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A review: Pharmaceutical induced ototoxicity

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ABSTRACT: Ototoxicity, defined as drug-induced damage to the auditory and vestibular systems, manifests as hearing loss, tinnitus, dizziness, and balance disorders, representing a critical challenge in clinical practice and pharmaceutical development. This review aims to consolidate advancements in the understanding of pharmaceutical-induced ototoxicity, focusing on its mechanisms, diagnostic methodologies, and preventive strategies. A comprehensive analysis of existing literature was conducted, encompassing clinical findings and experimental data on the ototoxic effects of major drug classes, including aminoglycosides, platinum-based chemotherapeutics, and loop diuretics. The review evaluates the underlying biochemical mechanisms and explores innovative approaches for mitigating ototoxic effects. Ototoxicity predominantly results from oxidative stress, mitochondrial dysfunction, disruption of calcium homeostasis, and activation of inflammatory pathways. Significant progress has been achieved in the development of therapeutic drug monitoring protocols, antioxidant therapies, and targeted drug delivery systems, including nanoparticles and hydrogels. Emerging technologies, such as gene-editing and caspase inhibitors, demonstrate potential for preserving hair cell integrity and mitigating auditory damage. Early detection and management of ototoxicity are paramount for maintaining auditory and vestibular function. This review provides a comprehensive framework for clinicians, researchers, and pharmaceutical professionals to address ototoxic effects effectively and highlights promising directions for future research and therapeutic development.

KEYWORDS: Hearing loss; cisplatin; aminoglycosides; oxidative stress; auditory hair cells.

1. INTRODUCTION

Ototoxicity refers to the damaging effects of certain pharmaceutical agents or chemicals on the auditory and vestibular systems, often resulting in hearing loss, tinnitus, or balance disorders. These effects significantly impact quality of life, making ototoxicity a key concern in clinical practice and pharmaceutical research. The use of ototoxic pharmaceuticals underscores the need for a more profound understanding of their adverse effects. This review consolidates current knowledge on pharmaceutical-induced ototoxicity, hearing physiology, major ototoxic drug classes, diagnostic and monitoring strategies, and preventive measures. Additionally, recent studies exploring ameliorative strategies and interventions to mitigate ototoxic effects are critically evaluated, providing insights for future research.

2. BACKGROUND

2.1. Physiology of Hearing

The auditory system enables sound perception by converting mechanical energy into electrical signals that the brain interprets, a process known as auditory transduction. This process involves the coordinated function of the outer, middle, and inner ear. The outer ear, comprising the pinna and auditory canal, collects sound waves and directs them to the tympanic membrane. The middle ear amplifies these sound waves via three ossicles (malleus, incus, stapes) and transmits them to the cochlea through the oval window. The Eustachian tube maintains pressure balance across the tympanic membrane. The cochlea, a spiral, fluid-filled structure, is the primary organ of hearing, as shown in Figure 1. It contains chambers filled with perilymph and endolymph, essential for establishing electrochemical gradients critical for hair cell function. The basilar membrane within the cochlea facilitates frequency discrimination, with high-frequency sounds activating the base and low-frequency sounds stimulating the apex.

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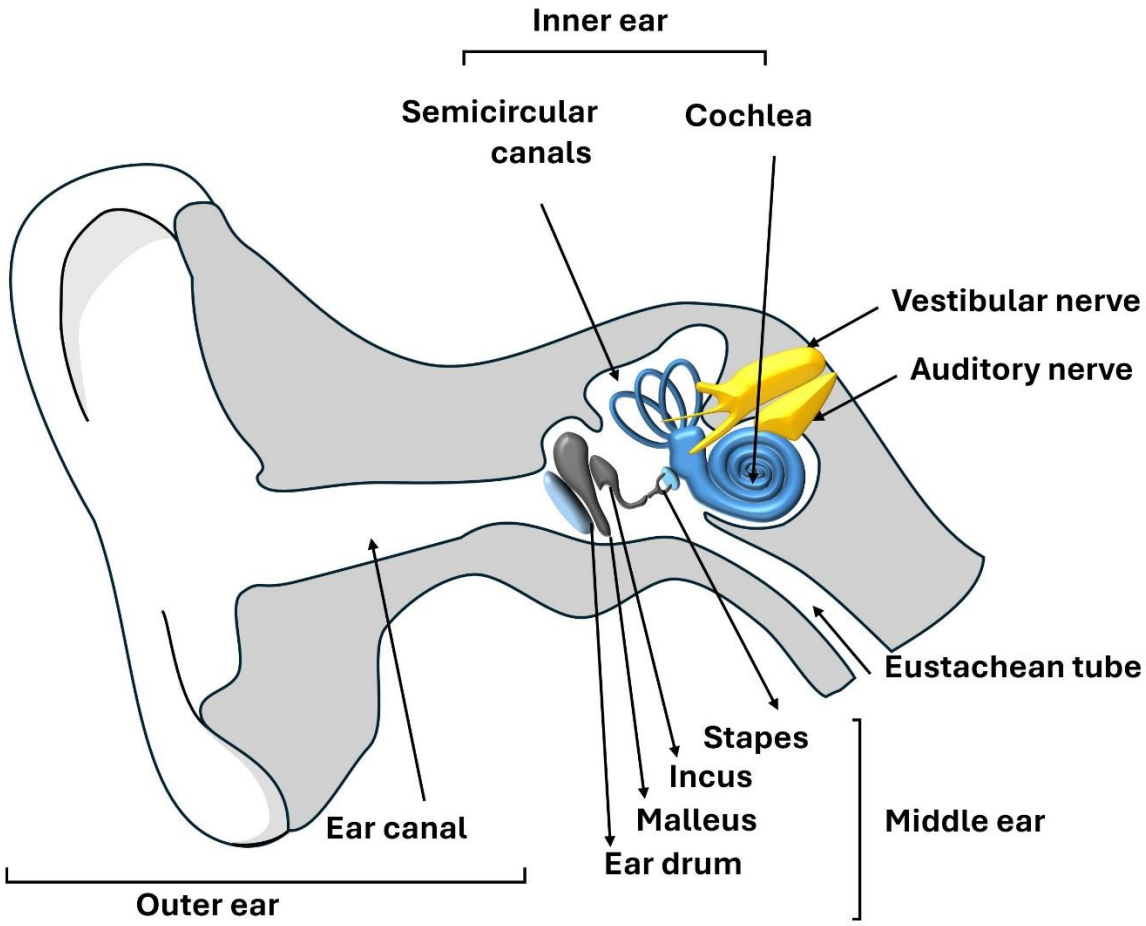

Figure 1. Illustration of the Ear anatomy

The organ of Corti (Figure 2), located on the basilar membrane, houses sensory hair cells. Inner hair cells (IHCs) convert mechanical vibrations into neural signals, while outer hair cells (OHCs) amplify these signals. Deflection of stereocilia on hair cells opens ion channels, allowing ion influx that generates receptor potentials. These signals are transmitted via the cochlear nerve to the auditory cortex through brainstem nuclei and the thalamus, where sound is ultimately interpreted as frequency discrimination. Sound waves entering the cochlea induce traveling waves along the basilar membrane, with specific regions responding to different frequencies—a phenomenon known as tonotopic organization. High-frequency sounds stimulate the base of the cochlea, while low-frequency sounds activate the apex.

