Review Article

Experimental animal models of drug-induced sensorineural hearing loss: a narrative review

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Objective: This narrative review describes experimental animal models of sensorineural hearing loss (SNHL) caused by ototoxic agents.

Background: SNHL primarily results from damage to the sensory organ within the inner ear or the vestibulocochlear nerve (cranial nerve VIII). The main etiology of SNHL includes genetic diseases, presbycusis, ototoxic agents, infection, and noise exposure. Animal models with functional and anatomic damage to the sensory organ within the inner ear or the vestibulocochlear nerve mimicking the damage seen in humans are employed to explore the mechanism and potential treatment of SNHL. These animal models of SNHL are commonly established using ototoxic agents.

Methods: A literature search of PubMed, Embase, and Web of Science was performed for research articles on hearing loss and ototoxic agents in animal models of hearing loss.

Conclusions: Common ototoxic medications such as aminoglycoside antibiotics (AABs) and platinum antitumor drugs are extensively used to induce SNHL in experimental animals. The effect of ototoxic agents *in vivo* is influenced by the chemical mechanisms of the ototoxic agents, the species of animal, routes of administration of the ototoxic agents, and the dosage of ototoxic agents. Animal models of drug-induced SNHL contribute to understanding the hearing mechanism and reveal the function of different parts of the auditory system in humans.

Keywords: Animal model; sensorineural hearing loss (SNHL); ototoxic agent

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Introduction

Approximately 466 million people worldwide experience hearing loss (World Health Organization, Deafness and Hearing Loss, 2019). Sensorineural hearing loss (SNHL) is the most common form of hearing loss. Individuals with hearing loss may experience impaired language development and suffer from depression (1,2). Understanding the mechanism of SNHL can improve clinical diagnosis, treatment, and prevention. Animal models mimicking the auditory impairments of SNHL are important in understanding the mechanism of SNHL in humans.

Common etiological factors of SNHL include genetic diseases, presbycusis, noise exposure, inflammation, ototoxic

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drugs, and chemicals. The pathological features of SNHL include damage to the cochlea, vestibulocochlear nerve (cranial nerve VIII), or the central processing center of the brain. The organ most regularly damaged by ototoxic drugs is the cochlea. The cochlea is a snail-shaped bony canal divided into three chambers known as the scala vestibuli, scala media, and scala tympani. The scala vestibuli and scala tympani are filled with perilymph, a solution rich in sodium (3). The scala media contains endolymph, a solution rich in potassium (4). The upper border of scala media is Reissner's membrane, and the lateral wall is composed of the spiral ligament, the thickened periosteum, and the stria vascularis, the upper portion of the spiral ligament containing an abundance of blood vessels. And the stria vascularis is mainly composed of three layers of epithelial cells known as marginal cells, intermediate cells, and basal cells. The intercellular space of the stria vascularis contains a rich capillary network, which is a component of the cochlear blood labyrinth barrier. The lower wall of the scala media is composed of the spiral edge and basilar membrane formed by the thickening of the periosteum on the spiral plate of bone. The Corti organ is located on the basilar membrane and consists of hair cells, supporting cells, and the tectorial membrane (5). One row of inner hair cells (IHCs) is located near the modiolus, and 3 rows of outer hair cells (OHCs) are located near the lateral wall of the cochlea. Spiral ganglion neurons (SGNs) are classified into types I and II, most of which are type I. The spiral ganglion is located in the spiral canal of the modiolus. One end of the nerve fiber of the spiral ganglion functions to establish synaptic connections with hair cells, while the opposite end extends into the central canal of the cochlear modiolus to form an acoustic nerve tract. This extension continues into the brain through the internal acoustic meatus and further in to reach the cochlear nucleus of the brainstem. When sound vibrates the tympanic membrane, the vibrations travel to the oval window through the ossicular chain, causing the perilymph and endolymph vibration, leading to the vibration of the basilar membrane. The basilar membrane and tectum, attached to the spiral plate of bone, move up and down along different modiolus. A shearing motion occurs between the tectum and the reticular plate, which causes the stereocilium of hair cells to bend or deflect. At this time, the potassium channel of the hair cells is open so the potassium ions in the endolymph can flow into the hair cells and cause depolarization. This depolarization leads to the opening of intracellular calcium channels, which allows calcium ions to flow into cells and stimulates hair cells to release neurotransmitters, causing nerve impulses at the cochlear nerve endings, which are attached to the bottom of hair cells. These nerve impulses are transmitted to the auditory cortex through the central conduction pathway to produce the sensation of hearing.

