Drug-induced Ototoxicity:

Mechanisms, Pharmacogenetics, and Protective Strategies

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Introduction

Drug ototoxicity limits quality of life of patients after treatment, having serious consequences especially for psychosocial development of children. While the ototoxicity of many drugs resolves after treatment discontinuation, the use of platinum derivatives and aminoglycosides is associated with permanent hearing loss. In this review, we have listed ototoxic drugs and the mechanisms by which they damage the ears. Moreover, possible protective strategies and important methods for early detection of ototoxic effects are discussed.

Ototoxic drugs

Ototoxic drugs and chemicals cause functional impairment and/or cellular degeneration of tissues of the inner ear. Cochleotoxicity is defined as damage affecting the auditory system and results in tinnitus and/or sensorineural hearing impairment. Vestibulotoxicity is caused by injury to the vestibular system and manifests in dizziness, vertigo, and loss of balance.[1] The symptoms can arise during or after the end of therapy. They are typically bilaterally symmetric or asymmetric, with one ear being affected later. More than 150 drugs are currently known to be ototoxic. These include aminoglycosides, glycopeptide and macrolide antibiotics, platinum-based anticancer drugs, loop diuretics, quinine, and salicylate analgesics.[2] Table 1 presents a selection of known ototoxic drugs.

Ototoxicity is usually permanent, however a mechanism of reversibility based on animal studies and some cases in humans proposes that initial damage to the marginal cells of the stria vascularis (for example through cisplatin) may recover if reparative processes are allowed to occur. If not, further accumulation of toxic medication exhausts the

chances of recovery, leading to permanent destruction of the outer hair cells and concomitant permanent hearing loss.[3]

Ototoxicity of loop diuretics, macrolide antibiotics, and quinine usually vanishes, when the treatment is stopped.[4–6] The ototoxicity of salicylate analgesics occurs in the event of an overdose and recedes after the medication is discontinued.[6] Platinum drugs, especially cisplatin, and aminoglycosides irreversibly damage the inner ear.[7] Permanent hearing impairment significantly impacts patients' quality of life and leads to considerable follow-up costs. Follow-up costs of $300,000 were estimated per adult who acquired permanent hearing impairment due to ototoxic medication.[8] Hearing impairment is especially deleterious for children before and during language acquisition, because it adversely affects their psychosocial development and education.[9] For children, who experienced permanent hearing impairment before speech acquisition the estimated follow-up costs increase to $1,000,000.[8] Deaf people or people with hearing impairment are often socially and economically disadvantaged.[10] Tinnitus can also be related to considerable distress, anxiety, depression and reduced quality of life.[11] Vestibular disorders are also often comorbid with panic, agoraphobia and depression.[12]

So far, research mainly focuses on ototoxicity induced by platinum-based anticancer drugs, especially cisplatin, and aminoglycosides. In-vivo studies on the pathomechanism of drug-induced ototoxicity were exclusively investigated in animal models and then related to the clinical manifestations of ototoxicity observed in humans.

Information on mechanisms of ototoxicity as well as common and individual risk factors for ototoxicity allows the development of strategies to prevent these life-long disabling side effects.

Physiological functions of the ear

The ears contain receptors for hearing and for balance and spatial orientation. These receptors are located in the cochlea and in the vestibular apparatus of the inner ear, respectively.

Sound perception is caused by compression and rarefaction of a medium, such as the air, to produce longitudinal waves, which travel through the medium. These waves (vibrations) are mainly characterized by a frequency, which is measured in hertz (Hz) and is perceived as pitch, and an amplitude or sound pressure, which is measured in pascals (Pa) and also decibel sound pressure level (dB SPL) and is perceived as loudness. Waves of a single frequency are pure tones, which are very rare in the environment. Music is an ensemble of harmonically related tones. Most acoustic events we experience are neither pure-tones nor harmonically-structured sound, but rather include many frequencies of the hearing range simultaneously.

Sound waves are collected by the external ear and transmitted to the tympanic membrane, which is connected to the malleus, the first of three small bones or ossicles (malleus, incus, and stapes), which are part of the middle ear. Because of the smaller area of the stapes compared with the tympanic membrane, and of the lever effect of the ossicles, the middle ear acts as an impedance transformer, strongly reducing reflection of sound energy. Vibrations of the stapes are transmitted to the fluid contained in the inner ear at the oval window. The part of the inner ear important for hearing function is the cochlea, a shell-like structure with two and half turns. The cochlea partition delimits the scala vestibuli, where the stapes inserts at the oval window, and the scala tympani, which ends at the round window (figure 1). The scala vestibuli and the scala tympani are filled with a fluid called perilymph, while the cochlea partition is filled with endolymph. Perilymph and endolymph have a different ionic composition: the perilymph is an

extracellular fluid similar to plasma, while the endolymph is similar to intracellular fluid containing high K^+ concentration up to 140 mM. The endolymph is positively charged with a potential of +85 mV. These electric properties of the endolymph are determined by the action of specialized cells constituting the stria vascularis and are of primary importance for hearing processes. The stria vascularis contains three different cell types: marginal, intermediate, and basal cells. The marginal cells are able to secrete K^+ in the endolymph. The first step of this process is the uptake of K^+ into the marginal cells, which is mediated by a basolateral (i.e. expressed on the membrane at the opposite site of the endolymph) $Na^+/K^+/2Cl^-$ cotransporter (NKCC1). The NKCC1 transports 1 Na^+, 2 Cl^-, and 1 K^+ ion (Na^+ according and Cl^- and K^+ against the electrochemical gradient) into the marginal cell. The secretion of K^+ into the endolymph is then mediated via apically expressed K^+ channels.[13]

The cochlear partition contains the hearing receptors (hair cells, outer hair cells –OHC- and inner hair cells –IHC- in a 3:1 ratio), which are organized together with other cells (supporting and Deiters cells) and structures (basilar and tectorial membranes) in the organ of Corti. The basilar membrane delimits the cochlear partition on the bottom from the scala tympani and the Reissner's membrane on the top from the scala vestibuli (figure 2). The hair cells derive their name from the presence of stereocilia on their top. These stereocilia are in close contact with the tectorial membrane in the OHCs, while stereocilia of the IHCs have a loose contact with this membrane. The space between the tectorial and the Reissner's membrane is filled with endolymph and is called scala media.

The vibrations transmitted by the stapes to the perilymph of the scala vestibuli press the cochlea partition toward the scala tympani and back causing a continuous up- and down movement of the basilar membrane and producing waves, which travel towards the

apex or apical part of the cochlea (figure 1). These waves are characterized by an amplitude, which reaches its maximum at different locations along the cochlea depending on their frequency. At these locations, hair cells are specifically stimulated. Tones of high frequency cause a traveling wave with a maximal amplitude near to the stapes. As tone frequency decreases, the maximal amplitude of the wave is reached towards the apex of the cochlea (tonotopy of the travelling wave). One reason for this is that the cochlear partition is more stiff at the base, near the oval window, and this stiffness decreases towards the apex. The other reason is the presence of a second mechanism called the cochlear amplifier. The vibrations of the basilar membrane bend the hair bundles of the OHCs and open or close ion channels, probably transient receptor potential (Trp) channels, depending on the direction of the bending.[13] The stereocilia on the apical membrane of hair cells are organized in an organ pipes fashion, and their bending toward the longer stereocilia opens the ion channels, whereas bending in the opposite direction closes them.[13] The opening of the ion channels drives positively charged ions (mostly K^+) from the endolymph to the cell cytoplasm, according to the electrochemical gradient for K^+. K^+ leaves the hair cells probably through basolaterally located Potassium Voltage-Gated Channel Subfamily Q Member 4 (KCNQ4, also known as Kv7.4) K^+ channels to be recycled in the cochlea. There are three models of K^+ recycling in the cochlea, which are well summarized by Zdebik et al., and are not discussed here.[13] The depolarization of OHCs induces the contraction of myosin filaments by acting on the protein prestin, which is expressed on the lateral membrane of these cells. This action of the OHCs requires a great deal of energy, which is provided by an abundance of mitochondria. The alternating shortening and elongation of OHCs strongly amplify vibrations at the site characteristic for a specific frequency, finally resulting in the depolarization of IHC followed by transmitter release and activation of

afferent nerve fibers. A detailed description of the cochlear amplifier was published in an excellent review by Ashmore.[14]

Another physiological function of the inner ear is the perception of linear and angular acceleration to inform the central nervous system dynamically about the spatial position and movement of the head. This function decisively contributes to maintaining balance. These spatial orientation and motion sensations are perceived in the vestibular organ, which is constituted by three semicircular canals roughly orthogonal to each other and two otolith organs. Each canal contains a cupula, which is able to sense angular acceleration. The two otolith organs, the utricle and the saccule, each contain a macula, which senses linear acceleration.[15] Both the semicircular canals and the otolith organs are filled with endolymph, which is also here rich in K^+ (145 mM). Excitation of the vestibular organ is transmitted to the brain by the vestibular nerve. The cupulae and the maculae contain hair cells, which are the sensory cells of the vestibular apparatus. These hair cells have stereocilia, which are embedded in a mucopolysaccharides-rich gelatinous mass, which, in the maculae only, is surmounted by calcium carbonate crystals. Besides stereocilia, the vestibular hair cells have one tallest cilium, named kinocilium. Linear and angular accelerations cause movement of the endolymph in the otolith organs and in the semicircular canals, respectively, which specifically "push" the maculae or cupulae, in this way bending the stereocilia. Bending of the stereocilia toward or away from the kinocilium, respectively, depolarizes or hyperpolarizes the cells opening or closing ionic K^+ channels and therefore modulating their afferent activity.