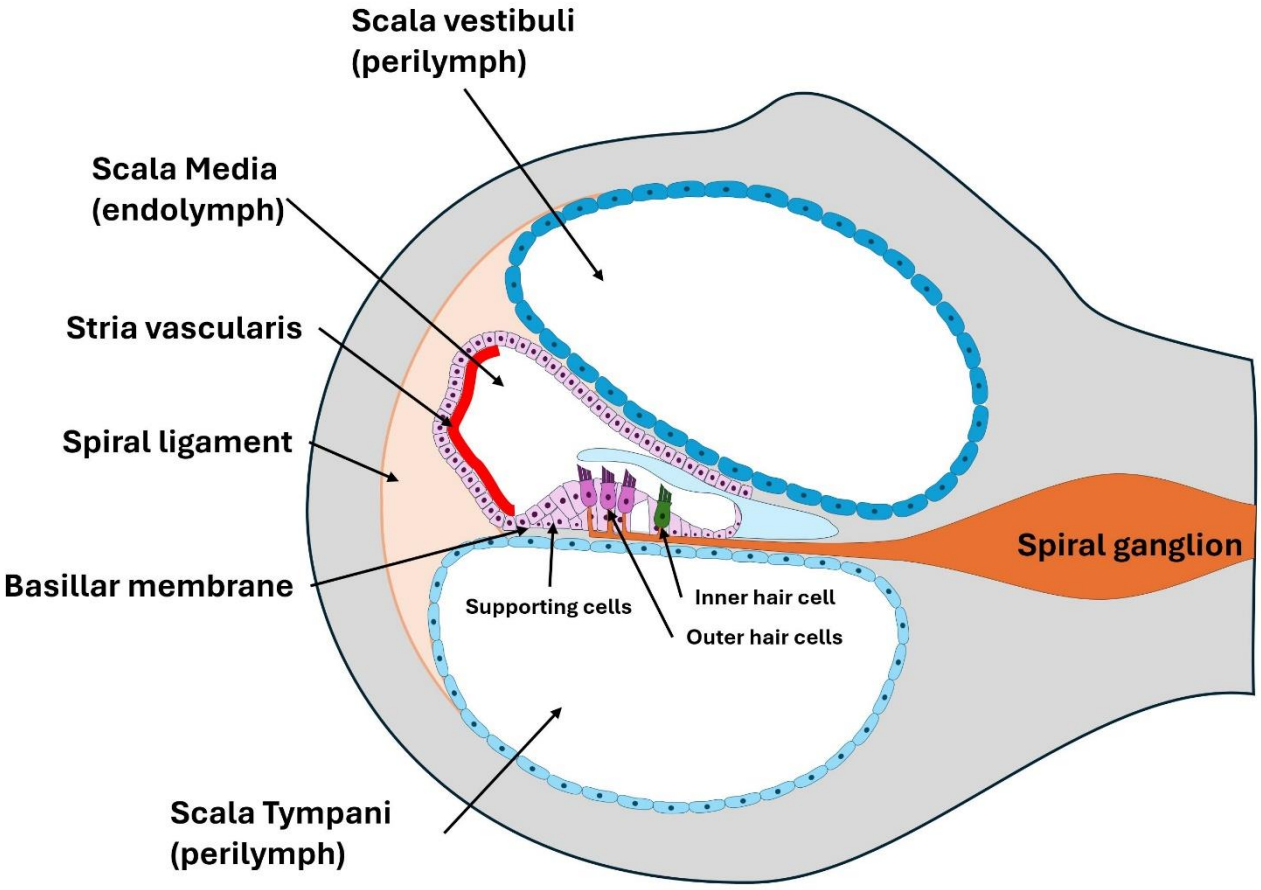

Figure 2. The organ of Corti Scheme

2.2. Mechanisms of Ototoxicity

The cochlear sensory hair cells, spiral ganglion neurons, and supporting cells are primary targets of ototoxic drugs. The mechanisms of ototoxicity are multifaceted and complex, involving biochemical and physiological processes that ultimately compromise the auditory system's functionality. A detailed examination of these mechanisms reveals the pathways through which drugs exert their deleterious effects, often leading to irreversible damage.

One of the most widely implicated mechanisms is oxidative stress. Ototoxic drugs such as aminoglycosides and cisplatin are known to generate reactive oxygen species (ROS) within cochlear cells [1]. These highly reactive molecules cause damage to lipids, proteins, and DNA, impairing cellular integrity and function. In normal conditions, endogenous antioxidant systems neutralize ROS. However, the overwhelming production of ROS during drug exposure surpasses the antioxidant capacity, tipping the balance toward oxidative damage [2]. Lipid peroxidation disrupts cell membranes, protein carbonylation impairs enzymatic and structural proteins, and oxidative DNA damage triggers cell cycle arrest or apoptosis [2]. Cochlear cells are susceptible to oxidative damage due to their high metabolic demands [3].

Mitochondrial dysfunction is another critical pathway in ototoxicity [4, 5]. Mitochondria, as the primary source of cellular energy, are particularly susceptible to oxidative damage. ROS generated by toxic compounds compromise mitochondrial DNA, respiratory chain complexes, and membrane potential. This process leads to impaired ATP synthesis, which is vital for the energy-demanding functions of cells [3-6]. Furthermore, mitochondrial damage activates intrinsic apoptotic pathways through the release of cytochrome c and subsequent activation of caspases (Figure 3). This cascade culminates in programmed cell death, a hallmark of ototoxicity [1, 7].

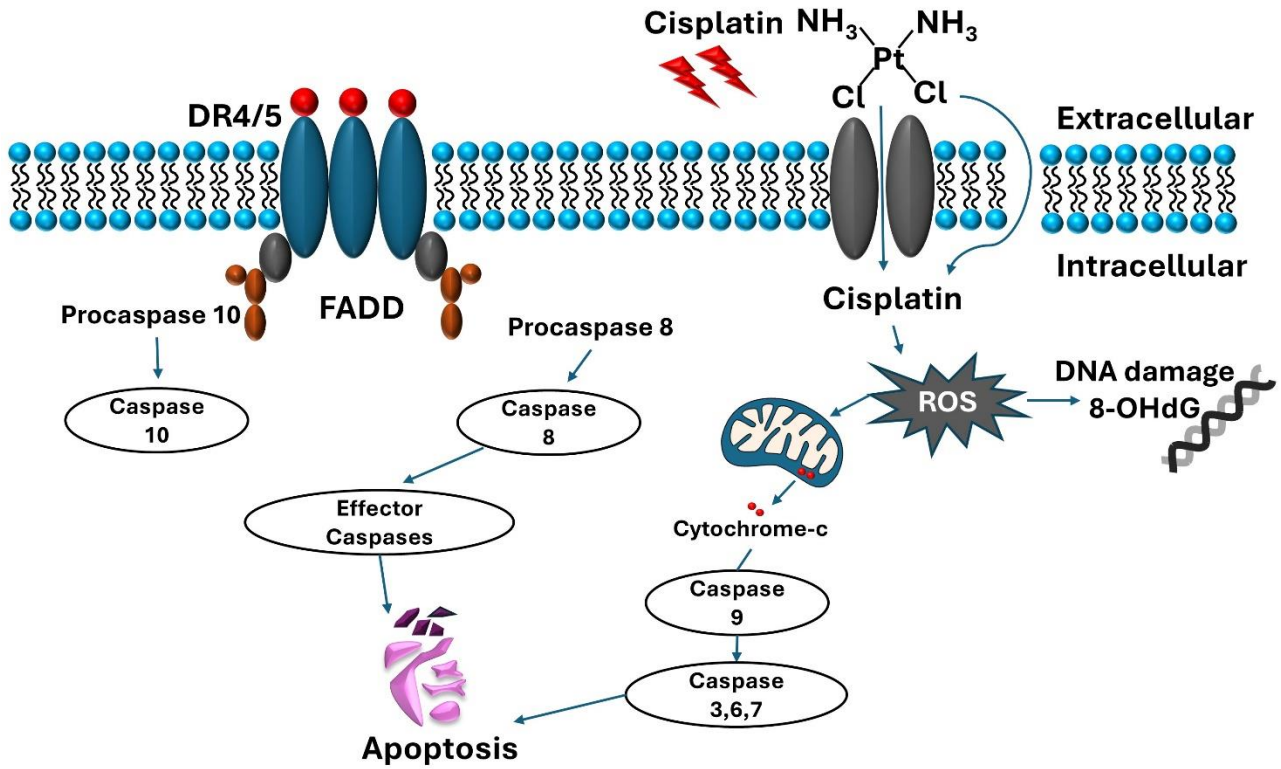

Figure 3. Cisplatin induced apoptosis mechanism

The dysregulation of intracellular calcium homeostasis is also pivotal in ototoxic damage. Calcium ions play essential roles in auditory signal transduction and hair cell function [8]. Ototoxic drugs disrupt calcium channel function, leading to abnormal intracellular calcium levels. Elevated calcium concentrations activate calcium-dependent proteases and endonucleases, which degrade cellular components and further amplify apoptotic signals. This disruption also impairs the synaptic transmission between inner hair cells and spiral ganglion neurons, compromising the propagation of auditory signals.