A common method of establishing animal models of SNHL with different damage characteristics is through ototoxic drugs. There are over 150 ototoxic agents, with aminoglycoside antibiotics (AABs), loop diuretics, and antitumor drugs being the most frequently used (6). Due to the various effects of ototoxic agents, those that induce specific inner ears lesions are often chosen for use in hearing research.

Methods of auditory assessment are predominantly comprised of cochlear microphonics (CM), summating potential (SP), auditory brainstem response (ABR), distortion product otoacoustic emissions (DPOAEs), compound action potentials (CAPs), and histological evaluation. CM involves a current primarily generated by the receptor currents of OHCs and can mirror the condition of OHCs (7). SP is a direct potential generated by the movement of the hair cells and basilar membrane (8-10). CAPs originate from the synchronous discharge of many primary afferent fibers in the auditory nerve and represent the total discharge of many single auditory neurons. CAPs are the compound nerve action potential of the eighth pair of brain nerve terminals (11). The main source of otoacoustic emissions in mammals is OHCs. These otoacoustic emissions can reflect their survival and mirror their functional status (12). The ABR reflects the transmission of auditory impulses induced by sound stimulation through the brainstem auditory pathway (13). The effectiveness of these methods in rodents was tested in studies (11,14-20).

The animal models of drug-induced SNHL can not only be used to study the ototoxic mechanisms of these drugs, but can also be used to study the relevant pathophysiological mechanisms of the lesions of the cochlear structures. This review introduces the commonly used animal models of SNHL induced by ototoxic drugs.

We present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/atm-21-2508).

Methods

We conducted a search of the PubMed, Embase, and Web of Science databases using the keywords "sensorineural hearing loss", "inner ear", "cochlea*", "animal model",

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"ototoxicity", "ototoxic agents", "ototoxic drugs", "Aminoglycoside antibiotics", "gentamicin", "kanamycin", "streptomycin", "amikacin", "tobramycin", "neomycin", "platinum", "cisplatin", "carboplatin", "oxaliplatin", "Doxorubicin", "Adriamycin", "aromatic solvent", "toluene", "ethylbenzene", "ouabain", "glutamic", "kainic acid", "2-hydroxypropyl-β-cyclodextrin", "HPβCD", "heavy metals", "manganese", "mercury", "cobalt", "cadmium", and "lead". By browsing the title and/or abstract, a total of 4,565 original articles and review articles related to ototoxic agent-induced SNHL and published in English between January 1980 and February 2021 were collected. Articles that were not animal experiments that did not focus on cochlear lesions and lacked morphological and/or functional tests were excluded. Relevant information was extracted for this review from the 375 articles remaining after applying the exclusion criteria.

Ototoxic agents

AABs

AABs are widely used in clinical practice; and include kanamycin, gentamicin, amikacin, neomycin, and tobramycin. The ototoxicity of AABs has long been confirmed by many studies (21-23). Long-term clinical therapy using these ototoxic medications is associated with irreversible hearing loss at high frequencies (24). Previous research has reported that gentamicin, streptomycin, and tobramycin are mainly vestibulotoxic; while kanamycin, amikacin, and neomycin are mostly cochleotoxic (25,26). Studies have demonstrated that AABs can pass through the blood-labyrinthine barrier (BLB) to the endolymph. The AABs subsequently enter hair cells through mechano-electrical transducer (MET) channels or endocytosis (27-29). AABs can inhibit depolarization of the hair cells and alter the concentration of perilymph ions, resulting in damage to the hair cell bundles, which causes permanent hearing loss (30). Also, AABs can produce free radicals and reactive oxygen species, which ultimately cause caspase activation and apoptosis (31-33).