Selected ototoxic drugs

Platinum-based anticancer drugs

The platinum-based anticancer drugs, cisplatin, carboplatin, and oxaliplatin, are amongst the most frequently used cytotoxic anticancer drugs.[16] All three platinum-drugs are ototoxic. However carboplatin and oxaliplatin are less ototoxic than cisplatin.[17] Cisplatin was the first platinum-based anticancer drug, which entered the clinic, and it is still the most frequently used platinum compound. Cisplatin is successfully used for the treatment of pediatric and adult solid tumors, like neuroblastoma, germ cell tumors, osteosarcomas, brain tumors, testicular cancer, ovarian cancer, endometrial cancer, lung cancer or head and neck cancer.[16]

Cisplatin is planar complex of a bivalent platinum cation with two cis-standing chloride and two cis-standing ammonia-ligands. Intracellular - at low levels of chloride anions - the chloride anions of the cisplatin complex are exchanged by water molecules. This results in a highly reactive aquo-complex, which binds to nucleophiles in DNA, RNA, proteins and peptides. In proliferating tumor cells the DNA is the main target of cisplatin. The toxicity of cisplatin correlates to the amount of platinum bound to DNA. It preferentially binds to the N-7 in guanine.[18] Cisplatin can induce inter- as well as intrastrand crosslinks. The interstrand crosslinks recruit high-mobility shift group (HMG) proteins, which activate numerous signaling cascades blocking DNA replication and transcription, arresting the cell cycle, and inducing DNA-repair.[18] Platination of mitochondrial DNA and proteins affects cell respiration and induces reactive oxygen species (ROS) formation, which additionally harms the cells.[19] Irreversible damage finally induces apoptosis in the affected cells. Cisplatin can enter cells by passive diffusion or via active transporters. The copper transporter 1 (CTR1, gene name *SLC31A1*) and the organic cation transporter 2 (OCT2, *SLC22A2*) were shown to mediate the cellular uptake of cisplatin.[20,21] Figure 3 shows the expression and localization of

these transporters in the scala media of the cochlea. In addition, endocytosis receptors might play a role in cellular cisplatin uptake.[22]

Cisplatin-induced ototoxicity

Treatment with cisplatin is associated with various side effects, including nausea, vomiting, neuro-, nephro- and ototoxicity. The neuro-, nephro-, and ototoxicity of cisplatin are dose-limiting. Cisplatin induces sensorineural hearing loss. Hearing impairment usually starts days to weeks after treatment and is mostly bilateral.[23] Initially the high frequency range is affected. If treatment is continued hearing loss progresses to lower frequencies and eventually leads to higher degrees of hearing loss. Hearing can even worsen after end of treatment, though a few cases of hearing improvement after end of therapy were reported.[3,24] Tinnitus has been identified as a common effect of cisplatin treatment, occurring in 25 – 50% of cases and persisting for at least one year in 38% of cases.[25] Apart from hearing impairment, tinnitus, vertigo with and without nausea were observed during cisplatin treatment.[17] Many years experience in the clinical use of cisplatin allowed the identification of risk factors for cisplatin-induced ototoxicity. Cisplatin ototoxicity depends on dose, route and duration of administration. Bolus infusions are more ototoxic compared to short or continuous infusions.[17] Up to know there is no evidence that continuous infusions are less ototoxic than short infusions.[26] The risk for cisplatin-induced ototoxicity increases with the level of the individual single dose as well as the cumulative cisplatin dose.[17] In addition, age ≤4 years, simultaneous cranial irradiation, noise exposure, co-medication with other ototoxic and nephrotoxic drugs, like loop diuretics or aminogylcosides, additional administrations of carboplatin, preexisting hearing impairment or renal insufficiency increase the risk for cisplatin-induced ototoxicity. The incidence rates for cisplatin-

induced ototoxicity also depend on the applied ototoxicity scales and the distribution of risk factors in the patient group studied. They can vary between 13 and 95%.[17]

Mechanism of cisplatin-induced ototoxicity

Damage of the hair cells of the cochlea is considered responsible for the cochleotoxicity of cisplatin. According to numerous studies in animal models the outer hair cells of the basal part of the cochlea are initially affected, which is consistent with the observed hearing impairment at high frequencies in humans. If treatment is continued, the damage progresses to the outer hair cells of the medial and apical parts of the cochlea and to the inner hair cells. Then lower frequencies become affected including speech frequencies. In rats the degeneration of the hair cells was accompanied by a loss of function of the blood-labyrinth barrier and of the endolymphatic potential.[27] The precise molecular mechanisms, which are responsible for the degeneration of the cochlear structures, are yet not fully understood. In mice both the cisplatin uptake transporters Ctr1 and OCT2 have been detected in the cochlea.[21] OCT2 seems to be expressed both in hair cells and in marginal cells of the stria vascularis.[21] Here, a stronger expression in the basal turn of the cochlea was observed, offering a possible explanation for initial high frequencies hearing impairment. After exposure of guinea pigs to either cisplatin or carboplatin Thomas and colleagues detected platin-DNA adducts in the hair cells of the cochlear and the marginal cells of the stria vascularis.[28] Because these cells do not proliferate, it is supposed that other mechanisms than inhibition of DNA replication might be responsible for cisplatin-induced ototoxicity, such as increased ROS formation in mitochondria followed by decompensation of the oxidative metabolism.[29] Mitochondrial DNA has no histones and is therefore more easily targeted by cisplatin as shown in preclinical studies with tumor cell lines.[30] In addition, platinated nucleotides are eliminated more slowly from the mitochondrial than from nuclear DNA. Thus,

mtDNA appears to be more vulnerable for DNA damage, which persists longer.[31] Thus, the inhibition of transcription and translation of mitochondrial proteins via platination of mtDNA and impairment of protein function via platination of proteins is supposed to negatively affect mitochondrial energy metabolism and to result in an increased formation of ROS. [30]

Pharmacogenetics of cisplatin-induced ototoxicity

The susceptibility of individual patients for cisplatin-induced ototoxicity is not exclusively related to clinical risk factors and strongly suggests the existence of genetic variations, which in addition determine patients' individual risk for cisplatin-induced ototoxicity.[17] Using a candidate gene or a high-throughput screening approach various genetic variants were identified, which could be related to patients' individual risk for cisplatin-induced ototoxicity. The candidate gene approach mainly focused on genes, which were supposed to play a central role in detoxification of cisplatin, like glutathione-S-transferases (GSTs), enzymes involved in DNA-repair, and proteins involved in the transport of cisplatin, like OCT2 or CTR1. Such studies identified protection from cisplatin-induced ototoxicity by a deletion of a *GSTT1* allele and/or the *GSTP1* SNP rs1695 among adults with testicular cancer. Among children with various cancers protection from cisplatin-induced ototoxicity was observed for patients carrying the *GSTM3* SNP rs1799735. Protection was also observed for children and adults, who carried the SNP rs316019 of the *OCT2* gene. On the other hand an increased risk for cisplatin-induced ototoxicity was linked to the LRP2 SNP rs2075252 or the Xeroderma pigmentosum group C (*XPC*) SNP rs228001. The first high-throughput screen, which analyzed variations in drug metabolizing genes attracted considerable attention. It identified an increased risk for cisplatin-induced ototoxicity amongst patients carrying the SNP rs12201199 in the thiopurine–S-methyl-transferase (*TPMT*) gene and/or the

SNP rs93323377 of the catechol-O-methyltransferase (*COMT*) gene in children with various cancers. Recently a genome wide-association study identified a significant association between the SNP rs1872328 of the acylphosphatase 2 gene (*ACYP2*) and an increased risk for cisplatin-induced ototoxicity amongst medulloblastoma patients. A detailed overview of the studies, which evaluated pharmacogenomic markers in connection with patients' individual susceptibility of cisplatin-induced ototoxicity, and respective references are provided in the supplement.

