

Aminoglycoside-Induced Ototoxicity

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Abstract: It has long been known that the major irreversible toxicity of aminoglycosides is ototoxicity. Among them, streptomycin and gentamicin are primarily vestibulotoxic, whereas amikacin, neomycin, dihydrostreptomycin, and kanamycin are primarily cochleotoxic. Cochlear damage can produce permanent hearing loss, and damage to the vestibular apparatus results in dizziness, ataxia, and/or nystagmus. Aminoglycosides appear to generate free radicals within the inner ear, with subsequent permanent damage to sensory cells and neurons, resulting in permanent hearing loss. Two mutations in the mitochondrial 12S ribosomal RNA gene have been previously reported to predispose carriers to aminoglycoside-induced ototoxicity. As aminoglycosides are indispensable agents both in the treatment of infections and Meniere's disease, a great effort has been made to develop strategies to prevent aminoglycoside ototoxicity. Anti-free radical agents, such as salicylate, have been shown to attenuate the ototoxic effects of aminoglycosides. In this paper, incidence, predisposition, mechanism, and prevention of aminoglycoside-induced ototoxicity is discussed in the light of literature data.

Key Words: Aminoglycosides, ototoxicity.

INTRODUCTION

Ototoxicity refers to medication-caused auditory and/or vestibular system dysfunction those results in hearing loss or disequilibrium [1]. Aminoglycoside antibiotics are the first ototoxic agents to highlight the problem of drug-induced hearing and vestibular loss [2]. It has long been known that the major irreversible toxicity of aminoglycosides is ototoxicity [3,4]. This finding first came to light shortly after the discovery of streptomycin [3]. Aminoglycosides have variable cochleotoxicity and vestibulotoxicity [2]. Among them, streptomycin and gentamicin are primarily vestibulotoxic, whereas amikacin, neomycin, dihydrostreptomycin, and kanamycin are primarily cochleotoxic [2]. Less is known regarding netilmicin ototoxicity but its ototoxic potential appears to be low [2]. Cochlear damage can produce permanent hearing loss, and damage to the vestibular apparatus results in dizziness, ataxia, and/or nystagmus [5]. Because it is possible to physiologically compensate for vestibular damage, cochleotoxicity is generally considered to be a far more serious problem [5]. In humans, the ototoxic effect of these drugs is reported as a sensorineural hearing loss, which is permanent because the hair cells in the cochlea do not regenerate [6]. Although beneficial effects of aminoglycosides in Meniere's disease (MD) made these antibiotics popular again, the relative ototoxicities of the aminoglycosides to the cochlea is important in making a choice between them [7]. In this paper, incidence, predisposition, mechanism, and prevention of aminoglycoside-induced ototoxicity is discussed in the light of literature data.

INCIDENCE OF AMINOGLYCOSIDE-INDUCED OTOTOXICITY

In a nationwide survey of 2235 otolaryngologists, it was reported that 94% of respondents used ototopicals in the presence of post-tympanostomy tube otorrhea, 84% used them in the presence of a draining perforation, and 75% used them with intraoperative packing [8]. Only 3.4% of the respondents reported irreversible inner ear damage caused by an ototopical drug [3,8]. In another report, the incidence of topical aminoglycoside toxicity was noted as only 1 in 10,000 [9]. However, in many of these earlier studies, ototoxicity was considered as hearing loss; vestibular symptoms and other complications were often not taken into account because they were either unrecognized or unreported [3].

Bath *et al.* [10] reported a large series of 29 patients with true, unmitigated ototoxicity caused by commercially available aminoglycoside-containing drops applied topically to the ear, in which 9 developed ataxia, 7 never returned to work, and 5 were confined to a wheelchair [10]. In the study of Black *et al.* [11] vestibular and auditory function test results of patients at least 1 year after discontinuation of gentamicin were investigated. It was reported that all subjects had vestibular function test results consistent with permanent gentamicin ototoxicity [11]. All complained of disequilibrium, 32 out of 33 described oscillopsia, and 23 had tinnitus. All 33 subjects had complained of symptoms consistent with ototoxicity within 1 to 3 weeks of initiation of gentamicin therapy; however, gentamicin vestibulotoxicity was not recognized before hospital discharge in 32 of 33 subjects [11].