Animals play an important role in establishing models of SNHL. Different species may present with different lesions following identical treatment. For example, the given dose of aminoglycosides that induces cochlear hair cell loss in guinea pigs and chinchillas does not have the same effects on inducing cochlear hair cell loss in mature mice (34). Only a small amount of hair cell damage was induced in the cochleae of adult mice and rats when administered conventional doses of gentamicin or kanamycin. Similar results were found when the same animals were administered high dosages that were close to a lethal concentration (35). The resistance to AABs in adult mice and rats may be attributed to the rapid clearance of the drugs in serum, suggesting the need to increase the dosage (36,37). The clearance half-life of kanamycin in 12-day-old rats was 2.5 times longer than in 25-day-old rats (36). Some experiments have attempted to use different dosages and frequencies of administration to induce auditory system lesions. One study administered a high dose (800 mg/kg) of kanamycin twice daily for 14 consecutive days and induced heavy hair cell loss in mice and rats (35). Concomitant administration of an additional ototoxic drug, such as a platinum anticancer drug, can enhance ototoxicity (38). Schweitzer *et al.* found that a combined cisplatin and kanamycin treatment resulted in more significant OHC loss and ABR threshold shifts than cisplatin treatment (38). In addition, concomitant loop diuretics have been shown to increase the ototoxic effect of AABs on the cochlea (39). A single subcutaneous injection of 1 mg/g body weight of kanamycin followed 40 minutes later by an intraperitoneal injection of 0.05 mg/g body weight of bumetanide resulted in almost the entire loss of OHCs within 48 hours (39). Loop diuretics include furosemide, ethacrynic acid, bumetanide, and torsemide (40). By blocking the capillaries on the lateral cochlear wall and inhibiting the arterial blood flow to the stria vascularis and spiral ligament, loop diuretics can cause ischemia and hypoxia in the stria vascularis epithelial cells and thus destruct the BLB (41). Combination therapy of AABs and loop diuretics can lead to irreversible deafness. For instance, a single dose of kanamycin (400–500 mg/kg) by subcutaneous injection accompanied by a dose of furosemide (100 mg/kg) by intraperitoneal injection can lead to permanent hearing loss in gerbils, with significant loss of both OHCs and IHCs as well as the injury of SGNs (42). In conclusion, animal species and dosage regimens are important factors in establishing AABs-induced animal models of SNHL, and selecting the appropriate animal species and adjusting dosage can obtain the expected model of specific lesions (*Table 1*) (39,43-54).

Platinum antitumor drugs

Cisplatin and carboplatin are robust antitumor drugs, but they can also be ototoxic or neurotoxic (55-64). Cisplatin enters hair cells mainly through copper transport channels

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Table 1 Animal models of SNHL induced by aminoglycoside antibiotics

Drug	Animal	Route	Dose	Schedule	Duration	Ototoxicity	Reference
Kana + bume	Mouse	s.c. + i.p.	1 mg/g + 0.05 mg/g	Single-dose	1 day	Loss of OHCs and delayed loss of IHCs	(39)
Kana	Guinea pig	s.c.	500 mg/kg	1× daily	7 days	Loss of OHCs, IHCs, and SGNs	(43)
kana	Mouse	s.c.	800 mg/kg	2× daily	15 days	Loss of OHCs. DPOAE thresholds shifted and ABR thresholds increased	(44,45)
Kana + furo	Mouse	s.c. + i.p.	1 g/kg + 300 mg/kg	Single-dose	1 day	Loss of OHCs and IHCs	(46)
Kana + furo	Rat	RWN	200 mg/mL + 50 mg/mL	Single-dose	1 day	ABR thresholds increased at 8–40 kHz	(47)
Kana + furo	Guinea pig	s.c. + RWN	200 mg/kg + 100 mg/kg	Single-dose	1 day	Loss of OHCs, IHCs, and SGNs. CAP thresholds shifted	(48)
Kana + furo	Mouse	i.p.	900 mg/kg + 50 mg/kg	2× daily	15 days	Loss of OHCs and IHCs, and SGNs damaged. ABR thresholds increased to 20 dB at 16 kHz, 35 dB at 22 kHz, and to maximum at 32 kHz	(49)
Gent	Mouse	i.p.	200 mg/kg	3× per week	1 week	ABR threshold increased	(50)
Gent + etha	Chinchilla	i.m. + i.v.	125 mg/kg + 40 mg/kg	Single-dose	1 day	Stereocilia and a cuticular plate of OHC and IHC damaged	(51)
Gent	Guinea pig	i.t.	0.1 mL	Single-dose	1 day	ABR thresholds increased	(52)
Neo	Mongolian gerbil	i.t.	40 mM	Single-dose	1 day	ABR average thresholds increased to 40 dB	(53)
Amik	Rat	i.m.	600 mg/kg	1× daily	15 days	Reduced amplitude of DPOAE	(54)