In addition to oxidative and calcium-mediated damage, inflammatory pathways contribute to ototoxicity. Cisplatin, for instance, activates pro-inflammatory signaling cascades, including the nuclear factor-kappa B (NF-κB) pathway. The upregulation of inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), exacerbates cochlear cell damage. These cytokines amplify oxidative stress and recruit immune cells that release additional cytotoxic mediators, creating a feedback loop of damage.

Genetic predisposition further influences the susceptibility to ototoxicity. Mutations in mitochondrial DNA or nuclear-encoded genes involved in antioxidant defenses or calcium signaling can heighten vulnerability.

The interplay of these mechanisms results in progressive damage to cochlear structures, often beginning with the outer hair cells, which are most susceptible due to their amplificatory role and high metabolic demands [3]. As damage progresses, inner hair cells, supporting cells, and spiral ganglion neurons are also affected. This cascade of cellular events ultimately leads to hearing loss, tinnitus, or vestibular dysfunction, depending on the drug and its pharmacokinetics. In Table 1, a summary of the pharmaceutical-induced ototoxicity mechanisms is presented.

Table 1. Summary of the pharmaceutical induced ototoxicity mechanisms

Primary Mechanism	Sub-mechanisms	Cellular/Molecular Events	Clinical Impact	Common Drug Examples	References
Oxidative Damage	•Free radical generation •Lipid peroxidation •Antioxidant depletion	•Mitochondrial DNA damage •Membrane disruption •Protein oxidation • Electron transport chain dysfunction	•Hair cell death •Progressive hearing loss •Vestibular dysfunction	•Cisplatin •Aminoglycosides •Loop diuretics	[2, 3]
Ion Homeostasis Disruption	•K+ transport interference •Ca2+ signaling dysfunction •Na+/K+ ATPase inhibition	•Altered endocochlear potential •Disturbed mechanotransduction •Changed membrane permeability	•Acute hearing loss •Tinnitus •Balance disorders	•Aminoglycosides •Loop diuretics •Salicylates	[8-10]
Cell Death Pathways	•Apoptosis activation •Necrosis •Autophagy dysregulation	•Caspase cascade activation •Cytochrome c release •PARP activation •JNK/p38 MAPK signaling	•Irreversible hair cell loss •Spiral ganglion degeneration	•Cisplatin •Aminoglycosides	[3, 11]
Neurotransmitter Disruption	•Glutamate excitotoxicity •Synaptic dysfunction •Neural transmission alteration	•Ca+2 overload •Receptor desensitization •Synaptic degeneration	•Neural hearing loss •Auditory processing issues	•Salicylates •Loop diuretics	[12, 13]
Vascular Effects	•Reduced cochlear blood flow •Stria vascularis damage •Microcirculation changes	•Endothelial dysfunction •Blood-labyrinth barrier disruption •Ischemia-reperfusion injury	•Metabolic stress •Tissue hypoxia •Chronic damage	•Loop diuretics •Cisplatin	[9, 11]
Inflammatory Response	•Cytokine upregulation •Immune cell infiltration •NF-κB activation	•Pro-inflammatory mediator release •Oxidative burst •Tissue remodeling	•Chronic inflammation •Progressive damage	•Cisplatin •Aminoglycosides	[7]
Mechanical/ Structural	•Hair cell stereocilia damage •Tectorial membrane changes •Basilar membrane alterations	•Cytoskeletal disruption •Motor protein dysfunction • Mechanical stress	•Altered frequency selectivity •Reduced sensitivity	•Aminoglycosides •Heavy metals	[14, 15]
Metabolic Stress	•ATP depletion •Protein synthesis inhibition •Mitochondrial dysfunction	•Energy crisis •Cellular stress response •Metabolic acidosis	•Cellular dysfunction •Compromised repair	•Cisplatin •Loop diuretics	[9, 11, 13]

3. MAJOR OTOTOXIC PHARMACEUTICALS

3.1. Aminoglycosides

Aminoglycosides, including drugs such as gentamicin, neomycin, tobramycin, kanamycin, amikacin, and streptomycin, are essential antibiotics widely used to treat serious infections, particularly those caused by gram-negative bacteria. These antibiotics, however, are well-known for their potential ototoxicity, which can affect both hearing and balance. The ototoxic effects of aminoglycosides were first recognized in 1945, with streptomycin-induced hearing loss documented as one of the earliest reports. The class of aminoglycosides remains a critical treatment option despite these adverse effects, especially in treating multidrug-resistant tuberculosis (MDR-TB) and other severe infections [5, 16].

The ototoxicity of aminoglycosides is primarily mediated through their selective accumulation in the cochlea and vestibular organs. The drugs preferentially damage the basal OHCs, the stria vascularis, and spiral ganglion neurons in the inner ear.

The mechanism of damage involves the accumulation of aminoglycosides in cochlear cells, where they generate ROS through Fenton-like reactions, leading to oxidative stress and cellular apoptosis. The drugs also disrupt mitochondrial function and calcium homeostasis, further exacerbating cellular damage. This multi-pronged attack on cochlear cells can lead to irreversible hearing loss, especially in the high-frequency region. Direct interaction of drugs with cellular structures also plays a role in ototoxicity. Aminoglycosides are known to bind to phosphoinositides in the hair cell membranes and interfere with mechanotransduction channels. This disrupts the normal ion flow required for the generation of receptor potentials. Additionally, aminoglycosides can accumulate within lysosomes, leading to lysosomal rupture and the release of hydrolytic enzymes that degrade cellular components [17].

Gentamicin is widely used to treat various serious infections, including meningitis, bloodstream infections, and urinary tract infections. The ototoxic effects of gentamicin are well-documented, with hearing loss, dizziness, and sometimes tinnitus being common side effects [16].

Amikacin Introduced as a semisynthetic derivative of kanamycin A, amikacin is widely used to treat MDR-TB. However, it carries a higher risk of ototoxicity compared to other aminoglycosides. Furthermore, tinnitus often accompanies high-frequency hearing loss in amikacin-treated patients. While the ototoxic effects of amikacin can be mitigated by once-daily dosing protocols, which have shown a reduced incidence of toxicity, the risk remains a significant concern [16].

Kanamycin has long been recognized for its ototoxic potential, especially in patients treated for MDR-TB. Kanamycin, like other aminoglycosides, causes significant loss of OHCs, particularly in the basal cochlea. Tinnitus is often a precursor to hearing loss, which can complicate the detection of early ototoxic effects [16] .

The pooled prevalence of ototoxic hearing loss in MDR-TB patients treated with aminoglycosides is approximately 40.62%. The prevalence varies by specific drugs, with kanamycin at 49.65%, amikacin at 38.93%, and capreomycin at 10.21%. Age also influences prevalence, with rates of 43.82% in adults, 32.34% in teenagers and adults combined, and 25.00% in children. Annually, about 50,000 cases of hearing loss in MDR-TB patients could be prevented by following WHO guidelines that prioritize non-aminoglycoside regimens [18].

Given the serious implications of aminoglycoside-induced ototoxicity, various strategies have been explored to mitigate this adverse effect. Therapeutic drug monitoring is crucial to ensure serum aminoglycoside levels remain within a safe range, particularly during prolonged therapy [19]. Nanocarrier-based drug delivery systems are also being investigated to reduce undesirable effects of aminoglycosides [20]. Despite these preventive efforts, the risk of ototoxicity remains a significant concern in clinical practice. Consequently, the development of newer aminoglycoside derivatives with reduced ototoxic potential, such as apramycin, has gained attention. These agents aim to retain antimicrobial efficacy while minimizing auditory and vestibular damage, thereby improving the therapeutic index of this indispensable antibiotic class [21].