In a MEDLINE search of the published literature from 1966 to the 2004, a total of 54 cases of gentamicin vestibular toxicity and, in 24 of these patients, cochlear toxicity was also documented [12]. In the same study, 11 cases of co-

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chlear and 2 cases of vestibular toxicity in neomycin-based ear drops were detected [12].

In a newborn study, of 8,333 children examined for hearing disorders, 134 (1.6%) had received previous treatment with gentamicin; only eight (6.0%) suffered from various extents of sensorineural hearing impairment, and all eight had a history of other risk factors of hearing loss such as perinatal asphyxia or meningitis [13]. In another study, 30 children with normal hearing had received gentamicin during the newborn phase, and 30 healthy children of similar age without previous gentamicin treatment were examined for vestibular function [14]. Neither in the number of spontaneous eye movements nor in the means of the nystagmus parameters of the rotatory test did the data show any significant difference between the groups [14]. The results indicated that gentamicin in controlled therapeutic doses has a less ototoxic and vestibulotoxic effect in newborns than it does in older children or in adults [13].

MECHANISM OF AMINOGLYCOSIDE OTOTOXICITY

Significant progress has been made in understanding aminoglycoside ototoxicity, which seems to be mediated by the disruption of mitochondrial protein synthesis, the overactivation of glutamatergic receptors (N-methyl-D-aspartate), and the formation of free radicals [4].

Studies performed in the last decade have helped to clarify, in part, the mechanisms by which the aminoglycosides damage the inner ear [15]. Drug-induced ototoxicity can be explained on a cellular level [16]. The cellular basis for aminoglycoside-induced hearing loss is a destruction of cochlear hair cells [5]. However, the biochemical and molecular mechanisms underlying this event are poorly understood [5].

Aminoglycosides appear to generate free radicals within the inner ear, with subsequent permanent damage to sensory cells and neurons, resulting in permanent hearing loss [15]. Histopathologic studies have shown that outer hair cells are more sensitive to ototoxic injury than are inner hair cells [17]. In animal models, histological findings resemble apoptotic cell death rather than necrosis [17]. In cases of aminoglycoside ototoxicity, a variety of free-radical species, including both oxygen and nitrogen free-radical species were detected in the inner ears, which are believed to initiate the apoptotic cascade [17]. There has been continued interest in discovering the cell signaling pathways by which aminoglycosides cause apoptosis of hair cells [15]. Apoptosis is primarily regulated by the activation of caspases through either internal or external pathways [15]. While in the internal pathway, mitochondria release apoptogenic factors into the cytoplasm to activate caspases, in the external pathway, caspases are activated by ligand binding to death receptors such as Fas and TNFR1 [15,18]. Bodmer *et al.* [18] revealed that gentamicin does not cause apoptosis *via* the Fas receptor. The mentioned study suggested that hair cell apoptosis is initiated *via* the intrinsic pathway in response to free radicals and stress signals [18]. However, Lei *et al.* [19] found a relationship between hair cell apoptosis and high expression of Fas protein in chicken inner ear following chronic kanamycin ototoxicity, thus, they concluded that Fas protein may contribute to regulate and control hair cells apoptosis.

Gentamicin causes condensation of the nuclei of outer hair cells followed by the loss of mitochondrial membrane potential and apoptosis [20]. Reactive oxygen species, known to play a role in gentamicin-induced ototoxicity, promote the opening of the mitochondrial permeability pore [21]. c-Jun N-terminal kinase (JNK) pathway also plays a role in gentamicin-induced cochlear and vestibular hair cell death *in vivo* [22].