SNHL, sensorineural hearing loss; kana, kanamycin; amik, amikacin; gent, gentamycin; neo, neomycin; bume, bumetanide; furo, furosemide; etha, ethacrynic acid; i.v., intraveneous; i.m., intramuscular; s.c., subcutaneous; i.p., intraperitoneal; RWN, round window niche; RWM, round window membrane; i.t., intratympanic; OHCs, outer hair cells; IHCs, inner hair cells; SGNs, spiral ganglion neurons; DPOAE, distortion product otoacoustic emission; ABR, auditory brainstem response; CAP, compound action potential.

to induce oxidative stress, leading to increased reactive oxygen species and inner ear damage (65,66). Cisplatin also stimulates the cell death factor receptor, which is located on the surface of the hair cell membrane, to activate caspase-8 and its downstream caspase-3 causing programmed death of hair cells (67). Experiments have indicated that cisplatin mainly injures cochlear hair cells, with OHCs being more susceptible than IHCs; while carboplatin chiefly affects cochlear IHCs, cochlear type I afferent neurons, and vestibular type I hair cells (56,68). Studies demonstrated that a single intraperitoneal dose (commonly 12–16 mg/kg) of cisplatin in rats, mice, or guinea pigs could cause time-

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Table 2 Animal models of SNHL induced by platinum antitumor drugs

Drug	Animal	Route	Dose	Schedule	Duration	Ototoxicity	Reference
Cis	C57BL/6 mouse	i.p.	12 mg/kg	Single-dose	1 day	OHCs were significantly reduced at 72 h after treatment in the apical, middle, and basal turns. IHCs remained intact. ABR thresholds increased 4 h and 72 h after treatment	(56)
Cis	Wistar rat	i.p.	16 mg/kg	Single-dose	1 day	OHCs and IHCs damaged. DPOAE values decreased	(57)
Cis	Guinea pig	i.p.	12 mg/kg	Single-dose	1 day	OHCs partially lost throughout the cochlea. ABR threshold shifted	(58,59)
Cis	Fischer 344/NHsd rat	i.p.	12 mg/kg	Single-dose	1 day	OHC loss increased from the apex to the base. ABR thresholds increased	(60)
Carb	Wistar rat	i.p.	256 mg/kg	Single-dose	1 day	ABR thresholds increased 4 days after treatment	(61)
Carb	Chinchilla	i.v.	400 mg/m^2	Single-dose	1 day	ABR and CAP thresholds increased	(62)
Carb	Chinchilla	i.v.	200 mg/m^2	Single-dose	1 day	Loss of IHCs. ABR thresholds increased	(63)
Carb	Chinchilla	i.p.	75 mg/kg	Single-dose	1 day	Loss of 40% of IHCs. The amplitude of SP and CAP declined	(64)

SNHL, sensorineural hearing loss; cis, cisplatin; carb, carboplatin; furo, furosemide i.v., intraveneous; i.p., intraperitoneal; OHCs, outer hair cells; IHCs, inner hair cells; ABR, auditory brainstem response; DPOAE, distortion product otoacoustic emission; CAP, compound action potential; SP, summating potential.

dependent damage of OHCs and the stria vascularis (*Table 2*) (56-60). Some studies also adopted regimens of lower single-dose multiple-administrations which resulted in low mortality rates (69). Death rates were lower in CBA/CaJ mice and C57BL/6J mice exposed to 48 mg/kg cisplatin and treated in 3 cycles of two 8 mg/kg doses every 10 days or 3 cycles of 4 mg/kg doses daily for 4 consecutive days, where each cycle was separated by 17 days than when exposed to three 16 mg/kg doses administered once per day, where each cycle was separated by 20 days (69).

Furthermore, the susceptibility to cisplatin ototoxicity in rats and mice can be affected by their circadian times, where the highest ABR threshold shift and the most severe OHC loss occur in the middle of the light circle (70,71). Additionally, concomitant administration of noise exposure or loop diuretics can enhance the ototoxicity of cisplatin (72,73). Cisplatin-treated rats were susceptible to noise exposure. Compared with cisplatin alone or noise exposure alone, a combination of the two resulted in rats showing greater threshold shifts and loss of OHCs (72). Guinea pigs treated with a co-administration of furosemide and cisplatin presented with more severe hearing loss and hair cell loss but lower mortality rates than those receiving cisplatin alone (73).