3.2. Platin Based Pharmaceuticals

Cisplatin, along with other platinum-based chemotherapeutic agents such as carboplatin and oxaliplatin, is among the most effective drugs in oncology, widely used to treat various malignancies, including testicular, ovarian, bladder, lung, and head and neck cancers. These agents achieve clinical success through their potent ability to form DNA crosslinks, thereby disrupting DNA replication and inducing

apoptosis in rapidly dividing cancer cells. However, their therapeutic efficacy is often accompanied by dose-limiting side effects, with ototoxicity being a significant concern, particularly for cisplatin. This ototoxicity, often irreversible, poses a substantial challenge to patient quality of life, especially in pediatric and long-term cancer survivors. While carboplatin is associated with a lower ototoxic risk compared to cisplatin, it is not without adverse effects, and oxaliplatin is more commonly linked to neurotoxicity, highlighting the diverse toxicological profiles of platinum-based therapies [22].

Cisplatin-induced ototoxicity results primarily from its preferential accumulation in the cochlea, specifically within the stria vascularis, outer hair cells, spiral ganglion neurons, and supporting cells. Once in the cochlea, cisplatin induces a cascade of cellular and molecular events, leading to auditory damage [22]. A key mechanism involves the generation of ROS and reactive nitrogen species (RNS). Cisplatin binds to mitochondrial DNA and impairs mitochondrial function, increasing ROS production. These free radicals damage lipids, proteins, and DNA, initiating oxidative stress that overwhelms the cochlear antioxidant defenses. Consequently, oxidative damage triggers apoptotic pathways, leading to the loss of cochlear hair cells and supporting cells as shown in Figure 3 [3]. Inflammation further exacerbates cochlear damage in cisplatin-treated individuals. Cisplatin activates pro-inflammatory pathways, notably NF-κB signaling cascade, which upregulates inflammatory cytokines [23]. This inflammatory response amplifies oxidative damage and contributes to cellular apoptosis, compounding the ototoxic effects [3].

Ion imbalance within the cochlea is another significant factor. Cisplatin disrupts the potassium cycling essential for generating endolymphatic potential, a critical driver of auditory signal transduction also creates an imbalance in homeostasis [24]. Damage to the stria vascularis, responsible for maintaining this ionic gradient, leads to endolymphatic dysfunction and hearing loss [24]. Additionally, the loss of outer hair cells in the basal turn of the cochlea typically results in high-frequency hearing loss, often one of the earliest detectable signs of cisplatin ototoxicity. The clinical manifestation of cisplatin ototoxicity is typically bilateral, sensorineural, and permanent. High-frequency hearing loss is a hallmark feature, though progression to mid- and low-frequency loss can occur with cumulative doses. Hearing loss was identified in 28% of the 117 patients evaluated who were undergoing cisplatin treatment for tumors in various locations [25].

3.3. Loop Diuretics

Loop diuretics, such as furosemide, bumetanide, torsemide, and ethacrynic acid, are widely used in clinical practice for their potent diuretic effects, particularly in managing conditions such as acute pulmonary edema, heart failure, chronic kidney disease, and hypertension. These drugs act by inhibiting the sodium-potassium-chloride (NKCC2) cotransporter in the thick ascending limb of the loop of Henle, thereby promoting the excretion of sodium, chloride, and water. While their efficacy in fluid overload conditions is undeniable, loop diuretics are associated with ototoxic effects, which can range from transient to permanent, depending on dosage, administration route, and individual susceptibility [5].

The ototoxicity of loop diuretics is primarily mediated by their action on the stria vascularis in the cochlea. This highly vascularized region is critical for maintaining the ionic composition of the endolymph, which is essential for normal auditory signal transduction. Loop diuretics inhibit NKCC1, a transport protein in the stria vascularis responsible for recycling potassium and chloride ions into the endolymph. Disruption of this ion transport mechanism leads to a rapid reduction in endocochlear potential, impairing the electromotive force necessary for hair cell depolarization. Consequently, the hair cells' ability to transduce sound into neural signals is diminished, resulting in temporary hearing loss [9].

The onset of loop diuretic-induced ototoxicity is typically rapid, with patients reporting symptoms such as tinnitus, vertigo, and hearing loss within hours of administration. These effects are usually dose-dependent and more common with intravenous administration, which leads to higher peak plasma concentrations compared to oral dosing. High doses, rapid infusion rates, and concurrent use of other ototoxic agents, such as aminoglycosides or cisplatin, significantly increase the risk [22]. Renal impairment, which slows the elimination of loop diuretics, also predisposes patients to ototoxicity.

Unlike the ototoxic effects of aminoglycosides or cisplatin, those associated with loop diuretics are often reversible [9]. Short-term exposure typically results in transient hearing loss, as the ionic imbalance in the cochlea resolves after the drug is cleared from the system. This reversibility makes loop diuretics a relatively safer option compared to other ototoxic drugs in patients requiring acute management of fluid overload. Additionally, the development of diuretics with improved safety profiles, such as those with lower affinity for cochlear NKCC1 transporters, represents a promising avenue for reducing ototoxic risk.

3.4. Salicylates

Salicylates, particularly acetylsalicylic acid (aspirin), are among the most well-studied non-steroidal anti-inflammatory drugs (NSAIDs) with respect to their ototoxic effects. These compounds are widely used for their anti-inflammatory, analgesic, and antipyretic properties, as well as their antithrombotic benefits at low doses. However, at higher doses, typically used in rheumatological conditions or during prolonged treatments, salicylates are associated with notable ototoxic effects, including tinnitus and hearing loss [5, 12]. The ototoxicity of salicylates is primarily attributed to their direct effects on cochlear structures, particularly the OHCs. One of the key mechanisms involves the inhibition of prestin, a motor protein exclusively expressed in OHCs. Prestin plays an essential role in cochlear amplification by enabling the electromotility of OHCs, which amplifies sound vibrations and enhances the sensitivity and frequency selectivity of auditory signals. When salicylates inhibit prestin function, the ability of OHCs to amplify sound waves is diminished, leading to a measurable reduction in auditory sensitivity [13].

Salicylates also impact the ionic homeostasis and metabolic activity of the cochlea [12]. These drugs disrupt the intracellular pH of cochlear cells, altering ion exchange and the endocochlear potential. The endocochlear potential is critical for the depolarization of sensory hair cells, and its reduction impairs the transduction of mechanical sound vibrations into electrical neural signals. This disruption results in the characteristic high-frequency hearing loss observed during salicylate exposure [10]. Central auditory mechanisms also play a role in salicylate-induced ototoxicity. Elevated concentrations of salicylates in the bloodstream lead to their accumulation in the cochlea and central auditory pathways. In the central auditory system, salicylates enhance excitatory neural activity, which is thought to underlie the perception of tinnitus. This increased activity is particularly prominent in the auditory cortex and other higher auditory processing centers, which become hyperactive in the presence of salicylates [26].

The auditory effects of salicylates are dose-dependent, with symptoms such as tinnitus typically appearing at higher therapeutic or supratherapeutic plasma concentrations. Notably, these effects are reversible in most cases, resolving within a few days of discontinuation of the drug as salicylate levels decline and normal cochlear and neural functions are restored [13]. However, repeated or prolonged exposure, especially at high doses, may exacerbate underlying auditory conditions or interact synergistically with other ototoxic agents, leading to more pronounced auditory damage.

3.5. Others

The category of "others" in ototoxic pharmaceuticals includes various drugs such as quinine and macrolides, which, although less frequently implicated compared to aminoglycosides or cisplatin, exhibit significant ototoxic potential under certain conditions. These agents affect auditory and vestibular systems through mechanisms that can range from direct cellular toxicity to secondary effects on cochlear homeostasis.

Quinine and its derivatives, historically used as an antimalarial agent and still occasionally employed in treating rheumatoid arthritis and lupus, are well-documented for their ototoxic properties [27]. Quinine's primary mechanism of auditory toxicity involves interference with cochlear blood flow and ionic homeostasis. Quinine reduces the oxygenation and perfusion of the cochlea by inducing vasoconstriction, particularly in the stria vascularis, which is responsible for maintaining the ionic gradient critical for hair cell function. This disruption impairs the electrochemical balance in the cochlea, resulting in temporary tinnitus and hearing loss, especially at high doses. The effects are mostly dose-dependent and usually reversible, but prolonged exposure or overdose can lead to permanent damage, particularly in individuals with pre-existing auditory conditions [27].