The majority of observed associations still require independent confirmation. The few replication studies produced varying results. The association between cisplatin ototoxicity and the *TPMT* SNP rs12201199 and the *COMT* SNP rs93323377 was confirmed in a separate cohort of pediatric cancer patients, but failed replication in three other cohorts of children with cancer. Likewise, the protective effect of *GSTM1* deletion, the *GSTP1* SNP rs1695, or the *GSTM3* SNP rs 1799735 were not confirmed by other groups.[17] There are many reasons for replication failure and replication failure does not necessarily mean that the observed association does not exist. The pharmacology of cisplatin is complex and numerous genes are involved in mediating cisplatin effects, resistance, and detoxification. Cancer chemotherapy is based on a combination of various anticancer drugs. Cytotoxic anticancer drugs often use the same mechanisms of detoxification as cisplatin and activate similar mechanism of resistance. The combination of anticancer drugs varies between different protocols. In different treatment protocols various detoxification and resistance pathways of cisplatin might be addressed to differing degrees. Thus, genetic variants in these genes might have different impacts on cisplatin-induced ototoxicity. In addition, the grading of ototoxicity and the distribution of other risk factors related to cisplatin-induced ototoxicity, like age

or co-medication with other ototoxic drugs might have impacted the significance of genetic variants for cisplatin-induced ototoxicity to varying degrees.

Aminoglycosides

Aminoglycosides are broad-spectrum antibiotics, which are highly active against aerobic, gram-negative bacteria, like enterobacteriaceae and pseudomonas.

Aminoglycosides (either alone or as part of combination therapy) are used for prevention and treatment of life-threatening sepsis in newborns or immunocompromised persons, i.e. after chemotherapy. In addition, they are used for eradication of *Pseudomonas aeruginosa* in patients with cystic fibrosis and for treatment of recurrent and resistant tuberculosis. Because of their low costs and the still high infection rates of tuberculosis in developing and emerging countries aminoglycosides are amongst the most frequently used drugs worldwide.[32]

Aminoglycosides bind to the aminoacyl-tRNA binding side of the 16S rRNA in the 30s subunit of bacterial ribosomes. They cause misreads during mRNA-translation and consequently compromise protein synthesis. The accumulation of truncated and malfunctioning proteins eventually kills sensitive bacteria.[7]

The therapeutic index of aminogylcosides is narrow with oto- and nephrotoxicity being dose limiting. Cochleotoxicity can manifest as tinnitus and/or sensorineural hearing loss and can lead to deafness. Vestibulotoxicity can occur as vertigo, nausea, nystagmus, and ataxia. The aminoglycosides differ in their ototoxic properties. Neomycin is considered most ototoxic followed by gentamicin, kanamycin, and tobramycin.[32] Streptomicin and gentamicin are mainly vestibulotoxic, while amikacin, neomycin and kanamycin are preferentially cochleotoxic.[33] Tobramicin is equally vestibulo- and cochleotoxic.[32,33]

Depending on the screening methods used, the nature and severity of the disease, and the dose and timing of aminoglycoside applications an incidence of ototoxicity between

3.2 to 47% was reported.[32] High doses of aminoglycosides, high plasma concentrations, frequent applications, and long therapy times are associated with an increased risk for aminoglycoside ototoxicity. In addition, renal dysfunction, higher age, noise exposure, preexisting hearing impairment, and co-administration with other ototoxic or nephrotoxic drugs also increase the risk of aminoglycoside-induced ototoxicity.[34] To identify elimination disorders and to adapt aminoglycoside doses accordingly therapeutic drug monitoring (TDM) of aminoglycoside plasma levels is recommended.[35]

Mechanism of aminoglycoside-induced ototoxicity

From animal studies it is known that aminoglycosides harm the hair cells of the cochlea. Firstly, the outer hair cells of the basal turn are affected, which impairs hearing at high frequencies. With ongoing exposure damage progresses to upper turns and the inner hair cells, which causes hearing impairment at speech frequencies or even deafness. Aminoglycosides also damage the stria vascularis, the marginal cells, and the spiral ganglion.[32] No accumulation of aminoglycosides in the peri- and endolymph of the inner ear was observed in animal studies. In these compartments, the concentration of aminoglycosides was only about one tenth of the aminoglycoside concentration in serum.[36] This suggests that aminoglycosides enter the inner ear by active transport mechanisms. As candidate transporters the endocytosis receptors, megalin and cubulin, TRP cation channel, and mechanoelectrical transducer (MET) channels were identified.[37,38] Uptake and transport of aminoglycosides were comprehensively reviewed and illustrated by Steyger.[39] Aminoglycosides are cleared slowly from the inner ear fluids. Elimination half-lives between 10-13 days had been observed after a single dose. After multiple doses the elimination half-life increased up to 30 days.[40] This

has to be considered, when other ototoxic drugs need to be given after aminoglycoside therapy.

The exact mechanism by which aminoglycosides damage the inner ear is yet not fully elucidated. A lot of evidence suggests that oxidative stress induces apoptosis and necrosis in the hair cells of the cochlea, the marginal cells, and the stria vascularis. Aminoglycosides interfere with bacterial ribosomes and inhibit bacterial protein biosynthesis. Mitochondrial ribosomes are structurally more related to bacterial ribosomes than the eukaryotic ribosomes in the cytosol.[41] By inhibition of mitochondrial protein biosynthesis aminoglycosides inhibit the enzyme aconitase, which in turn impairs cellular respiration and leads to an accumulation of ferric cations. Aminoglycosides complex these cations and form ROS by Fenton reaction. [42] In addition, the $Fe^{2+}/^{3+}$-Aminoglycoside-complex forms a ternary complex with arachidonic acid, which promotes ROS formation via lipidperoxidation.[43] Due to the high prevalence of mitochondria in the cells of the inner ear these cells are considered especially sensitive for aminoglycoside toxicity.[7]

Pharmacogenetics of aminoglycoside-induced ototoxicity

The A1555G mutation in the mitochondrial genome has been associated with an increased risk for aminoglycoside ototoxicity.[41] This mutation is located in the gene, which codes for the mitochondrial 12S rRNA. By replacement of the adenine by guanine in position 1555 the guanine can form an additional base pair with the cytosine in position 1494 in the ternary structure of the 12S rRNA. This renders the mutated 12S rRNA more similar to the bacterial 16S rRNA, the key target of aminoglycosides.[41] Aminoglycosides bind with higher affinity to the mutated 12S rRNA than to the wild type 12S rRNA. This is supposed to be the reason for the increased risk of aminoglycoside-

induced ototoxicity in patients carrying the A1555G mtDNA mutation.[44] Among Asian patients, who experienced aminoglycoside ototoxicity, 10-33% carried this mutation. In 17% of Caucasians, who suffered from aminoglycoside-induced hearing impairment, this mutation was detected, while the overall prevalence of the A1555G mutation in Caucasians is only 0.2%.[45,46] Apart from an increased risk to experience sensorineural hearing loss from aminoglycoside treatment, these patients have also an increased risk for presbyacusis – age-related hearing loss.[41] Additional mutations in the mitochondrial genome have been identified, which also predispose for aminoglycoside-induced hearing impairment. Many of these polymorphisms are located in the gene coding for the 12S rRNA suggesting that this is a hot spot for non-syndromic aminoglycoside-induced hearing loss. A detailed summary on genetic variants related to patients individual risk to experience ototoxicity by treatment with aminoglycosides is given in the supplement.

Other Ototoxic drugs

Salicylate analgesics are amongst the most commonly used drugs worldwide. High doses of salicylate can induce mild to moderate hearing loss. Hearing loss occurs bilaterally symmetric and is accompanied by tinnitus and suprathreshold changes.[47] It usually recedes within 24h to 72h after cessation of the drug.[6] However, individual cases with permanent hearing loss have been reported, too.[47] Salicylates rapidly enter the cochlea and diffuse all parts of the cochlear duct. In animals serum salicylate levels correlated with perilymph salicylate concentrations and hearing loss.[48] In addition they suppressed cochlear function preferentially at high and low frequencies and enhanced the neural activity in the auditory thalamus, cortex, and amygdala.[49] Cyclooxygenase inhibition is considered one cause of salicylate-induced ototoxicity.[6] Decreased

prostaglandins and increased leucotrienes result in vasoconstriction and reduced cochlear blood flow. This increases the permeability and reduces the mobility of the outer hair cells. In addition salicylates reduced hair cell mobility by reversible inhibition of MET channels in rat cochlear explants.[50] The changes reverted, if the cyclooxygenase and MET channels were no longer inhibited.