Schacht [23] demonstrated that aminoglycosides can interact fairly efficiently with transition metals, including iron and copper, which can form free radicals. Aminoglycosides also have a very high affinity for binding to various types of phosphate lipids, and this affinity correlates fairly well with their potential ototoxicity [17]. Both *in vivo* and *in vitro* evidence suggests that aminoglycosides can interact with iron and lipids in the formation of reactive oxygen species and the expression of toxicity [17,23,24]. *In vitro* observations revealed that aminoglycosides can catalyze the formation of reactive oxygen species [25,26] in the presence of transition metals such as iron and copper [27,28]. Arachidonic acid can serve as an electron donor in the formation of reactive oxygen species by gentamicin and iron cell-free systems and lipid peroxidation occurs in the early stages of aminoglycoside-induced hearing loss in inner ear tissues [25,26,29].

Another study demonstrated that gentamicin causes a dose-dependent increase in intracellular calcium in hair cells of chick tissue cultures of sensory epithelium [30]. Ding *et al.* [31] hypothesized that the mechanism of hair cell death caused by gentamicin is cellular calcium influx and calpain activation.

Although the traditional explanation for their ototoxicity in cochlear and vestibular hair cells has been the inhibition of mitochondrial protein synthesis, evidence is accumulating to suggest that many aminoglycosides cause excitotoxicity in hair cells as a result of their agonist action at the polyamine site on the N-methyl-D-aspartate (NMDA) receptor [32-34].

Aminoglycoside antibiotics enhance the function of NMDA receptors by interaction with a polyamine modulatory site because they can mimic the positive modulatory actions of endogenous polyamines [33-35]. Accordingly, high doses of aminoglycosides may increase calcium entry through the NMDA receptor-associated channel and promote degeneration of hair cells and cochlear nerve fibers [34].

Round window membrane (RWM) permeability is an important factor in topical aminoglycoside ototoxicity [36]. Both substance and anatomic factors affect it [36]. Substance factors include molecular size and configuration, concentration, electrical charge, and lipid solubility [36]. Substances with a molecular weight of less than 1,000 such as gentamicin, streptomycin, neomycin, and tetracycline are transported actively through the RWM over a short period of time [36-38]. Among the anatomic factors are the thickness of the RWM and the presence of false membranes, tissue plugs, and bony obliteration [36].

It should be noted that during middle ear infections, there is often an abundance of mucosal edema, microorganisms, and fluid that might occlude the round window niche and further inhibit the absorption of topical medications [36]. However, it is suspected that as the infection clears with therapy, the round window becomes more permeable in the

new-normal middle ear space, which increases the ototoxic potential of continued application of aminoglycoside drops [3].

The presence or absence of facilitator drugs is another factor; their presence appears to increase the permeability of the round window membrane. Substances that have been shown to increase permeability include pontocaine, histamine, prostaglandins, leukotrienes, and, perhaps most notably, staphylococcal and streptococcal exotoxins [39-42].

PREDISPOSITION TO AMINOGLYCOSIDE OTOTOXICITY

Ototoxicity occurs both in a dose-dependent and idiosyncratic fashion [43]. The idiosyncratic pathway is presumably due to genetic predispositions [43]. Two mutations in the mitochondrial 12S ribosomal RNA gene have been previously reported to predispose carriers to aminoglycoside-induced ototoxicity [43-45]. One of these mutations, A1555G, has been found to be associated with non-syndromic deafness and aminoglycoside-induced deafness [46]. This finding suggests that in some carriers of this mutation aminoglycoside exposure is sufficient, but not necessary, for hearing loss to occur, and additional environmental and/or genetic risk factors likely exist [44]. These antibiotics exert their detrimental effect through an alteration of mitochondrial protein synthesis, which exacerbates the inherent defect caused by the mutation, reducing the overall translation rate down to and below the minimal level required for normal cellular function (40–50%) [46]. It was reported that 17-33% of patients with aminoglycoside ototoxicity carry the mentioned mutation. Ke *et al.* [47] reported that mitochondrial DNA from 62 members of 9 aminoglycoside induced deafness families revealed the nucleotide A1555G mutation in 12S rRNA gene of mtDNA in 20 members of 5 families. In another genetic study carried out in order to determine the frequency of the mutations A1555G in the mitochondrial DNA and 35delG in the connexin-26 gene in 21 patients from 21 non-consanguineous unrelated families affected by late-onset bilateral non-syndromic sensorineural hearing loss revealed that the A1555G mutation was found in 6 patients [48]. Five of these 6 patients had been treated with aminoglycosides, and in all of them the auditory impairment affected mainly the high frequencies [48].