Macrolide antibiotics, such as erythromycin, azithromycin, and clarithromycin, also exhibit ototoxic potential, particularly in individuals with renal or hepatic impairment, where drug clearance is reduced. The ototoxic effects of macrolides are thought to arise from their impact on the cochlear sensory epithelium and inner ear fluid dynamics [28]. These antibiotics can accumulate in perilymph and endolymph, leading to disruptions in ionic homeostasis and oxidative stress within the cochlea. These macrolide group antibiotics have been reported to cause temporary hearing loss, which generally resolves upon discontinuation of the drug [28-30]. However, in rare cases, prolonged use or high systemic concentrations, or even normal conditions can lead to more severe and possibly irreversible auditory damage [30].

Other drugs occasionally implicated in ototoxicity include antineoplastic agents like vincristine [31], antitubercular drugs such as ethambutol [32], and even some antihypertensive agents under specific conditions. In 2007, a case of profound sensorineural hearing loss linked to sildenafil use led to FDA

warnings about potential hearing risks with PDE5 inhibitors. While the causal relationship remains unconfirmed, animal studies have shown mixed results, with some indicating no hearing threshold changes and others suggesting inner ear effects like hydrops. Clinical data indicate these drugs may negatively impact hearing, potentially causing sudden sensorineural hearing loss, which can be reversible, partially recoverable, or permanent [5].

Each of these agents operates through distinct mechanisms, including disruption of mitochondrial function, generation of ROS, or interference with neurotransmission in the auditory pathway. Despite their relatively lower prevalence compared to aminoglycosides or cisplatin, the ototoxic effects of quinine, macrolides, and other lesser-known agents warrant attention, particularly in populations with predisposing risk factors or when used in combination with other ototoxic drugs.

4. RISK FACTORS AND SUSCEPTIBILITY

Susceptibility to ototoxicity depends on genetic, physiological, and pharmacological factors that determine the severity and permanence of auditory damage [5]. Mitochondrial DNA mutations, including the A1555G mutation in MT-RNR1, heighten vulnerability to aminoglycosides by mimicking bacterial ribosomal RNA. Polymorphisms in genes like GSTTI and GSTP1 influence susceptibility to cisplatin by affecting oxidative stress regulation and detoxification pathways (Figure 4) [5]. Other genetic factors, including OCT2 and SOD2, further modulate cochlear vulnerability [33].

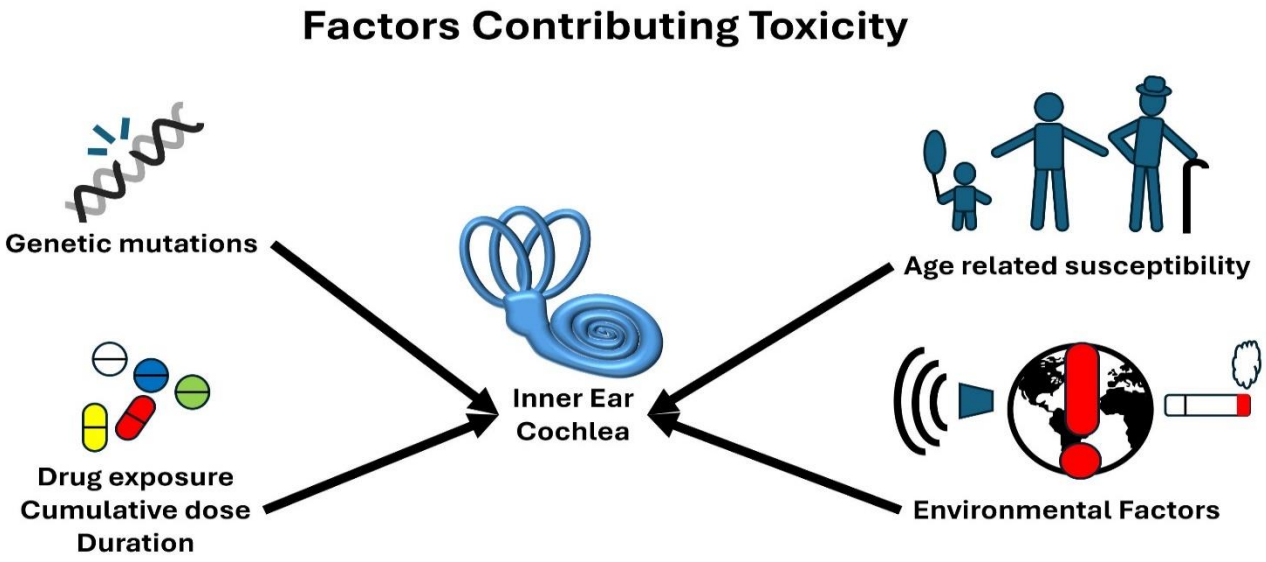

Figure 4. Risk Factors

Age-related degeneration of cochlear structures increases risk in older adults, while the immature auditory system in neonates is particularly susceptible. Comorbidities, including diabetes and chronic kidney disease, impair cochlear blood flow and drug clearance, compounding the effects of ototoxic agents [3]. Concurrent administration of multiple ototoxic drugs, like loop diuretics or NSAIDs, amplifies cochlear damage through synergistic toxicity.

Higher cumulative doses and prolonged exposure to aminoglycosides or cisplatin elevate the risk of irreversible auditory damage [11]. Environmental and lifestyle factors, including noise exposure, smoking, and nutritional deficiencies, exacerbate vulnerability by depleting antioxidant defenses and reducing cochlear microcirculation [3].

5. DIAGNOSIS AND MONITORING OF OTOTOXICITY

Audiological testing plays a crucial role in the detection, monitoring, and management of ototoxicity. These assessments help to identify early auditory changes, often before symptoms like hearing loss become clinically apparent. A range of tests, including audiometry and otoacoustic emissions (OAEs), is utilized to evaluate the functional integrity of the auditory system in patients exposed to ototoxic drugs [14, 15, 31].

5.1. Audiometry

Audiometry is the most widely used diagnostic tool in audiology, offering a quantitative measure of hearing thresholds across a range of frequencies. Pure-tone audiometry (PTA) assesses the softest sounds a person can hear at specific frequencies, typically between 250 Hz and 8,000 Hz [31]. High-frequency audiometry extends this range to frequencies up to 20,000 Hz, which is particularly valuable in ototoxicity monitoring, as high-frequency hearing loss often precedes damage at lower frequencies. In ototoxicity, audiometric testing can reveal early changes in hearing sensitivity, even when patients are asymptomatic. Serial audiograms, taken at regular intervals, allow for the detection of progressive hearing loss and provide critical data for determining whether treatment modifications are necessary to prevent further auditory damage [31].

5.2. Otoacoustic Emissions (OAEs)

OAEs are sounds generated by the OHCs of the cochlea in response to acoustic stimuli, reflecting the functional status of these cells. This non-invasive test is highly sensitive to early cochlear damage, making it a key tool in the early detection of ototoxicity.

- Transient-Evoked Otoacoustic Emissions (TEOAEs) involve short acoustic stimuli, such as clicks, to evoke emissions. A reduction or absence of TEOAEs indicates damage to OHCs, often before hearing loss is detected by audiometry [31].
- Distortion-Product Otoacoustic Emissions (DPOAEs) use two simultaneous pure-tone stimuli to produce emissions at a third frequency, which can be measured. DPOAEs are particularly useful for monitoring specific frequency regions, including the high frequencies most affected by ototoxic drugs.

  Because OAEs are objective and do not require active participation from the patient, they are particularly suitable for populations that are difficult to test with audiometry, such as infants or individuals with cognitive impairments [34].

5.3. Combined Audiological Monitoring

Audiological monitoring often involves a combination of audiometry and OAE testing to provide a comprehensive assessment of auditory function. While audiometry evaluates the patient's perception of sound, OAEs provide direct insight into the physiological integrity of cochlear OHCs. This dual approach enhances the sensitivity and specificity of ototoxicity monitoring [13].