The anti-malaria drug *quinine* experienced a renaissance over the recent years because of increased resistance of plasmodium falciparum to the semisynthetic chloroquine.[51] Administration of quinine can induce a transient increase in hearing threshold of about 10 dB.[52] The precise mechanisms, which are responsible for this hearing threshold shift, are yet not fully understood. Though quinine does not inhibit cyclooxygenases, it, like salicylates, caused vasoconstriction in the cochlea, decreased the cochlear blood flow, and induced reversible alterations of the outer hair cells in guinea pigs.[6] A reversible inhibition of MET channels along with reduction hair cell motility is also supposed to be involved in the increase of hearing thresholds.[50] This increase is dose dependent and already occurs at therapeutic doses in the high frequency range. To avoid an increase in ototoxicity co-administration of quinine with other ototoxic drugs should thus be avoided.[48]

Loop diuretics, like etacrynic acid, furosemide, torasemide, or bumetanide are established drugs in the treatment of cardiac and renal insufficiency, and high blood pressure. Loop-diuretics caused a dose dependent transient hearing loss through a reversible reduction of the endocochlear potential in animal studies.[53] They reversibly block Na-K-Cl cotransporter (NKCC1 and NKCC2) in the thick ascending limb of loop of Henle. Similar transporters are also expressed in the inner ear, regulating the ionic composition of the endolymph and mechanical transduction.[54] In addition the reduction in blood flow temporarily impairs the barrier function of the endothelium, which

facilitates the transition of other ototoxic drugs, like cisplatin and aminoglycosides, into the inner ear. Thus, loop diuretics increase the ototoxicity of cisplatin and aminoglycosides and co-administration has to be avoided.[5,53]

Macrolide antibiotics, like erythromycin, clarithromycin, and azithromycin, are widely used for the treatment of bacterial infections of the respiratory tract and the skin. They are also successfully used for the treatment of chlamydia infections and syphilis. Macrolide antibiotics can induce hearing loss, tinnitus, and vertigo. Ototoxicity usually manifests in bilateral, symmetrical hearing loss of 40-50 dB within 2 to 7 days after treatment start. It increases with higher dose and serum levels.[4,55] In most cases it resolves within 1 to 3 weeks after cessation of treatment. Occasionally irreversible hearing loss was reported after administration of erythromycin as well as azithromycin.[4,56] The mechanisms underlying the observed ototoxicity are still unknown and preclinical studies are controversial. Some animal studies observed an effect of macrolides on outer hair cells and transiently evoked otoacoustic emissions, while others did not. Measuring the transepithelial short circuit current Liu et al. observed a reversible reduction of K^+-secretion in explanted gerbil cochleas exposed to erythromycin. They postulated that an inhibition of ion transport in the stria vascularis and the vestibular dark cells might contribute to macrolide-induced ototoxicity. However, they did not further specify, which transporters might have been inhibited by erythromycin.[57] Because some patients also reported on psychiatric complications, like confusion, lack of control, dizziness, it is also considered that erythromycin induced hearing loss through the impairment of central nervous system function.[58]

Glycopeptide antibiotics, like vancomycin, are labeled ototoxic. They are potent antistaphylococcal antibiotics and successfully used in case of methicillin-resistant infections. Tinnitus and sensorineural hearing loss were reported after administration

of vancomycin.[59] The establishment of a causal link was difficult. Preclinical studies on vancomycin-induced ototoxicity came to contradictory results. Tinnitus and sensorineural hearing loss were partly attributed to impurities in the fermentation product. The first vancomycin in clinical use was also known as Mississippi mud because of 30% impurities. Today technological improvements allow the production of vancomycin with a purity of 92-95%.[60] Many patients, who developed ototoxicity after vancomycin were also treated with other ototoxic drugs, like aminoglycosides or loop-diuretics, or suffered from infections, which were also known to cause sensorineural hearing loss.[61] Subsequent prospective clinical trials observed no ototoxicity after therapeutic doses of vancomycin.[62] Glycopeptide antibiotics are nephrotoxic and can impact the elimination of other ototoxic drugs. In view of the reports on ototoxicity after administration of vancomycin and other ototoxic drugs vancomycin should not be combined with other ototoxic drugs like aminoglycosides.

Evaluation and Grading of ototoxicity

Drug-induced hearing loss typically affects primarily the high frequencies. Performing high frequency (HF) audiometry discovers ototoxic damage on a subclinical stage. At this stage, the patient usually does not realize any hearing impairment. Since the audiograms before and after treatment, which are necessary for the evaluation of drug-induced ototoxicity, are often not available, the actual rate of ototoxic drugs might be underestimated.[63] Given the large number of potentially ototoxic drugs it is also unrealistic to monitor each ototoxic medication. However, timely recognition of ototoxicity, even before the patients become aware of the problem, allows early interventions (i.e. consideration of treatment alternatives, dose modifications, application of otoprotective substances) and can minimize or even prevent the

progression of ototoxicity. Thus, audiological monitoring is standard care for patients who will receive cisplatin or aminoglycosides, because these drugs can induce permanent hearing impairment.[23]

Evaluation of ototoxicity requires baseline audiograms before treatment.[63] The basic audiologic assessment comprises ear microscopy and pure tone audiometry in air and bone conduction to rule out a middle ear problem. Conventional pure tone audiometry is performed from 250 to 8000 Hz in air and from 250 to 6000Hz in bone conduction. With HF audiometry, which extends measurements up to 16 kHz, damages of the inner ear can be detected at an early stage.[64] In infancy and childhood age-specific hearing tests are performed like Visual Reinforcement Audiometry and conditioned play audiometry.[65] Speech audiometry in noise is an important indicator of speech comprehension in daily life, especially at school or workplace.[66] The middle ear status is checked by impedance audiometry. Transient evoked otoacoustic emissions (TEOAE) result from movements of the cochlear outer hair cells in response to an acoustic stimulus. Usually they are recorded within a range of 1–4 kHz and can determine minor cochlear dysfunction. Distortion product emissions (DPOAE) are evoked by simultaneous stimulation with two tones at different frequencies. DPOAE can be recorded within a frequency range from 1 to 8 kHz and are useful for the detection of early alterations in auditory function documented by their amplitude changes. DPOAE measurements are recommended for monitoring of cisplatin-induced ototoxicity.[23] Auditory brainstem response (ABR) and auditory steady state response (ASSR) audiometry record auditory evoked potentials, potentially needed as an objective measurement tool in case of infants, psychically and mentally diseased persons. In conventional devices using clicks as stimulus, the measurement is restricted to 500-4000Hz but using chirps recordings are possible up to 6000 Hz. Further high frequency

solutions concerning OAE, ABR and ASSR are in development, e.g. combining DPOAE recordings with ASSR measurements reaching frequencies up to 6000 Hz.[67]

For reporting of ototoxicity in clinical trials the WHO Common Toxicity Criteria and the National Cancer Institute Common Criteria for Adverse Events (CTCAE) are used. (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-0614_QuickReference_5x7.pdf)

These grading scales were found inadequate for monitoring of cisplatin-induced ototoxicity especially in children. Brock and colleagues were the first, who established a grading system for cisplatin-induced ototoxicity. They used standard pure-tone audiogram frequencies and defined hearing loss at a threshold ≥ 40 dB.[68] Chang and Chinosornvantana refined this grading system and introduced 20 dB HL as hearing threshold.[69] This classification was adopted by the SIOP Boston Ototoxicity Scale, which is actually validated against existing scales with respect to correlation to final outcomes, feasibility, and inter-rater reliability.[23] The Muenster Classification of high frequency hearing loss with multi-level discrimination within the hearing loss categories and considering tinnitus and minimal hearing losses (>10 - ≤20 dB) was validated by Schmidt et al.[70] It was developed for early detection of beginning ototoxicity and early intervention. A hearing loss between 10-20 dB after the second cisplatin cycle according to the Muenster classification had the highest predictive value concerning the need for hearing aids at end of chemotherapy (sensitivity: 67%, specificity, 87%).[71] A recently published review by Waissbluth provides an excellent overview of ototoxicity grading scales. [72]

Prevention of ototoxicity

Up to now neither cisplatin nor aminoglycosides can be replaced completely by equally effective and less ototoxic drugs. In case of life-threatening infections, the use of

aminoglycosides is often without alternative. In addition, for their activity against recurrent and resistant tuberculosis and their low price they are still amongst the most frequently used drugs world-wide.[32] Because the antitumor effects of cisplatin, carboplatin, and oxaliplatin are not identical, cisplatin is only little, if at all, replaced by the less ototoxic carboplatin and oxaliplatin. Thus, the risk of ototoxicity is still taken on. If treatment with these drug is planned or probable i.e. as part of anticancer treatment, regularly audiologic monitoring is part of standard care. With appropriate audiological testing at high frequencies early damage can be detected and alternative treatments might be considered. In addition, recording and consideration of individual risk factors (like age, preexisting hearing loss, renal insufficiency, and validated pharmacogenomics markers) are essential to identify patients at risk, who require a closer monitoring, possibly alternative medications, or otoprotectants.