In contrast, carboplatin shows less ototoxicity to rodents, such as mice, than cisplatin (74). Gersten *et al.* treated mice with equimolar doses of cisplatin, oxaliplatin, and carboplatin; and found cisplatin-induced OHC-loss in only the middle and basal regions, elevated ABR thresholds across frequencies, and decreased DPOAE amplitudes (74). It is suggested that the lower ototoxicity of carboplatin and oxaliplatin is associated with a reduced uptake within the inner ear (74). Dose- and time-dependent oxidative damage of carboplatin to the cochlea in rats was reported (61,75). The high doses (192 or 256 mg/kg, intraperitoneal injection) of carboplatin led to increased cochlear lipid peroxidation and decreased antioxidant enzyme activity (75). Carboplatin caused elevated ABR threshold shifts, increased levels of nitric oxide, reactive oxygen species, increased *manganese superoxide dismutase* activity, and decreased antioxidant enzyme activity 4 days post-treatment in rats (61). A single intraperitoneal dose of carboplatin can induce selective damage of IHCs in chinchillas, providing an ideal IHC loss model (*Table 2*) (62-64,68,76-81). Additionally, studies have demonstrated that a low dose of carboplatin (38–125 mg/kg) selectively damages IHCs, while a high dose of carboplatin (≥200 mg/kg) causes extensive IHC loss and damage of

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OHCs in chinchillas (76).

Overall, cisplatin is more ototoxic than carboplatin and oxaliplatin; and platinum modeling is affected by dose, administration frequency, circadian rhythm, melanin, and drug combination.

Doxorubicin

DOXO is a common antitumor medication that intercalates into the DNA strand to interfere with DNA-directed mRNA synthesis (82). Studies have demonstrated that DOXO can cause demyelination secondary to Schwann cell degeneration (83), and thus utilized DOXO as an experimental demyelinating agent to create animal models of SGNs and auditory nerve demyelination (84). Intraneural injection of DOXO inside the internal auditory canal of chinchillas caused severe myelin injury in SGNs and in fibers of the Rosenthal's canal; and reduced ABR, CAP amplitude, and inferior colliculus-evoked potentials after 2 months. OHCs and IHCs were mostly intact, which was demonstrated by presenting regular cochleograms and the preservation of cubic DPOAEs and CMs (84). Hearing functional testing results of models demonstrating demyelination in SGNs and auditory nerve fibers (ANFs) exclusively resembled those of patients with auditory neuropathy, which is a type of SNHL resulting from auditory synaptopathy and neuropathy (85-87). Overall, the injection of DOXO through the internal auditory canal provides animal models demonstrating the demyelination of auditory nerves without damaging cochlear hair cells.

Aromatic solvents

Aromatic solvents are widely used in industries such as plastics, textiles, and pharmaceuticals (88). Exposure to aromatic solvents like toluene, ethylbenzene, and styrene is ototoxic to both animals and humans (89-92). Studies have shown that ototoxic aromatic solvents mainly damage OHCs, with different degrees of ototoxicity for OHCs and IHCs (90,93-96). Rats were exposed to 1,750–2,000 ppm toluene vapor for 6 consecutive weeks, resulting in OHC damage and preservation of IHCs (96). Studies have demonstrated that exposure to 2,500 ppm toluene or 1,600 ppm styrene for 8 hours/day for 5 days in rats can lead to a significantly increased auditory threshold, matching the lesions of OHC loss (97).

Additionally, rats exposed to ethylbenzene at 400 ppm or 550 ppm for 8 hours/day for 5 consecutive days exhibited reduced DPOAEs and high OHC loss after 3–6 weeks (98). Studies have shown that chinchillas are less susceptible to toluene than mice and rats, which may be due to the higher amount of hepatic cytochrome P-450s and glutathione in the cochleae of chinchillas compared to rats and mice (99,100). Although the underlying mechanism of ototoxicity is undetermined, findings suggest that aromatic solvents including toluene, ethylbenzene, and styrene are useful in inducing selective OHC loss. Moreover, mice and rats may be more suitable for models of toluene ototoxicity.

Ouabain

Ouabain is a kind of cardiac glycoside and can selectively inhibit the α subunit of the sodium-potassium adenosine triphosphatase (Na^+/K^+-ATPase) (101). The Na^+/K^+-ATPase consists of the 3 subunits, α, β, and γ. Within the α subunit, the subunit α 3 is abundant in spiral ganglion somata, the type I afferent ending, and the medial efferent endings (102). Studies have found that an intraperitoneal dose of 50 mg/kg or 10 mM ouabain, or applying 5–40 µL of a 1 mM ouabain solution on round window niche (RWN) can selectively destroy cochlear type I spiral ganglion cells and their ANFs in experimental animals such as mice, gerbils, and rats (15,103-106). Experiments have shown that ouabain damages the spiral ganglion through mitochondrial apoptosis and by demyeli[na]tion of ANFs (105). However, Hamada and Kimura found OHC degeneration, type I SGN loss, and edema of the stria vascularis in some severe cases after ouabain was applied on a round window membrane in guinea pigs (107).

