular procedures
Tobramycin	Administered IV for lower respiratory infections, osteomyelitis, some recurrent urinary tract or abdominal infections, meningitis, and skin and bone infections. Nebulized for infections in cystic fibrosis.	
Amikacin	Administered IV or IM for some highly drug resistant gram-negative organisms, as well as meningitis and uncomplicated urinary tract infections.	
Netilmicin *	Given IV or IM for <i>Pseudomonas</i> infections, skin, bone, or abdominal infections, gonorrhea, lower respiratory infections, and urinary tract infections.	
Spectinomycin	Administered IM for gonorrhea.	

* Now infrequently used

The chart was compiled from information in Gilbert (2004) and Avent et al. (2011) and other sources.