A second mutation, the deletion of the T nucleotide at position 961 of the mitochondrial 12S rRNA gene, combined with a heteroplasmic, variable increase in the number of C nucleotides surrounding position 961, has been reported in one family and in an isolated patient with hearing loss and a history of exposure to aminoglycoside antibiotics [44,49,50].

In the study of Tang *et al.* [44], the A1555G mutation was found in 1 specimen out of 1,161 anonymized dried blood spot cards for the newborn screening laboratory and the 961delT + C[n] mutation was found in 7 specimens. Two previously unreported mutations, T961G and 956–960insC, were found in 6 and 5 specimens, respectively [44]. These results suggested that genetic susceptibility to aminoglycoside ototoxicity may be more common than suspected [44]. Interestingly, a report on three patients with a tympanic membrane perforation who developed severe ototoxicity after use of eardrops containing 0.35 per cent neomycin

showed no A1555G point mutation in these patients [51]. This finding indicated that application of low concentration neomycin to the middle ear can cause severe inner ear damage even in humans who are not hypersusceptible to aminoglycosides [51].

Phenotypically, there is a difference in the way this gene is expressed in Asian populations and in Caucasian populations [17]. In Chinese patients with this defect, hearing loss caused by aminoglycoside-induced ototoxicity is usually rapid and severe [17]. Among whites in the United States, hearing loss tends to be less severe and slowly progressive, sometimes occurring over a period of years after aminoglycoside administration [17]. It has been speculated that the difference between these two populations may be attributable to another genetic mutation--either in the mitochondrial gene or possibly in the nuclear gene--that modulates or modifies the severity of the defect [17].

The documentation of these susceptibility mutations raises questions about whether and how to screen for susceptibility to aminoglycoside-induced hearing loss. One way to assess individual risk is by careful evaluation of family history [44]. What this means clinically is that a good history might allow us to prevent ototoxicity in some cases [17]. If you determine that a patient's first-degree relative has experienced an aminoglycoside-induced hearing loss, this is a clear suggestion that perhaps the patient should not receive an aminoglycoside [17].

Fischel-Ghodsian *et al.* [52] emphasized the importance of thoroughly assessing a patient's family history for hearing loss prior to the administration of aminoglycoside antibiotics by demonstrating that with careful assessment of family history, many cases could perhaps be prevented. However, some studies did not support the necessity of screening [45]. The study of Fischel-Ghodsian *et al.* [52] also demonstrated that family history alone is not a perfect preventive method since three of the seven patients with hearing loss after aminoglycoside treatment had no known family history of hearing loss.

Another approach may be screening only at-risk populations [44]. At-risk populations might be defined as those individuals with increased risk of exposure to aminoglycoside antibiotics because of some other, unrelated condition such as cystic fibrosis, an immunological dysfunction, or individuals in whom the use of aminoglycoside antibiotics is under consideration for treatment of infectious disease [44].