Additionally, Schomann *et al.* applied ouabain on a round window niche and observed OHC loss in a dose-dependent manner, without edema in the stria vascularis (108). High doses of ouabain damaged type I spiral ganglion cells and their ANFs and hair cells, whereas low or medium doses did not damage cochlear hair cells. It is assumed that ouabain directly affects Na^+/K^+-ATPase in OHCs, and indirectly affects OHCs by changing the endolymph content; however, the mechanisms remain undetermined (109,110). The selective damage to SGNs caused by low or medium dosages of ouabain suggests that the ouabain model is an effective model for research into of SGN death and SGN protection (111).

Glutamic acid and Glutamate analogs

Glutamate is released excessively in cochlear tissues during ischemia and noise injury (112). Excess glutamate can cause

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excitotoxicity. The mechanism of excitotoxicity involves increased activity in neurons, the maintenance of neurons' depolarization, and increased concentration of chloride ions in cells, which causes more calcium ions to flow in, thus increasing the osmotic pressure in cells. As water enters the cell, the tension of the cell membrane increases, and the cell lyses (113). The ionotropic receptors of glutamate are classified as N-methyl-d-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainite. AMPA receptors mainly mediate the rapid excitatory neurotransmission between IHCs and AFNs, and the late component of neurotransmission is mediated by NMDA and kainite (114). AMPA/kainite receptors are activated first and may play a role in synaptic transmission at low and moderate intensity. The NMDA receptor is activated by high-intensity sound (112). In cultured spiral ganglion explants, a high glutamate concentration in the synaptic cleft can cause swelling and collapse of the afferent nerve endings and damage to type I SGNs (115).

The glutamate toxicity-induced apoptosis in SGNs is initiated by apoptosis-inducing factors rather than caspase-3 (116). Kainic acid, a glutamate agonist, has been used to damage type I afferent nerve endings in animals. The swelling of afferent synapses was rapidly induced by low concentrations of kainic acid and high concentrations of kainic acid in the perilymph of chicken cochlea (117). The swelling of synapses damaged by the low concentrations of kainic acid disappeared, and the synaptic area returned to normal within 1 day, while the synapses damaged by the high concentrations of kainic acid were irreversibly damaged, and the number of spiral ganglion cells decreased. The morphology and function of hair cells in both groups were normal. Low doses of kainic acid applied to the round window of chinchillas also caused reversible damage to the postsynaptic terminals of the auditory nerve. Three-5 days after treatment, the nerve endings underwent swelling, degeneration, and recovery (118). However, high concentrations of kainic acid-induced the loss of 34% of SGNs, with no toxic effect on cochlear hair cells or supporting cells (119). Henry *et al.* demonstrated that kainic acid caused a decrease in wave I amplitude of ABR, with waves II-V unaffected or slightly changed; suggesting that kainic acid can selectively damage the afferent neurons of the auditory nerve (120).

The glutamate analogs of SGNs can induce selective SGN loss in animal models to explore the causes of initial excitotoxic injury to the postsynaptic neuron of SNHL and the regeneration of afferent nerve terminals (118).

2-Hydroxypropyl-β-cyclodextrin

HPβCD is a cholesterol-chelating agent used to solubilize lipophilic drugs and is employed to treat Parkinson's disease, atherosclerosis, and Niemann-Pick disease type C (121-124). It is reported to cause ototoxicity in cats and patients undergoing long-term therapies (125,126). A single subcutaneous dose of HPβCD (8,000 mg/kg) in mice led to elevated ABR, the elimination of DPOAEs, and loss of OHCs in the basal half of the cochlea; without affecting IHCs after 1 week (127). In another study, HPβCD was subcutaneously administered to rats at high doses of 2,000 or 4,000 mg/kg, leading to remarkably reduced DPOAE amplitudes and loss of OHCs (128). Studies also indicated that when using a high dosage, the IHC loss and lesions in other cochlear tissues occurred 4-8weeks post-treatment (129,130). The mechanisms of the selective damage to OHCs remain undetermined. One factor at plays may be that prestin, the OHC motor protein and the main component of the OHC lateral membrane, is sensitive to changes in cholesterol levels (131-133). Compared to AABs' protocols, HPβCD seems to be a more effective agent to induce loss of OHCs selectively. HPβCD requires only a single systemic administration to induce OHC loss in mice, and its systemic toxicity is low (121). A greater understanding of the pharmacokinetics and mechanism of HPβCD can lead to its improved application in experimental hair cell ablation in research animals. Based on this research, HPβCD can induce OHC loss quickly and affect other cochlear sensory cells after weeks.