5.4. Advanced Audiological Tests

In cases where more detailed analysis is required, advanced tests such as auditory brainstem response (ABR) testing may be employed. ABR evaluates the neural transmission of sound from the cochlea to the brainstem, offering insight into retrocochlear pathology that may accompany ototoxicity [34]. Similarly, electrocochleography (ECoG) can assess cochlear function at the level of the hair cells and auditory nerve [35].

6. BIOMARKERS IN OTOTOXICITY

6.1. Oxidative Stress Biomarkers

Biomarkers of oxidative stress provide critical indicators of cochlear injury severity and progression. Key ROS, including superoxide anions and hydrogen peroxide, initiate damage by disrupting cellular homeostasis. Secondary products like peroxynitrite induce extensive modifications in lipids, proteins, and DNA, exacerbating cochlear injury. Although these molecules are short-lived, their presence can be indirectly measured through stable byproducts [2, 36].

Lipid peroxidation markers, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), accumulate in cochlear tissues during oxidative stress, correlating with auditory dysfunction [37]. Protein oxidation biomarkers, such as protein carbonyls, and DNA oxidation products like 8-hydroxy-2'-deoxyguanosine (8-OHdG), reflect cumulative damage, providing insights into genotoxic stress and its downstream effects on hair cells and spiral ganglion neurons [13]. The single-cell electrophoresis (Comet Assay) is also a critical genotoxic evaluation method, enabling the detection and quantification of DNA

damage. Notably, it can be applied to the widely used House Ear Institute–Organ of Corti 1 (HEI-OC1) cell line, which requires specialized incubation conditions of 33°C and 10% CO_2 [38].

The cochlear antioxidant system interacts with oxidative toxicants, initiating a cascade of oxidative processes. Activated toxicants generate superoxide anions (O_2^-) within cells, which are subsequently converted into hydrogen peroxide (H_2O_2) and oxygen (O_2) by the enzyme superoxide dismutase (SOD). Hydrogen peroxide is then further broken down into water (H_2O) by the action of catalase (CAT). Activated toxicant readily interacts with the antioxidant reduced glutathione (GSH), oxidizing it to form oxidized glutathione (GSSG). Glutathione peroxidase (GPx) facilitates the conversion of GSH to GSSG and plays a crucial role in reducing H_2O_2 to water. Glutathione reductase (GR) regenerates GSH from GSSG while simultaneously oxidizing reduced nicotinamide adenine dinucleotide phosphate (NADPH) to $NADP^+$. Diagram of this cycles are shown in Figure 5.

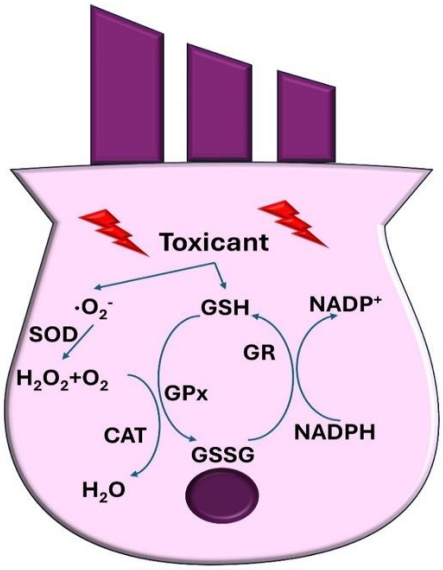

Figure 5. Interaction of the Cochlear Antioxidant System with Oxidative Toxicants

Antioxidant defense biomarkers, including SOD, GSH, CAT, GR and GPx, reflect the cochlea's intrinsic capacity to counteract oxidative insults [33]. Reduced activity of these antioxidants signals increased vulnerability to ototoxic damage [2, 7].

6.2. Inflammatory Biomarkers

Inflammatory pathways exacerbate oxidative stress and directly contribute to ototoxicity by promoting sensory hair cell apoptosis, strial vascularis dysfunction, and neuronal injury. Monitoring inflammatory biomarkers provides insights into cochlear pathology and therapeutic targets. Pro-inflammatory cytokines, including TNF-α and interleukins (IL-1β, IL-6, and IL-8), play a vital role in the pathogenesis of neuroinflammation [39]. TNF-α activates apoptotic pathways in cochlear cells, while IL-6 mediates microvascular dysfunction and promotes hair cell death [26, 40]. IL-8 exacerbates tissue injury by facilitating neutrophil recruitment into the cochlea [41]. Nuclear factor-kappa B (NF-κB), a master regulator of inflammation, drives the expression of adhesion molecules like intercellular adhesion molecule-1 (ICAM-1), which facilitates leukocyte infiltration [42].

6.3. Apoptosis-Related Biomarkers

Apoptosis, or programmed cell death, is a hallmark of ototoxicity, leading to irreversible loss of sensory hair cells. Caspase activation, particularly caspase-3 and caspase-9, is a key event in apoptosis. These proteases degrade structural proteins and essential cellular components, dismantling hair cells and spiral ganglion neurons. Mitochondrial dysfunction, marked by cytochrome c release and changes in the Bcl-2/Bax ratio, initiates intrinsic apoptotic pathways [3, 4]. Annexin V, which binds phosphatidylserine during early apoptosis, and DNA fragmentation assays like TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labelling), provide tools for detecting and quantifying apoptotic events in cochlear tissues [40, 43].

In addition to apoptosis, ferroptosis has emerged as a key contributor to ototoxicity. This iron-dependent programmed cell death pathway is characterized by the depletion of GPx4, a central enzyme that

prevents lipid peroxidation, and the disruption of key regulators such as system xc- (SLC7A11), responsible for glutathione synthesis. Dysregulated iron homeostasis, involving proteins transferrin, ferroportin, and ferritin, leads to an accumulation of reactive ferrous ions (Fe^{2+}), which catalyze lipid peroxidation via the Fenton reaction [44, 45]. This process damages mitochondrial and cellular membranes. Key markers of ferroptosis include ACSL4 and LPCAT3 activation, along with the accumulation of oxidized phospholipids. These changes destabilize cochlear outer hair cells and spiral ganglion neurons, causing mitochondrial dysfunction and cell death. Ferroptosis also interfaces with other pathways, such as p53-mediated suppression of SLC7A11, while FSP1 provides a GPx4-independent protective mechanism [44]. This cascade is triggered by ototoxic agents like cisplatin and aminoglycosides, highlighting ferroptosis as a target for mitigating hearing loss.

6.4. Metabolic and Synaptic Biomarkers

Metabolic disruptions are central to ototoxicity, with biomarkers such as ATP levels and the NAD+/NADH ratio providing insights into mitochondrial dysfunction [46]. Depletion of ATP compromises energy-dependent processes in hair cells, impairing electromotility and contributing to hearing loss [47].

OHC-specific proteins like prestin and RIBEYE are essential for cochlear function, and their altered expression indicates early dysfunction [26, 48, 49]. Synaptic biomarkers, including glutamate and synaptophysin, reveal excitotoxicity and synaptic damage, advancing our understanding of ototoxic injury. Stress proteins such as heat shock proteins mitigate protein misfolding during cellular stress. Elevated levels of these chaperones indicate ongoing injury and cellular attempts at protection [50].

7. PREVENTION AND MANAGEMENT

Reducing auditory damage caused by ototoxic drugs requires a multifaceted approach that begins with optimizing drug dosage. Tailoring doses to patient-specific factors, including age, weight, and renal function, is critical for minimizing risk. Therapeutic drug monitoring (TDM) ensures drug concentrations remain within safe ranges, especially for drugs with narrow therapeutic windows like aminoglycosides and cisplatin. Studies have shown that cumulative cisplatin doses increase the risk of high-frequency hearing loss. Additionally, slower infusion rates can reduce cochlear toxicity, while dose modifications for patients with renal impairment prevent excessive drug accumulation and prolonged clearance. When dose optimization alone proves insufficient, substituting ototoxic drugs with safer alternatives can further reduce risks.

For high-risk populations, safer substitutes such as third-generation cephalosporins may replace aminoglycosides, while carboplatin offers reduced cochlear toxicity compared to cisplatin in certain cancers. However, carboplatin's lower efficacy in specific malignancies necessitates careful evaluation. Advances in drug delivery systems, including liposomal formulations and intratympanic administration, provide innovative solutions by limiting systemic exposure while maintaining therapeutic efficacy. Preclinical studies have shown liposomal cisplatin formulations to minimize auditory damage without compromising anticancer effectiveness.