In view that effective alternatives are not always available, efforts were undertaken to protect the inner ear from cisplatin and/or aminoglycoside ototoxicity. Because ROS are supposed to play a critical role in mediation of cisplatin as well as aminoglycoside ototoxicity, administration of free radical scavengers are evaluated as otoprotectants. Amifostine is a prodrug of the radical scavenger WR-1065 and is approved for the prevention of neutropenia, nephrotoxicity, and xerostomia. The concerns that amifostine also protects tumor cells from platinum drugs and alkylating agents have not been confirmed so far.[73] However, up to now the few clinical trials, which evaluated the use of amifostine for prevention of cisplatin-induced ototoxicty, do not support its use as an otoprotectant.[74] More randomized controlled trials will be needed to answer this question. Further antioxidants, like N-acetylcysteine and vitamin E were evaluated preclinically with promising results. In addition, drugs which can induce the endogenous production of anti-oxidants (sodium thiosulfate (STS), D-methionine, ebselen) or which

prevent the production of ROS (i.e. allopurinol, erdosteine) were tested, too.[29] Free radical scavengers impair the antitumor activity of cisplatin in vitro. This raises major concerns, when radical scavengers are to be applied systemically for the protection of cisplatin-induced ototoxicity. Presumably, the effects of antioxidants on the activity of anticancer drugs have not been addressed in randomized controlled clinical trials so far for this reason. In contrast, intratympanic administration of free radical scavengers promises a selective protection of the inner ear and minimal interaction with the antitumor activity of cisplatin. For both - the systemic and the intratympanic approach - it is critical that the sufficient amounts of radical scavengers reach the inner ear either by crossing the blood-inner-ear barrier or diffusion through the round window membrane.[75] Protection from cisplatin-induced ototoxicity by systemic or intratympanic administration of N-acetylcysteine and STS are actually being evaluated in clinical trials. (https://clinicaltrials.gov)

Inhibition of transporter, which selectively mediate uptake of the ototoxic drug into the inner ear, is another approach to prevent or reduce drug-induced ototoxicity. The human OCT2 and the CTR1 were shown to transport cisplatin in the cells.[20,21] Inhibitors of OCT2 or CTR1 can reduce the uptake of cisplatin into the inner ear. If the tumor cell does not express the transporters, as demonstrated by the lack of OCT2 expression in pediatric tumor types, OCT2 expressing non-malignant tissues can be selectively protected from cisplatin-induced toxicity.[21] Such strategies are actually evaluated preclinically.

Despite their intention to protect from this usually lifelong disabling side effect these protective strategies are not without risk. Thus, it will be crucial to identify patients at risk by careful monitoring of clinical, audiologic, and pharmacogenomic risk factors, because these patients will benefit most from such intervention strategies.

References

1. Roland, P. S. & Rutka, J. A. *Ototoxicity* (Decker, Hamilton, Ontario, 2004).
2. Cianfrone, G. *et al.* Pharmacological drugs inducing ototoxicity, vestibular symptoms and tinnitus: a reasoned and updated guide. *European review for medical and pharmacological sciences* **15**, 601–636 (2011).
3. Truong, M. T., Winzelberg, J. & Chang, K. W. Recovery from cisplatin-induced ototoxicity: a case report and review. *International journal of pediatric otorhinolaryngology* **71**, 1631–1638 (2007).
4. Brummett, R. E. Ototoxic liability of erythromycin and analogues. *Otolaryngologic clinics of North America* **26**, 811–819 (1993).
5. Ikeda, K., Oshima, T., Hidaka, H. & Takasaka, T. Molecular and clinical implications of loop diuretic ototoxicity. *Hearing research* **107**, 1–8 (1997).
6. Jung, T. T., Rhee, C. K., Lee, C. S., Park, Y. S. & Choi, D. C. Ototoxicity of salicylate, nonsteroidal antiinflammatory drugs, and quinine. *Otolaryngologic clinics of North America* **26**, 791–810 (1993).
7. Schacht, J., Talaska, A. E. & Rybak, L. P. Cisplatin and aminoglycoside antibiotics: hearing loss and its prevention. *Anatomical record (Hoboken, N.J. : 2007)* **295**, 1837–1850 (2012).
8. Mohr, P. E. *et al.* The societal costs of severe to profound hearing loss in the United States. *International journal of technology assessment in health care* **16**, 1120–1135 (2000).
9. Knight, K. R. G., Kraemer, D. F. & Neuwelt, E. A. Ototoxicity in children receiving platinum chemotherapy: underestimating a commonly occurring toxicity that may influence academic and social development. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **23**, 8588–8596 (2005).
10. Stelmachowicz, P. G., Pittman, A. L., Hoover, B. M., Lewis, D. E. & Moeller, M. P. The importance of high-frequency audibility in the speech and language development of children with hearing loss. *Archives of otolaryngology--head & neck surgery* **130**, 556–562 (2004).
11. Baguley, D., McFerran, D. & Hall, D. Tinnitus. *Lancet (London, England)* **382**, 1600–1607 (2013).
12. Yardley, L. Overview of psychologic effects of chronic dizziness and balance disorders. *Otolaryngologic clinics of North America* **33**, 603–616 (2000).
13. Zdebik, A. A., Wangemann, P. & Jentsch, T. J. Potassium ion movement in the inner ear: insights from genetic disease and mouse models. *Physiology (Bethesda, Md.)* **24**, 307–316 (2009).
14. Ashmore, J. Cochlear outer hair cell motility. *Physiological reviews* **88**, 173–210 (2008).
15. Day, B. L. & Fitzpatrick, R. C. The vestibular system. *Current biology : CB* **15**, R583-6 (2005).
16. Dilruba, S. & Kalayda, G. V. Platinum-based drugs: past, present and future. *Cancer chemotherapy and pharmacology* **77**, 1103–1124 (2016).
17. Langer, T., Am Zehnhoff-Dinnesen, A., Radtke, S., Meitert, J. & Zolk, O. Understanding platinum-induced ototoxicity. *Trends in pharmacological sciences* **34**, 458–469 (2013).
18. Wang, D. & Lippard, S. J. Cellular processing of platinum anticancer drugs. *Nature*