Heavy metals

In daily life, excess exposure to heavy metals such as manganese (Mn), mercury (Hg), cobalt (Co), cadmium (Cd), and lead (Pb) in food or air can cause various organ toxicity effects. Studies found that heavy metals can damage cochlear structures and lead to SNHL (134-139). Research has demonstrated that heavy metals enter cells, including cochlea cells, through divalent metal transporter 1 (DMT1), zinc transporter ZIP8, and ZIP14 (140-145). Heavy metals mainly affect mitochondrion by inhibiting calcium uptake and enhancing calcium release, thus altering the mitochondrial permeability and causing a release of cytochrome c. Consequently, heavy metals can induce oxidative stress leading to cell death (146-148). In research into cochlear organotypic cultures, Cd can induce apoptosis in the OHCs and IHCs of rats in a time-dependent manner (135); Mn

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can induce time- and dose-dependent OHC, IHC, SGN, and ANF damage (149), where ANFs are more vulnerable to Mn than hair cells and IHCs are more susceptible to Mn than OHCs (138); Hg tends to affect sensory epithelium in the apical regions of the cochlea and seldom damages the basal regions (150); there is an increase in the damage caused by Co to OHCs, IHCs, and SGNs from the base to the apex of the cochlea as dose and time increase, and the OHCs are more vulnerable to Co damage than IHCs (139); and Pb primarily injures cochlear nerve fibers and SGNs, rather than hair cells (151). Experimental animals have been successfully used to investigate the ototoxicity of heavy metals. Rats co-administered 5 mg/kg $CdCl_2$ (i.p.) and 200 mg/kg furosemide (i.p.) showed damaged OHCs in the apical and middle cochlear regions, extensive loss of IHCs and OHCs in the basal turn, and significantly increased ABR thresholds after 1 week (152). However, oral administration of 30 to 300 μM Cd in adult CBA/CaJ mice for 11 weeks presented normal ABR thresholds (153). Rats exposed to 10 mg Manganese chloride ($MnCl_2$)/liter water for 90 days showed no significant threshold shifts of DOPAE, CAP, and ABR; while rats exposed to noise simultaneously presented threshold shifts (154). Mice treated with 1.0 mg/kg mercuric sulfide per day through gastric gavage for 7 consecutive days demonstrated in an elevation of ABR threshold and prolongation of interwave latencies I–V (155). C57BL/6 mice given 2 mM Pb in water for 28 days presented 8–12 dB shifts in ABR thresholds (156). Rats subjected to 4.0 mg/kg Pb acetate by gavage for 30 days demonstrated prolonged latencies of waves I–V and increased wave amplitudes, implying the deterioration of the neural reflex and damaged hearing (157). In another experiment, guinea pigs treated with Pb (20 mg/mL, i.p.) exhibited in OHC damage and elevated ABR thresholds (158). In conclusion, the animal models of SNHL induced by heavy metals were established using gavage, drinking water exposure, and intraperitoneal injection. Drinking water exposure required a long period.

Discussion

The hearing loss induced by ototoxic drugs can be avoided in the clinic. By establishing the animal model of drug-induced hearing loss, we can not only study the damage mechanism of drugs but also improve treatment and prevent the occurrence of ototoxic deafness. In addition, animal models can also be used to explore the molecular mechanism of deafness, hair cell regeneration, and cochlear cell replantation.

We searched the literature for 8 kinds of toxic cochlear drugs and ototoxic chemicals that have been studied in auditory diseases. These included AABs, platinum antitumor drugs, doxorubicin (DOXO), aromatic solvent, ouabain, glutamic acid, and Glutamate analogs, 2-Hydroxypropyl-β-cyclodextrin (HPβCD), and heavy metals. The tendency of these ototoxic agents to damage the cochlea can be divided into 3 types: the types which included those which tend to damage auditory hair cells, those which tend to damage SGNs and nerve fibers, and those which tend to damage auditory hair cells and neurons.