Data are lacking on the general population frequency of these mutations in most countries including United States [44]. In the Swiss population, the mentioned mutation was not found in patients with ototoxicity due to aminoglycosides, but in only one of the five patients with ototoxicity had two sequence alterations in 12S rRNA of uncertain pathogenicity [45]. In New Zealand, one A1555G carrier in a sample of 206 persons was reported [53]. Data in literature suggest that the A1555G mutation is not an uncommon cause of hearing loss, and that a variety of populations may be at risk [44]. Genetic testing for susceptibility to aminoglycoside ototoxicity is complicated by the fact that the A1555G mutation carries a risk for hearing loss without exposure to aminoglycoside antibiotics, albeit with reduced penetrance [44].

It is fairly clear that patients who experience ototoxicity-induced hearing loss as a result of aminoglycoside administration have a genetic defect [17]. Among those who do not have such a defect, the hearing loss appears to be a dose-related phenomenon [17].

COCHLEOTOXIC EFFECTS OF DIFFERENT AMINOGLYCOSIDES-LITERATURE REVIEW

The morphological changes induced by different aminoglycosides have been extensively studied, and the pattern of toxicity has been found to vary greatly within this group of antibiotics [7,54,55]. The toxicity of these antibiotics can also vary depending on whether they are administered systemically or locally. The cochleotoxic lesion involves the destruction of the sensory hair cells in the cochlea, which can be quantified functionally or histologically in experimental animals [56].

It is known that aminoglycoside-induced changes in the cochlea move from the base to the apex, and from the outer hair cells to supporting cells, to more central neural structures such as spiral ganglion cells [1]. The only exception is the case reported by Lindsay *et al.* [57], in whom the outer hair cells less affected than the inner ones. In their study with tobramycin, Aran *et al.* [58] found total loss of outer hair cells outside the apex in an animal, and found that the population of inner hair cells was almost normal at the base but completely damaged from there towards the apex, where a small population of inner hair cells remained. In common with many authors, in our comparison study of different aminoglycosides (streptomycin, gentamicin, amikacin, and netilmicin) on guinea pigs we observed degeneration in the basal turn moving towards the apex, occurring first in outer hair cells and then in inner hair cells, supporting cells, and spiral ganglia and nerve fibres [7]. The situation was a little different in the gentamicin and amikacin groups [7]. With gentamicin, the histopathological changes in the spiral ganglia and nerve fibres were more severe than the damage in hair cells, and with amikacin, histopathological changes were more likely to be observed in the spiral ligament and stria vascularis [7]. It was reported that <http://www.jimonline.net/content/admin/blank.asp?ArticleID=303&type=full-5> type I hair cells were more sensitive to gentamicin than type II cells, and that hair cells were more sensitive than support cells [59]. However, in our mentioned study, systemic gentamicin caused equal toxicity in the basal, middle and apical turns of the cochlea [7]. We also found that it produced vacuolar degeneration and cellular loss in the organ of Corti, especially in the outer hair cells, and severe degenerative changes in spiral ganglia and nerve fibres [7]. Similar changes in the topical gentamicin group were noted [7].

The transtympanic route has been shown to induce ototoxic damage in many species, and unilateral transtympanic application is believed to have little or no systemic effect on the untreated ear [60]. Studies of the local effects of gentamicin have given varying results depending on the method used. Kimura *et al.* [61] found that transtympanic gentamicin caused severe damage in both cochlear and vestibular systems. Occlusion of the round window with a fat graft reduced the damage in an inconsistent manner [61].

There is little consensus in the literature about the relative effects on the inner ear of different aminoglycosides. Some studies have found gentamicin more cochleotoxic than streptomycin [62,63], while others have found vice versa [60,64,65]. Many factors may contribute to these discrepancies: the route of administration; the dose; the species; and the frequency and duration of treatment. In our mentioned study, gentamicin showed greater cochlear ototoxicity than streptomycin [7].