reviews. Drug discovery **4**, 307–320 (2005).
19. Hellberg, V. *et al.* Cisplatin and oxaliplatin toxicity: importance of cochlear kinetics as a determinant for ototoxicity. *Journal of the National Cancer Institute* **101**, 37–47 (2009).
20. Howell, S. B., Safaei, R., Larson, C. A. & Sailor, M. J. Copper transporters and the cellular pharmacology of the platinum-containing cancer drugs. *Molecular pharmacology* **77**, 887–894 (2010).
21. Ciarimboli, G. *et al.* Organic cation transporter 2 mediates cisplatin-induced oto- and nephrotoxicity and is a target for protective interventions. *The American journal of pathology* **176**, 1169–1180 (2010).
22. Riedemann, L. *et al.* Megalin genetic polymorphisms and individual sensitivity to the ototoxic effect of cisplatin. *The pharmacogenomics journal* **8**, 23–28 (2008).
23. Brock, P. R. *et al.* Platinum-induced ototoxicity in children: a consensus review on mechanisms, predisposition, and protection, including a new International Society of Pediatric Oncology Boston ototoxicity scale. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **30**, 2408–2417 (2012).
24. Weissenstein, A., Deuster, D., Knief, A., Am Zehnhoff-Dinnesen, A. & Schmidt, C.-M. Progressive hearing loss after completion of cisplatin chemotherapy is common and more pronounced in children without spontaneous otoacoustic emissions before chemotherapy. *International journal of pediatric otorhinolaryngology* **76**, 131–136 (2012).
25. Vermorken, J. B., Kapteijn, T. S., Hart, A. A. & Pinedo, H. M. Ototoxicity of cis-diamminedichloroplatinum (II): influence of dose, schedule and mode of administration. *European journal of cancer & clinical oncology* **19**, 53–58 (1983).
26. van As, J. W., van den Berg, H. & van Dalen, E. C. Different infusion durations for preventing platinum-induced hearing loss in children with cancer. *The Cochrane database of systematic reviews*, CD010885 (2014).
27. Laurell, G., Viberg, A., Teixeira, M., Sterkers, O. & Ferrary, E. Blood-perilymph barrier and ototoxicity: an in vivo study in the rat. *Acta oto-laryngologica* **120**, 796–803 (2000).
28. Thomas, J. P., Lautermann, J., Liedert, B., Seiler, F. & Thomale, J. High accumulation of platinum-DNA adducts in strial marginal cells of the cochlea is an early event in cisplatin but not carboplatin ototoxicity. *Molecular pharmacology* **70**, 23–29 (2006).
29. Rybak, L. P., Whitworth, C. A., Mukherjea, D. & Ramkumar, V. Mechanisms of cisplatin-induced ototoxicity and prevention. *Hearing research* **226**, 157–167 (2007).
30. Yang, Z., Schumaker, L. M., Egorin, M. J., Zuhowski, E. G., Guo, Z. & Cullen, K. J. Cisplatin preferentially binds mitochondrial DNA and voltage-dependent anion channel protein in the mitochondrial membrane of head and neck squamous cell carcinoma: possible role in apoptosis. *Clinical cancer research : an official journal of the American Association for Cancer Research* **12**, 5817–5825 (2006).
31. Olivero, O. A., Chang, P. K., Lopez-Larraza, D. M., Semino-Mora, M. C. & Poirier, M. C. Preferential formation and decreased removal of cisplatin-DNA adducts in Chinese hamster ovary cell mitochondrial DNA as compared to nuclear DNA. *Mutation research* **391**, 79–86 (1997).
32. Xie, J., Talaska, A. E. & Schacht, J. New developments in aminoglycoside therapy and ototoxicity. *Hearing research* **281**, 28–37 (2011).
33. Hain, T. C. Pharmacodynamics of Gentamicin as they relate to ototoxicity. http://www.dizziness-and-balance.com/disorders/bilat/PCD.html.

34. Gatell, J. M. *et al.* Univariate and multivariate analyses of risk factors predisposing to auditory toxicity in patients receiving aminoglycosides. *Antimicrobial agents and chemotherapy* **31**, 1383–1387 (1987).
35. Avent, M. L., Rogers, B. A., Cheng, A. C. & Paterson, D. L. Current use of aminoglycosides: indications, pharmacokinetics and monitoring for toxicity. *Internal medicine journal* **41**, 441–449 (2011).
36. Tran Ba Huy, P., Bernard, P. & Schacht, J. Kinetics of gentamicin uptake and release in the rat. Comparison of inner ear tissues and fluids with other organs. *The Journal of clinical investigation* **77**, 1492–1500 (1986).
37. Vu, A. A. *et al.* Integrity and regeneration of mechanotransduction machinery regulate aminoglycoside entry and sensory cell death. *PloS one* **8**, e54794 (2013).
38. Nagai, J. & Takano, M. Entry of aminoglycosides into renal tubular epithelial cells via endocytosis-dependent and endocytosis-independent pathways. *Biochemical pharmacology* **90**, 331–337 (2014).
39. Steyger, P. S. Cellular uptake of aminoglycosides. *The Volta Review* **105**, 299–324 (2005).
40. Henley, C. M. 3. & Schacht, J. Pharmacokinetics of aminoglycoside antibiotics in blood, inner-ear fluids and tissues and their relationship to ototoxicity. *Audiology : official organ of the International Society of Audiology* **27**, 137–146 (1988).
41. Prezant, T. R. *et al.* Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. *Nature genetics* **4**, 289–294 (1993).
42. Sha, S. H. & Schacht, J. Stimulation of free radical formation by aminoglycoside antibiotics. *Hearing research* **128**, 112–118 (1999).
43. Lesniak, W., Pecoraro, V. L. & Schacht, J. Ternary complexes of gentamicin with iron and lipid catalyze formation of reactive oxygen species. *Chemical research in toxicology* **18**, 357–364 (2005).
44. Hong, S., Harris, K. A., Fanning, K. D., Sarachan, K. L., Frohlich, K. M. & Agris, P. F. Evidence That Antibiotics Bind to Human Mitochondrial Ribosomal RNA Has Implications for Aminoglycoside Toxicity. *The Journal of biological chemistry* **290**, 19273–19286 (2015).
45. Usami, S. *et al.* Prevalence of mitochondrial gene mutations among hearing impaired patients. *Journal of medical genetics* **37**, 38–40 (2000).
46. Vandebona, H. *et al.* Prevalence of mitochondrial 1555A--G mutation in adults of European descent. *The New England journal of medicine* **360**, 642–644 (2009).
47. Cazals, Y. Auditory sensori-neural alterations induced by salicylate. *Progress in neurobiology* **62**, 583–631 (2000).
48. Alvan, G., Berninger, E., Gustafsson, L. L., Karlsson, K. K., Paintaud, G. & Wakelkamp, M. Concentration-Response Relationship of Hearing Impairment Caused by Quinine and Salicylate: Pharmacological Similarities but different molecular mechanisms. *Basic & clinical pharmacology & toxicology* (2016).
49. Chen, G.-D., Stolzberg, D., Lobarinas, E., Sun, W., Ding, D. & Salvi, R. Salicylate-induced cochlear impairments, cortical hyperactivity and re-tuning, and tinnitus. *Hearing research* **295**, 100–113 (2013).
50. Alharazneh, A. *et al.* Functional hair cell mechanotransducer channels are required for aminoglycoside ototoxicity. *PloS one* **6**, e22347 (2011).
51. Achan, J. *et al.* Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. *Malaria journal* **10**, 144 (2011).
52. Berninger, E., Karlsson, K. K. & Alvan, G. Quinine reduces the dynamic range of the human auditory system. *Acta oto-laryngologica* **118**, 46–51 (1998).

53. Rybak, L. P. Ototoxicity of loop diuretics. *Otolaryngologic clinics of North America* **26**, 829–844 (1993).
54. Delpire, E., Lu, J., England, R., Dull, C. & Thorne, T. Deafness and imbalance associated with inactivation of the secretory Na-K-2Cl co-transporter. *Nature genetics* **22**, 192–195 (1999).
55. Brown, B. A., Griffith, D. E., Girard, W., Levin, J. & Wallace, R. J., JR. Relationship of adverse events to serum drug levels in patients receiving high-dose azithromycin for mycobacterial lung disease. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **24**, 958–964 (1997).
56. Ress, B. D. & Gross, E. M. Irreversible sensorineural hearing loss as a result of azithromycin ototoxicity. A case report. *The Annals of otology, rhinology, and laryngology* **109**, 435–437 (2000).
57. Liu, J., Marcus, D. C. & Kobayashi, T. Inhibitory effect of erythromycin on ion transport by stria vascularis and vestibular dark cells. *Acta oto-laryngologica* **116**, 572–575 (1996).
58. Cohen, I. J. & Weitz, R. Psychiatric complications with erythromycin. *Drug intelligence & clinical pharmacy* **15**, 388 (1981).
59. Brummett, R. E. & Fox, K. E. Vancomycin- and erythromycin-induced hearing loss in humans. *Antimicrobial agents and chemotherapy* **33**, 791–796 (1989).
60. Bailie, G. R. & Neal, D. Vancomycin ototoxicity and nephrotoxicity. A review. *Medical toxicology and adverse drug experience* **3**, 376–386 (1988).
61. Forouzesh, A., Moise, P. A. & Sakoulas, G. Vancomycin ototoxicity: a reevaluation in an era of increasing doses. *Antimicrobial agents and chemotherapy* **53**, 483–486 (2009).
62. Gendeh, B. S., Gibb, A. G., Aziz, N. S., Kong, N. & Zahir, Z. M. Vancomycin administration in continuous ambulatory peritoneal dialysis: the risk of ototoxicity. *Otolaryngology--head and neck surgery : official journal of American Academy of Otolaryngology-Head and Neck Surgery* **118**, 551–558 (1998).
63. American Academy of Audiology. Ototoxicity Monitoring. Position Statement and Clinical Practice Guidelines. http://audiology-web.s3.amazonaws.com/migrated/OtoMonGuidelines.pdf_539974c40999c1.58842217.pdf.
64. Abujamra, A. L. *et al.* The use of high-frequency audiometry increases the diagnosis of asymptomatic hearing loss in pediatric patients treated with cisplatin-based chemotherapy. *Pediatric blood & cancer* **60**, 474–478 (2013).
65. Kerkhofs, K. & Smit, M. de *Paediatric Behavioural Audiometry (0 – 6 years)* (Springer, , in press).
66. Am Zehnhoff-Dinnesen, A., Langer, T. & Zolk, O. *Ototoxicity in children.* (Springer, , in press).
67. Rosner, T., Kandzia, F., Oswald, J. A. & Janssen, T. Hearing threshold estimation using concurrent measurement of distortion product otoacoustic emissions and auditory steady-state responses. *The Journal of the Acoustical Society of America* **129**, 840–851 (2011).
68. Brock, P. R., Bellman, S. C., Yeomans, E. C., Pinkerton, C. R. & Pritchard, J. Cisplatin ototoxicity in children: a practical grading system. *Medical and pediatric oncology* **19**, 295–300 (1991).
69. Chang, K. W. & Chinosornvatana, N. Practical grading system for evaluating cisplatin ototoxicity in children. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **28**, 1788–1795 (2010).