In addition to preventive strategies, otoprotective agents address the molecular mechanisms underlying ototoxicity. Antioxidants like N-acetylcysteine and D-methionine strengthen the cochlea's defense against ROS, while iron chelators mitigate oxidative damage by inhibiting Fenton reactions [3, 51]. Calcium channel blockers stabilize intracellular calcium levels, reducing excitotoxic damage to cochlear hair cells. Corticosteroids like dexamethasone remain a cornerstone therapy due to their dual anti-inflammatory and antioxidant effects [52, 53]. These agents modulate the NF-κB pathway, suppressing the production of pro-inflammatory cytokines and interleukins, which exacerbate oxidative stress and apoptotic processes in cochlear cells [43]. Corticosteroids also stabilize the blood-labyrinth barrier (BLB), reducing vascular permeability and preventing the infiltration of harmful inflammatory mediators into cochlear tissues. Furthermore, they enhance the activity of endogenous antioxidant enzymes like SOD, counteracting the accumulation of ROS and mitigating mitochondrial damage. By targeting both inflammation and oxidative stress, corticosteroids preserve the structural and functional integrity of the cochlea, offering critical protection against progressive auditory damage [53, 54]. Intratympanic delivery of corticosteroids achieves high cochlear concentrations with minimal systemic effects, while sustained-release formulations, such as hydrogels, optimize therapeutic outcomes by prolonging drug action and reducing administration frequency [54-56].

Emerging therapies, including caspase inhibitors and neurotrophic factors, offer additional avenues for protecting and regenerating cochlear hair cells. Gene therapy, utilizing advanced techniques like CRISPR-Cas9, represents a promising frontier by targeting genetic predispositions associated with ototoxic susceptibility.

For cases of irreversible auditory damage, supportive measures focus on improving quality of life. Cochlear implants bypass damaged hair cells to restore auditory function in individuals with profound hearing loss. Advances in implant technology, such as multi-electrode arrays and refined signal processing algorithms, have significantly improved outcomes [57]. For milder impairments, modern hearing aids equipped with adaptive noise reduction and Bluetooth connectivity enhance communication. Tinnitus, a common consequence of ototoxicity, can be managed effectively through sound therapy, cognitive-behavioral interventions, and pharmacological treatments to alleviate its psychological and physical burden.

8. IN VITRO AND IN VIVO STUDIES ABOUT PHARMACEUTICAL INDUCED OTOTOXICITY

Gentamicin-induced ototoxicity, largely attributed to oxidative stress, can be mitigated through targeted therapeutic strategies. A study demonstrated the efficacy of 2,3-dihydroxybenzoate, an iron chelator, in preventing the formation of the toxic gentamicin-iron complex responsible for hydroxyl radical production [51]. When combined with mannitol, a hydroxyl radical scavenger, this approach provided significant protection against hearing loss and cochlear damage in guinea pigs treated with gentamicin (120 mg/kg/day for 19 days). Co-treatment with 2,3-dihydroxybenzoate (30–300 mg/kg/day) reduced auditory threshold shifts, while its combination with mannitol (100 mg/kg/day and 15 mg/kg/day, respectively) completely preserved hearing thresholds and cochlear hair cell integrity as shown in Figure 6. Importantly, these interventions did not compromise gentamicin's antimicrobial activity, underscoring the potential of oxidative stress-targeted therapies for safer aminoglycoside use in clinical settings.

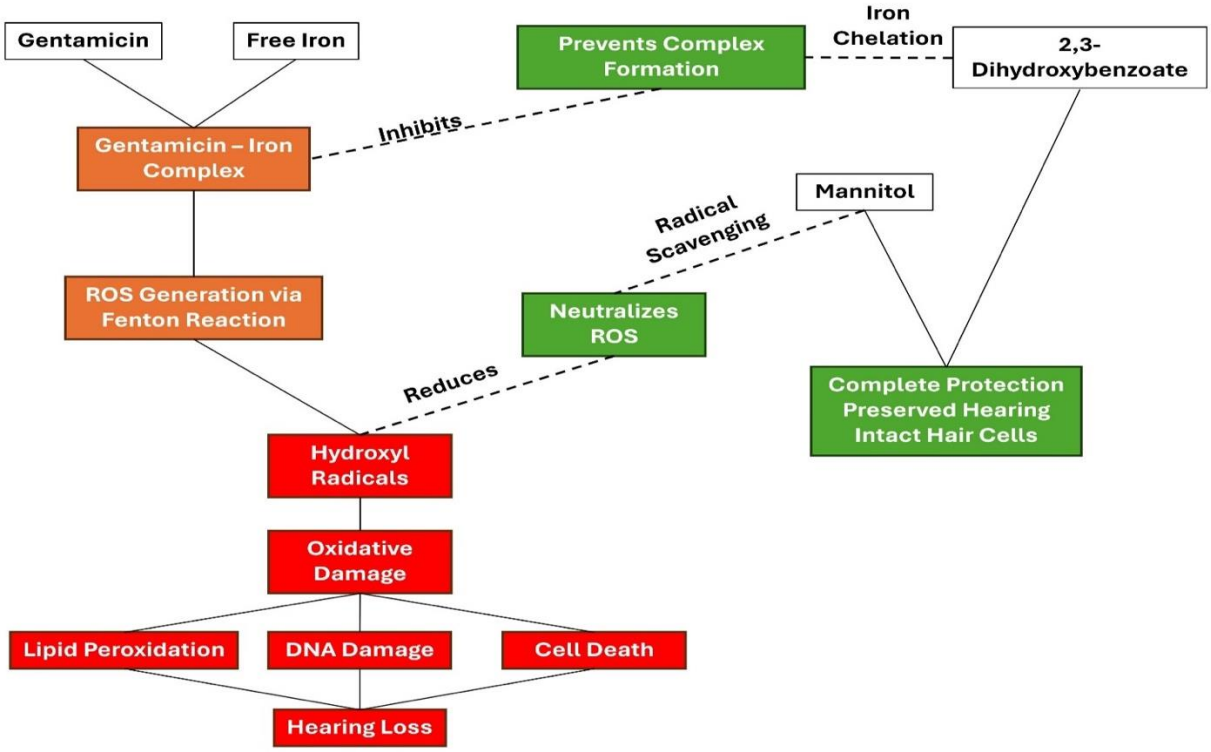

Figure 6. Mechanism and Experimental Outcomes of Gentamicin-Induced Ototoxicity Prevention through Iron Chelation and Hydroxyl Radical Scavenging

The potential protective effects of intratympanic memantine, an NMDA receptor antagonist, against cisplatin-induced ototoxicity were evaluated in guinea pigs. Animals were divided into groups receiving cisplatin alone, memantine alone, cisplatin with memantine, cisplatin with physiological serum (PS), or no treatment (control). Cisplatin (12 mg/kg) was administered intraperitoneally, with intratympanic memantine (0.2 mL) or PS delivered 30 minutes prior. Electrophysiological assessments, including DPOAE and ABR, showed significant hearing threshold shifts and reduced DPOAE signal-to-noise ratios across all

cisplatin-treated groups, with no notable auditory protection from memantine. However, histopathological analyses demonstrated protective effects in the cisplatin + memantine group, including fewer hair cell losses, less spiral ganglion degeneration, and better-preserved stria vascularis thickness compared to cisplatin-only groups. While the functional auditory outcomes were inconclusive, these structural findings suggest that memantine may reduce cochlear damage caused by cisplatin. Further studies are required to validate these results and explore the underlying mechanisms of protection [34].