Among them, AABs damage both hair cells and SGNs. Cisplatin tends to damage cochlear hair cells, particularly OHCs; and carboplatin tends to damage IHCs and SGNs. DOXO can damage the myelin. Aromatic solvents and cyclodextrin can cause more damage to OHCs than IHCs. Ouabain tends to damage SGNs and auditory nerve myelin. Glutamate analogs damage afferent nerve endings. Heavy metals mostly damage nerves but also damage auditory hair cells.

The establishment of animal models of drug-induced hearing loss may be affected by animal selection, route of drug administration, and dosage regimen.

Animal selection is an important aspect of the study of drug-induced deafness. Animals most commonly used in hearing research include rodents, such as guinea pigs, mice, rats, chinchillas, and gerbils. The auditory anatomy of rodents, including cochlear turns, sensory hair cells, and central auditory system, is similar to humans (159,160). Different animals have different auditory physiology and anatomical structure, thus providing different advantages in auditory research. The hearing frequency of mice, rats, and guinea pigs is 100 kHz, 250 Hz to 80 kHz, and 150 Hz to 50 kHz. In chinchillas and gerbils, their hearing frequencies are ~50 Hz to 33 kHz and 100 Hz to 50 kHz, respectively; which demonstrates that the hearing range of chinchillas is closer to humans (who have a range of 20 to 20 kHz) (161-164).

Moreover, guinea pigs, chinchillas, and rats have bigger ear bulla, allowing for greater efficiency of procedures such as injection through the tympanic membrane, retroauricular sulcus, or semicircular canal. Additionally, animals showed different susceptibilities to ototoxic drugs, which affected the dosage regimen. For example, guinea pigs and chinchillas showed higher susceptibility to AABs, while adult rats and mice showed lower susceptibility. Although adult mice and rats are resistant to some ototoxic drugs, they are still widely used in studies of drug-induced hearing

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loss. Particularly mice, which possess various strains such as C57BL/6 mice, BALB/c mice, and CBA/CaJ mice, and gene knockout varieties, allow research into hearing loss with certain lesions. C57BL/6J mice are also widely used as models of presbycusis due to their Ahl/Ahl2 genes which accelerate age-related hearing loss (20,165-171).

CBA/CaJ mice show stable hearing thresholds in advanced age (12–18 months), so they are suitable for experiments involving chronic exposure to ototoxic agents (172). Solute Carrier Family 19 Member 2 (SLC19A2)-deficient mice showed selective loss of both IHCs and intact supporting cells around IHCs (173). Aside from rodents, zebrafish are increasingly used in hearing research, such as hair cell regeneration studies, due to the susceptibility of their sensory epithelial cells along the lateral line to toxic agents (174-176).

The route of drug administration is another important factor affecting the establishment of animal models. The majority of the drugs were delivered to the cochlea by systemic administration, including subcutaneous injection and intraperitoneal injection, local administration involving transtympanic injections, cochleostomy with perilymphatic perfusion, and the round window niche technique. The direct methods of cochlear medication can avoid systemic toxicity such as nephrotoxicity and peripheral neuropathy; and can avoid the BLB, which is conducive to a faster and greater accumulation of drugs that do not easily cross the BLB in the inner ear (177,178). Nevertheless, local delivery requiring perforations of the tympanic membrane, or incisions behind the ear or on the retroauricular groove, need to be performed on large rodents such as rats, guinea pigs, chinchillas or gerbils; and may increase the risk of infection. the perforation of the tympanic membrane may affect the detection of auditory function in animals (179). Moreover, additional training is required to perform these operations.

Various drug dosage regimens are adopted to induce SNHL. These include increasing the dosages, which may increase the mortality rate of animals; prolonging the treatment duration by multiple administrations; combining administration of loop diuretics, such as furosemide and *ethacry*nic acid, which can open the BLB; combining administration with noise or another ototoxic drug.

Conclusions

Over 150 ototoxic agents have been reported to date. Common ototoxic medications such as AABs and platinum antitumor drugs are extensively used to induce SNHL in experimental animals. The effect of ototoxic agents *in vivo* is generally influenced by the chemical mechanisms of the agents themselves, type of anima, routes of drug administration, and drug dosage levels. Studies involving experimental animal models of SNHL explore the underlying mechanisms involved in drug-induced hearing loss to discover effective interventions for clinical practice.

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