70. Schmidt, C.-M., Bartholomaus, E., Deuster, D., Heinecke, A. & Dinnesen, A. G. The "Muenster classification" of high frequency hearing loss following cisplatin chemotherapy. *HNO* **55**, 299–306 (2007).
71. Lafay-Cousin, L. *et al.* Early cisplatin induced ototoxicity profile may predict the need for hearing support in children with medulloblastoma. *Pediatric blood & cancer* **60**, 287–292 (2013).
72. Waissbluth, S., Peleva, E. & Daniel, S. J. Platinum-induced ototoxicity: a review of prevailing ototoxicity criteria. *European archives of oto-rhino-laryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery* (2016).
73. Hospers, G. A., Eisenhauer, E. A. & Vries, E. G. de. The sulfhydryl containing compounds WR-2721 and glutathione as radio- and chemoprotective agents. A review, indications for use and prospects. *British journal of cancer* **80**, 629–638 (1999).
74. van As, J. W., van den Berg, H. & van Dalen, E. C. Medical interventions for the prevention of platinum-induced hearing loss in children with cancer. *The Cochrane database of systematic reviews*, CD009219 (2014).
75. van den Berg, J H, Beijnen, J. H., Balm, A. J. M. & Schellens, J. H. M. Future opportunities in preventing cisplatin induced ototoxicity. *Cancer treatment reviews* **32**, 390–397 (2006).

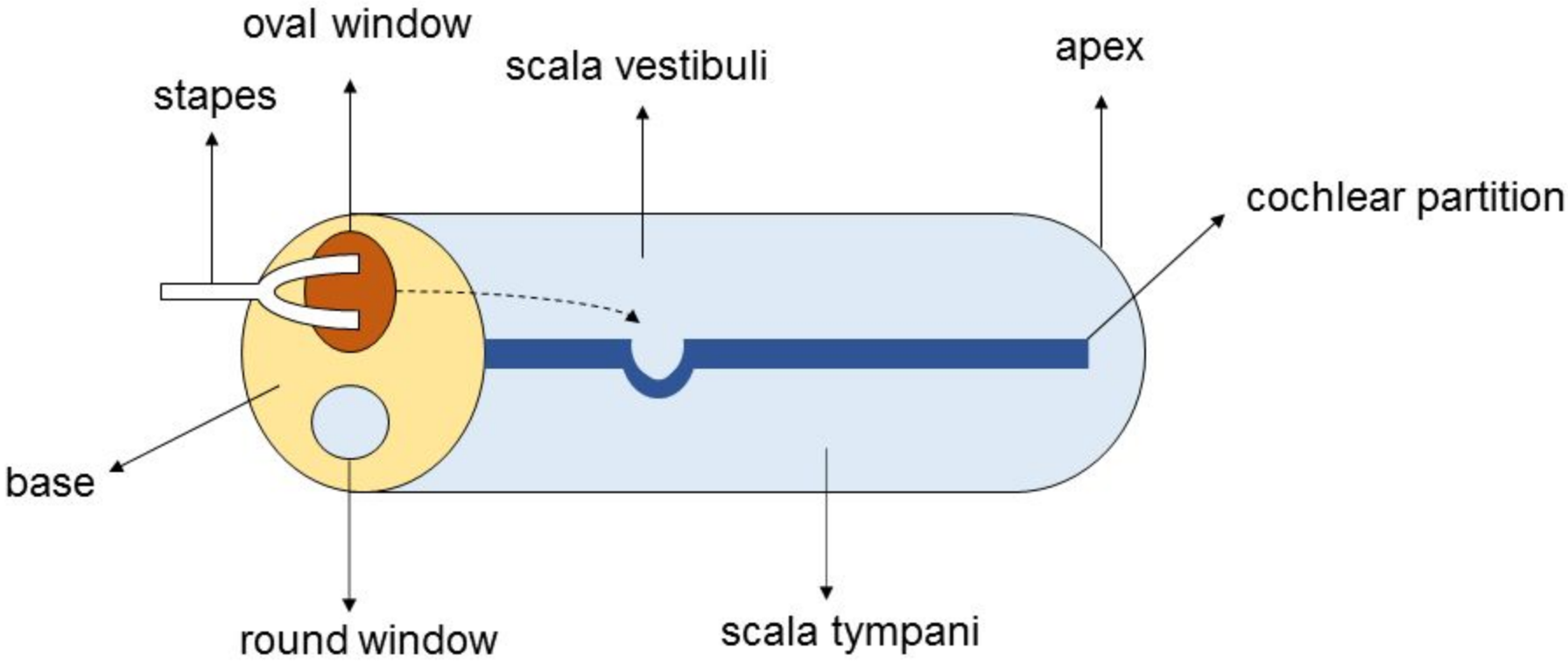
Legends:

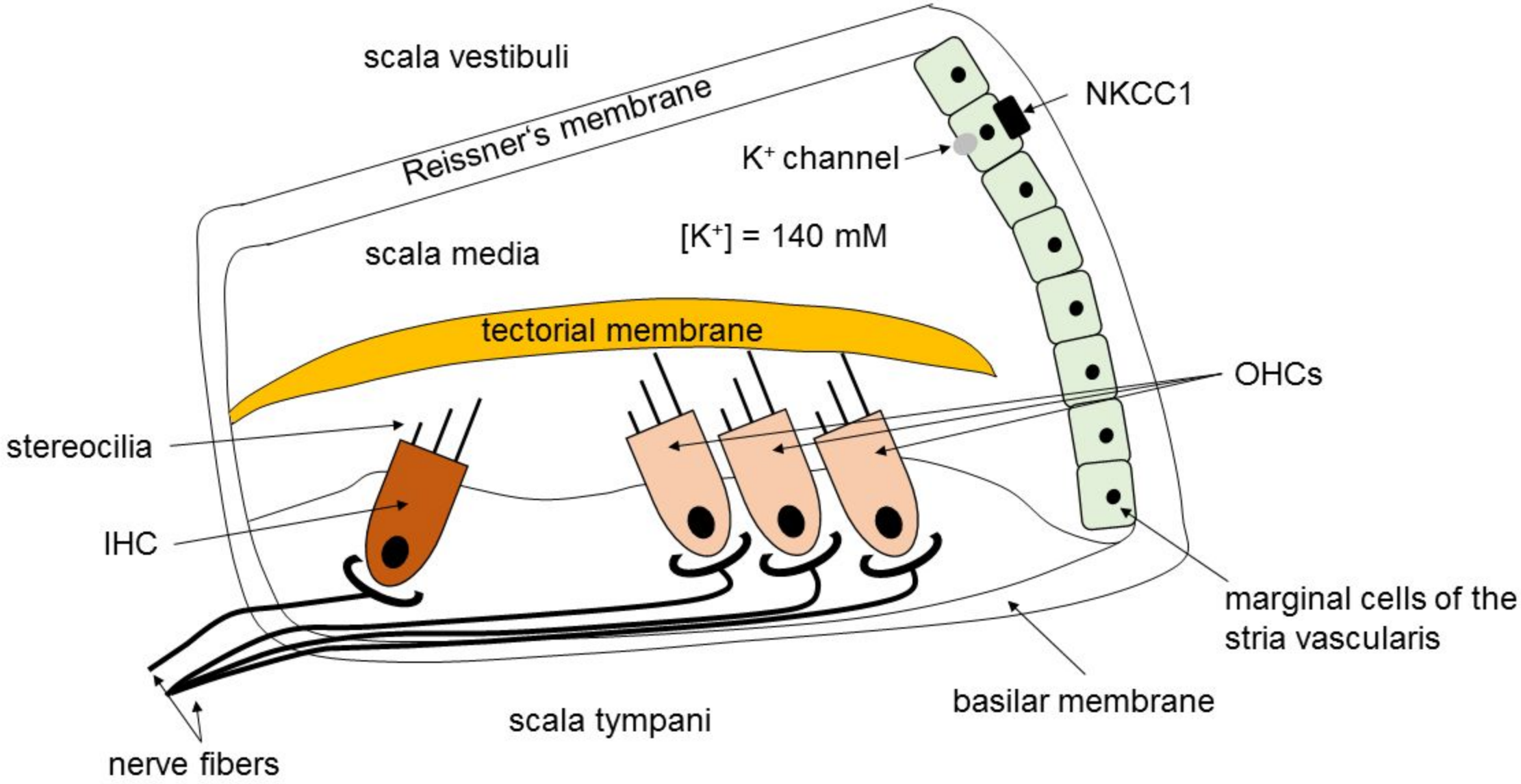
Figure 1. This figure shows a schematic representation of the longitudinal section of the cochlea. Vibrations of the stapes are transmitted to the perilymph of the scala vestibuli at the oval window and cause a deflection of the cochlear partition at a site dependent on the tone frequency (dashed arrow). The part of the cochlea, which is in contact with the round windows is the scala tympani. The part of the cochlea containing the oval and round windows is the base, while the part where the scala vestibuli pass into the scala tympani is the apex.