Another study evaluated the protective effects of ascorbic acid against cisplatin-induced ototoxicity using electrophysiological tests (TEOAE and ABR) and ultrastructural analyses (light and electron microscopy) in guinea pigs [15]. Thirty ears from 15 guinea pigs with normal auditory thresholds were divided into three groups: control (Group I), cisplatin-only (Group II), and cisplatin + ascorbic acid (Group III). Groups II and III received a single intraperitoneal dose of cisplatin (1.5 mg/kg/day), with Group III also receiving ascorbic acid (300 mg/day) for 8 days. Electrophysiological evaluations revealed significant deteriorations in otoacoustic emissions (reproducibility and response values) and ABR thresholds in the cisplatin-only group (Group II) after treatment ($p < 0.05$). In the cisplatin + ascorbic acid group (Group III), reductions in reproducibility and response values were observed but were less pronounced compared to Group II, though not statistically significant ($p > 0.05$). Ultrastructural analyses showed clear dystrophic changes and hyperactive organelles in cochlear cells of the cisplatin-only group, while no significant dystrophic changes were observed in the ascorbic acid-treated group. These findings suggest that ascorbic acid may provide protective effects against cisplatin-induced cochlear damage, as evidenced by less severe electrophysiological and structural deterioration.

Another study highlights the protective role of allicin, another antioxidant compound, against cisplatin-induced damage in the stria vascularis of the cochlea. Allicin mitigates apoptosis by reducing the expression of cleaved caspase-3, PARP-1, and AIF nuclear translocation, effectively preventing cochlear damage through modulation of apoptotic pathways [58].

Bulut et al. examined the effects of salicylate-induced DNA methylation on the prestin gene during acute and chronic phases of ototoxicity in guinea pigs [13]. Fifteen animals were divided into three groups: control, saline-treated, and salicylate-treated. Salicylate (200 mg/kg) was administered intramuscularly twice daily for two weeks, with cochlear function assessed using DPOAEs and prestin gene methylation analyzed via methylation-specific PCR (MSP). The results showed significant reductions in DPOAE signal-to-noise ratios (S/N-R) during the acute phase (0–8 hours) of salicylate administration, correlating with increased prestin gene methylation. During the chronic phase (1–2 weeks), DPOAE S/N-R returned to baseline, and prestin gene methylation was reduced. These findings suggest that salicylate-induced changes in prestin gene methylation influence cochlear function and may contribute to the reversible nature of salicylate ototoxicity.

Chen et al. explored the protective effects of a dexamethasone-loaded silk-polyethylene glycol hydrogel against cisplatin-induced ototoxicity. This study employed both in vitro cochlear organ cultures and in vivo models to assess the cytoprotective effects of the hydrogel formulation. The findings revealed that the DEX-SILK hydrogel significantly reduced intracellular ROS production, which is a key contributor to cisplatin-induced cellular damage. In vitro results showed that the hydrogel provided partial protection against cisplatin-induced cytotoxicity at various concentrations, while in vivo assessments indicated improved auditory thresholds in treated animals compared to untreated controls. This study underscores the potential of biocompatible hydrogels as a delivery system for otoprotective agents, paving the way for future clinical applications in preventing hearing loss associated with chemotherapy [54].

Another study conducted by Wang et al. investigated the use of A666-conjugated nanoparticles as a targeted drug delivery system to prevent cisplatin-induced ototoxicity [55]. A666 is a peptide specifically designed to bind to prestin, a motor protein uniquely expressed in the OHCs of the cochlea. By targeting prestin, these nanoparticles deliver therapeutic agents directly to OHCs, enhancing efficacy and minimizing off-target effects. In vitro experiments using HEI-OC1 cell cultures showed that A666-conjugated nanoparticles loaded with dexamethasone (A666-DEX-NP) exhibited enhanced cellular uptake compared to non-targeted nanoparticles. Cells treated with A666-DEX-NP displayed reduced apoptosis, lower ROS levels, and improved viability following cisplatin exposure. In vivo experiments in a guinea pig model demonstrated significant hearing preservation in animals treated with A666-DEX-NP. Intratympanic administration through the round window membrane effectively protected hearing thresholds at 4, 8, and 16 kHz after cisplatin treatment. Histological analysis confirmed better preservation of cochlear structures in animals receiving A666-DEX-NP compared to controls. This study underscores the potential of A666-

conjugated nanoparticles as a novel therapeutic strategy for cisplatin-induced hearing loss. By delivering drugs specifically to OHCs, this system mitigates ototoxicity while reducing systemic exposure. These findings suggest that nanoparticle-based targeted delivery may also offer promising clinical applications in protecting auditory function during chemotherapy.

Kim et al. investigated the protective effects of cyclic adenosine monophosphate (cAMP) signaling in preventing cisplatin-induced ototoxicity. The study utilized both in vitro and in vivo models to demonstrate that cAMP-mediated intercellular communication through gap junctions could inhibit the apoptotic pathways activated by cisplatin exposure. The researchers found that treatment with rolipram, a phosphodiesterase-4 inhibitor that increases intracellular cAMP levels, significantly improved the survival of spiral ganglion neurons and reduced hearing loss in a cisplatin-treated rat model. The results suggest that enhancing cAMP signaling may represent a viable therapeutic strategy to protect against cisplatin-induced auditory damage, highlighting the potential for pharmacological interventions targeting intracellular signaling pathways [59].

In the study by Guan et al., the authors investigated the role of Apoptosis Repressor with a CED-4 Homology (ARC) in protecting auditory hair cells from neomycin-induced damage. Using the HEI-OC-1 cell line, which serves as a model for cochlear hair cells, the researchers demonstrated that ARC expression was significantly decreased following neomycin exposure. The study employed various assays to measure cell viability, apoptosis, and ROS levels. The results indicated that the inhibition of ARC led to increased apoptosis and decreased survival of HEI-OC-1 cells after neomycin treatment, suggesting that ARC plays a crucial role in protecting hair cells from aminoglycoside-induced ototoxicity. The findings highlight the potential for targeting ARC as a therapeutic strategy to enhance hair cell survival in the context of aminoglycoside treatment [60].

A study investigated the role of ACSL4-catalyzed lipid peroxidation in cisplatin-induced ototoxicity using in vitro and in vivo models, including HEI-OC1 cells, mouse cochlear explants, and zebrafish. The study assessed lipid peroxidation markers (MDA and 4-HNE) and the protective effects of ferroptosis inhibitors, ferrostatin-1 (FER-1) and vitamin E (Vit-E). Cisplatin exposure increased lipid peroxidation and decreased cell viability. FER-1 and Vit-E partially protected hair cells, reducing lipid peroxidation levels and improving survival. Further, metabolomic analysis identified significant changes in the arachidonic acid metabolic pathway, with ACSL4 being a key enzyme. Inhibiting ACSL4 with rosiglitazone reduced lipid peroxidation and improved hair cell survival more effectively than FER-1 or Vit-E. This indicates that targeting ACSL4 may provide novel protective strategies against cisplatin-induced ototoxicity by addressing lipid peroxidation at its source [37].

A novel study highlights ferroptosis, a form of regulated cell death driven by iron-dependent lipid peroxidation, as a contributor to cisplatin-induced ototoxicity. Cisplatin-treated mice exhibited upregulated TfR1, a ferroptosis biomarker, in outer hair cells, and RNA sequencing of cochlear explants revealed altered expression of key ferroptosis regulators. Using Gpx4 and Fsp1 knockout mouse models, severe outer hair cell loss and synaptic damage in inner hair cells were observed in Atoh1-Gpx4−/− mice, while Fsp1−/− mice showed no significant hearing deficits. These findings underscore the critical role of Gpx4 in maintaining cochlear function. Furthermore, luteolin, an FDA-approved compound, inhibited ferroptosis by reducing transferrin expression and intracellular ferrous ion concentrations, alleviating cisplatin-induced ototoxicity. This study suggests that targeting ferroptosis through modulation of intracellular iron levels could be a promising therapeutic strategy for preventing cisplatin-induced hearing loss [45].

9. CONCLUSION

Pharmaceutical-induced ototoxicity, driven by oxidative stress, mitochondrial dysfunction, and inflammation, continues to pose challenges in health quality. Recent research has identified key signaling pathways and molecular mechanisms such as the activation of apoptotic cascades and disruption of ion homeostasis that contribute to ototoxicity. However, novel approaches, such as conjugated nanoparticles and drug-loaded hydrogels, offer targeted solutions to preserve auditory function. These advancements, alongside antioxidant strategies and modulators of molecular pathways, represent significant progress toward mitigating ototoxicity's impact on undergoing essential pharmacological treatments.

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