Using organ culture, Anniko [66] found by light microscopy that there were no morphological changes in the hair cells of those incubated in 1 mg/ml and 10 mg/ml netilmicin solution for 5 days, but there were ultrastructural changes. With the use of 100 mg/ml and 1000 mg/ml netilmicin, cochlear toxicity was determined while vestibular toxicity was more apparent in fetal mice [66]. In histological and functional studies, it was shown that ototoxicity of netilmicin in guinea-pigs was very low or absent [67,68]. However, in our mentioned study [7], we observed cochlear toxicity in the group given 75 mg/kg per day, which decreased from the basal turn to the apex. Proctor and el-Kashef [69] found significant damage in the basal turn of the cochlea with application of streptomycin into the tympanum of rats. We observed cochlear toxicity greater than that in the netilmicin group [7].

In a histological study performed on guinea-pigs, Kitasato *et al.* [70] found cochlear toxicities in the order gentamicin, amikacin and netilmicin, with gentamicin being the most toxic. Bamonte *et al.* [68] compared kanamycin, netilmicin, gentamicin and sisomicin in guinea-pigs, and reported that the least toxic of this group was netilmicin. In our study [7], cochlear toxicity was in the order gentamicin, amikacin, streptomycin, netilmicin, where gentamicin was the most toxic. In that study, no statistically significant difference between the severity of cochlear damage resulting from the systemic and topical applications was detected [7].

VESTIBULOTOXIC EFFECTS OF DIFFERENT AMINOGLYCOSIDES-LITERATURE REVIEW

Vestibular ototoxicity is defined as a chemical substance that has a destructive or damaging effect on the structure and function of the labyrinthine hair cells and their connections through the eighth nerve to the central nervous system [1,71]. The damage can vary from being minimal to the complete loss of vestibular function [1,71]. It may present early with positional nystagmus. If severe, vestibular toxicity can lead to disequilibrium and oscillopsia [2].

With vestibular toxicity, the initial and most extensive hair cell damage occurs in the apex of the cristae and the striolar regions of the maculae [2]. There may be hair cell loss extending toward the periphery of the vestibular receptor, and additional damage to the otoconial membrane and the otolith structures themselves [1,72,73]. It is known that streptomycin preferentially affects vestibular system rather than the auditory system. Some studies reported that systemic streptomycin administration caused dose dependent necrosis in the vestibular hair cells particularly in the epithelium of the cristae ampullaris [74,75]. In our study performed on guinea-pigs to compare the vestibulotoxic effects of streptomycin, gentamicin, amikacin, and netilmicin, we found that streptomycin was the most vestibulotoxic amino-

glycoside particularly when used topically [2]. Systemic administration caused moderate to severe degeneration in utriculus, sacculus and cristae ampullaris [2]. Cristae ampullaris was the most affected region in both systemic and topical administration as demonstrated by Lindeman [76]. Moderate to severe degeneration was reported in experimental studies using transtympanic streptomycin in guinea pigs [69]. Wanamaker *et al.* [59] reported that its vestibulotoxicity was similar to that of gentamicin. It is well known that gentamicin causes a greater degree of vestibulotoxicity than cochleotoxicity [3]. Kitasato *et al.* [70] found mild to moderate degeneration in the cristae ampullaris and moderate to severe degeneration in the utriculus of gentamicin-treated guinea-pigs. Aran *et al.* [58] reported that a large proportion of hair cells missing both in ampulla and in the utricule and the sacculus appeared to be slightly affected. In our mentioned study [2], cristae ampullaris, utriculus, and the sacculus were equally affected. Experimental studies on transtympanic gentamicin administration in different animals demonstrated some degeneration in the vestibule [62,77,78]. Amikacin is a derivative of kanamycin and has very little vestibular toxicity. However, in our study [2], mild to moderate degeneration was observed in the vestibular system in the amikacin group and the vestibulotoxic effects of netilmicin and amikacin were similar. Wersall *et al.* [79] reported no degeneration in systemic netilmicin group but a significant degeneration in the amikacin group. Comparative studies showed that the netilmicin appears to be the safest among aminoglycosides, with the lowest incidence of ototoxicity [2,66]. While Kitasato *et al.* [70] found mild degeneration in the cristae ampullaris and utriculus with 150 mg/kg netilmicin, we found mild to moderate degeneration with 75 mg/kg dose [2]. While the ototoxicity from systemic administrations of aminoglycosides is well documented, there is still controversy regarding the existence and significance of ototoxicity from the topical preparations [2]. Human studies are lacking, however, in our animal study [2], we demonstrated that pathological alterations after topical administration were similar to those observed after systemic administration. In our mentioned study the severity of vestibular damage was in the order of streptomycin, gentamicin, amikacin, and netilmicin [2].