Figure 2. Schematic representation of the cochlear partition in cross-section. Inner (IHC) and outer (OHC) hair cells with the stereocilia are sustained by supporting and Deiters cells (not shown), which lay on the basilar membrane. The stereocilia of the OHC are in direct contact with the tectorial membrane. The Reissner's membrane separates the cochlear partition from the scala vestibule and the basilar membrane from the scala tympani. The marginal cells of the stria vascularis secrete K^+ into the endolymph via NKCC1 and K^+ channels on their basolateral and apical membrane, respectively. As a result of this secretion process, the K^+ in the endolymph is 140 mM. The innervation of the hair cells is also shown.

Figure 3. Schematic representation of the scala media in cross-section, with indication of the possible expression of the transporters (Ctr1 and OCT2) implied in the uptake of cisplatin. Both Ctr1 and OCT2 are expressed in the stria vascularis, where OCT2 seems to be expressed both on the apical and basolateral membrane. The localization of Ctr1 is not clear. Here we speculate that Ctr1 is expressed on the basolateral cell membrane. In the outer hair cells (OHC) OCT2 seems to be expressed on the apical part of the plasma

membrane, while Ctr1 localizes rather on the lateral plasma membrane. The inner hair cells (IHC) express only the OCT2 overall on the plasma membrane. OHC express also ATP7B, a cisplatin efflux transporter, in intracellular vesicles (not shown). Ctr1 and OCT2 are also expressed in the spiral ganglion (not shown). In this figure, the tectorial membrane is missing. The empty squares represent Ctr1; the grey circles with black border represent OCT2; the bombs represent cisplatin.





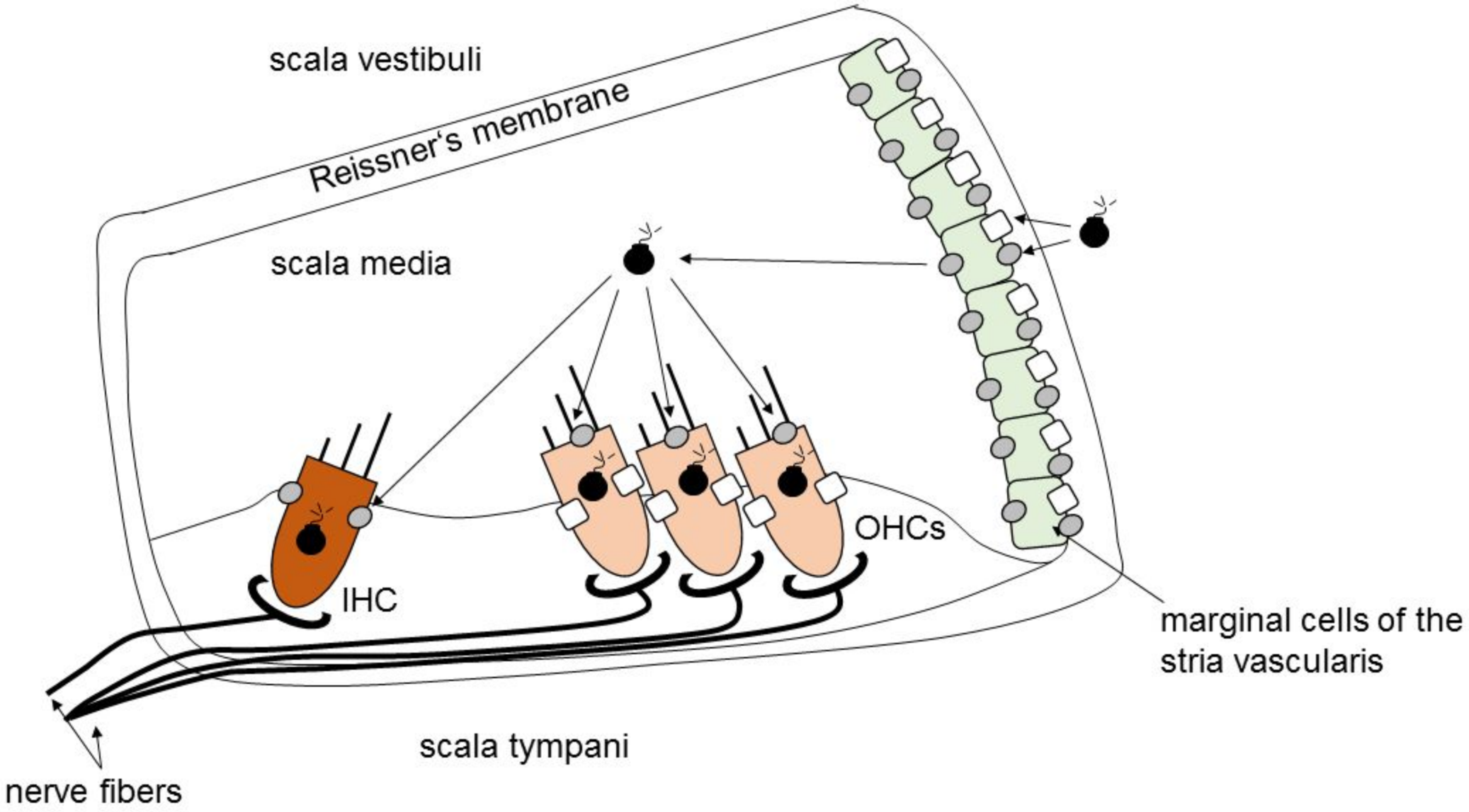


Table 1. List of ototoxic drugs with cochlear or vestibular toxicity. Reversible means that the toxicity disappears after drug withdrawal.

Drugs	Type of ototoxicity		References
	Cochlear toxicity	Vestibular toxicity	
Antibiotics			
Aminoglycosides			
Neomycin	✔ (irreversible)	✔	2,32
Gentamycin	✔ (irreversible)	✔	2,32
Kanamycin	✔ (irreversible)	✔	2,32
Tobramycin	✔ (irreversible)	✔	2,32
Amikacin	✔ (irreversible)	✔	2,32
Glycopeptides			
Vancomycin	✔ (irreversible)*		2,60
Macrolides			
Erythromycin	✔ (reversible)	✔	2
Azithromycin	✔ (reversible)	✔	2
Antimalarics			
Quinine	✔ (reversible)		2
Chemotherapeutic agents			
Cisplatin	✔ (irreversible)	✔	17
Carboplatin	✔ (irreversible)	✔	17
Oxaliplatin	✔ (irreversible)	✔	17
Loop Diuretics			
Furosemide	✔ (reversible)		2,53
Torasemide	✔ (reversible)		2,53
Bumetanide	✔ (reversible)		2,53
Non-steroidal anti-inflammatory drugs (NSAIDs)			
Salicylates	✔ (reversible)		47

* Ototoxicity was related to impurities in the fermation product, which are no longer present due to technical improvements.