The extent to which animal toxicity data can be reasonably applied to clinical treatment regimens for humans is unclear [39]. Nonetheless, there are clearly differences between the ototoxic effects of aminoglycosides placed into the middle ear of experimental animals and their effects on humans [39,80,81]. Otherwise, the widespread use of ototoxic medications in the middle ear and mastoid would have produced dramatic and widespread hearing loss and balance disturbance [17]. There are at least two reasons that account for such a discrepancy between animal and human data [82]. Brummett *et al.* [83] suggested that topical ototoxicity might not be recognized because it is not looked for since many clinicians do not obtain serial audiograms during topical therapy, and when sensorineural hearing loss is recognized after therapy, it usually is considered a squeal of chronic otitis media [83]. Additionally there are many anatomic differences between animals and humans that account for the discrepancy in susceptibility to topical ototoxicity [82]. The RWM in chinchillas is thinner than in humans, much deeper

and is positioned high on the superior surface of the niche in humans [82,84]. Thus, findings from animal research should be applied to humans with caution [82].

TREATMENT REGIMENS AND OTOTOXICITY

In several studies, safety and efficacy of single and multiple daily dose regimens were compared and found no loss of efficacy and no relationship between auditory and vestibular toxicity using single versus multiple dose regimens [85-89]. In a meta-analysis, including 24 studies, it was found that there was no significant difference between single and multiple dose regimens in the primary ototoxicity outcomes [90]. The pooled ototoxicity rates for studies that provided auditory testing results were 2.3% (10 of 436 cases) in the single dose regimen and 2.0% (8 of 406 cases) in the multiple dose regimen [90]. The fixed-effects risk ratio was 1.06 (95% CI: 0.51-2.19) [90]. In the same meta-analysis, studies that provided clinical vestibular function testing results revealed no toxicity among 209 patients given single dose and 206 patients given multiple doses [90]. In the mentioned study, ototoxicity based on pure tone audiometry, brainstem auditory evoked responses, or otoacoustic emissions for neonates and infants, vestibular testing, or clinical impression [90]. The results were consistent with meta-analyses of adult data, which showed no difference in ototoxicity rates between single or multiple dose regimens [91-93]. However, Blaser *et al.* [94] investigated those regimens for amikacin, netilmicin, and gentamicin in 24 randomized, clinical trials including a total of 3,181 patients, and found that ototoxicity occurred less frequently during single daily dosing (4.2% vs. 5.8%, respectively) [94].

PREVENTION

A great effort has been made to develop strategies to prevent aminoglycoside ototoxicity, e.g. by the co-administration of NMDA receptor antagonists or neurotrophins [4]. A number of antioxidant enzymes appear to reduce ototoxicity-induced hearing loss, which would appear to confirm the free-radical model of injury [17]. It was shown that both cochlear and vestibular toxicity of aminoglycosides could be attenuated by cotherapy with a wide spectrum of antioxidants or iron chelators [95]. Anti-free radical agents have a protective effect against the ototoxicity caused by aminoglycosides in animals [97-101]. These agents include deferoxamine [96], 2,3 dihydroxybenzoate [97,98], alpha-lipoic acid [99], and salicylate [100]. D-methionine, an amino acid with chelating and antioxidant properties, also attenuated the ototoxic effects of gentamicin [101].

Megalin, an endocytic receptor predominantly expressed in the kidney proximal tubule cells, has been shown to be present also in the cochlear sensory cells [102]. It is thought to mediate aminoglycoside toxicity in both the kidney and inner ear [17,103]. Receptor antagonists are candidate preventive measures for ototoxicity [17].

AMINOGLYCOSIDE USAGE IN MENIERE'S DISEASE (THERAPEUTIC TOXICITY)

Schuknecht used intratympanic streptomycin for vestibular ablation in patients with Meniere's disease (MD) in 1957 [104]. Since then, others have reported success with the use

of transtympanic gentamicin to ablate vestibular function in Meniere's patients [105,106]. Over the past decade, intratympanic gentamicin has become a major treatment modality for intractable MD [107]. Gentamicin is the preferred aminoglycoside for transtympanic vestibular ablation because it is more vestibulotoxic than cochleotoxic and therefore it may ablate vestibular function while preserving hearing [3].

The application of intratympanic gentamicin is an effective therapy for MD patients with intractable attacks and non-serviceable hearing in the affected ear or vestibular drop attacks [4]. As low-dose gentamicin therapy (30-40 mg weekly until signs of unilateral vestibular hypofunction appear) has been demonstrated to be effective, it should be recommended as the standard procedure [4].

Kaplan *et al.* [106] used the commercial gentamicin/betamethasone preparation to treat 20 patients with incapacitating MD. Most patients experienced ototoxicity by day 12, and half of them exhibited no response to ice-water calorics [106]. None of the 20 patients experienced any hearing loss [106].

Botrill *et al.* [108] published 2 years follow-up results of 50 patients with unilateral MD treated with 1 and 6 intratympanic injections of gentamicin. Control or significant improvement of definitive Meniere's attacks was achieved in 92% of patients and hearing preserved or improved in 76% [108]. Only one patient experienced profound sensorineural hearing loss [108].

In a meta-analysis including fifteen trials with 627 patients, it was shown that with intratympanic gentamicin treatment, complete vertigo control was achieved in 74.7% of patients, and complete or substantial control was achieved in 92.7% [107]. The success rate was not affected by gentamicin treatment regimen [fixed vs. titration] [107]. Toxic effects of intratympanic administration of gentamicin on hearing and word recognition were not significant [107]. It was also reported that patients who were administered the drug on a titration regimen experienced a worsening of their hearing and word recognition [0.02 dB and 0.4%, respectively] to a lesser extent than those who had the drug administered on a fixed dose regimen (5.4 dB and 6.5%, respectively). In the mentioned study [107], it was concluded that it is safer to avoid the short, high-dose regimen. It should be remembered that each patient must be evaluated individually and should be informed of all possible therapeutic options and consequences [107].

CONCLUSION

Despite their important side effects, aminoglycosides are likely to remain an important component of antibiotic treatment worldwide. Thus, a means of limiting the ototoxic effects of these drugs is desirable [5]. For that reason, limiting the duration of treatment to what is essential to resolve the middle ear process, choosing the topical agents that appear to be less toxic, and monitoring for ototoxicity with audiometry, especially in patients in whom prolonged topical therapy is necessary are essential [82]. Physicians prescribing topical aminoglycoside ear drops should be aware of their potential ototoxicity, particularly in the presence of a tympanic membrane defect [10]. In such circumstances, netilmicin, the least

ototoxic aminoglycoside, should be recommended and, especially in patients with a tympanic membrane defect, aminoglycosides should be used for the shortest duration possible [7]. Aminoglycoside eardrops should not be used in an open infected ear for more than 7 days [3]. Although transtympanic gentamicin and streptomycin are alternative therapies in MD, they should be used cautiously because of their severe cochleotoxicity [2,7].

A great effort has been made to develop strategies to prevent aminoglycoside ototoxicity. Among them, anti-free radical agents, such as salicylate, seem to be candidate preventive measures for ototoxicity.

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