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Review Article

Hearing loss during chemotherapy: prevalence, mechanisms, and protection

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Abstract: Ototoxicity is an often-underestimated sequela for cancer patients undergoing chemotherapy, with an incidence rate exceeding 50%, affecting approximately 4 million individuals worldwide each year. Despite the nearly 2,000 publications on chemotherapy-related ototoxicity in the past decade, the understanding of its prevalence, mechanisms, and preventative or therapeutic measures remains ambiguous and subject to debate. To date, only one drug, sodium thiosulfate, has gained FDA approval for treating ototoxicity in chemotherapy. However, its utilization is restricted. This review aims to offer clinicians and researchers a comprehensive perspective by thoroughly and carefully reviewing available data and current evidence. Chemotherapy-induced ototoxicity is characterized by four primary symptoms: hearing loss, tinnitus, vertigo, and dizziness, originating from both auditory and vestibular systems. Hearing loss is the predominant symptom. Amongst over 700 chemotherapeutic agents documented in various databases, only seven are reported to induce hearing loss. While the molecular mechanisms of the hearing loss caused by the two platinum-based drugs are extensively explored, the pathways behind the action of the other five drugs are primarily speculative, rooted in their therapeutic properties and side effects. Cisplatin attracts the majority of attention among these drugs, encompassing around two-thirds of the literature regarding ototoxicity in chemotherapy. Cisplatin ototoxicity chiefly manifests through the loss of outer hair cells, possibly resulting from damages directly by cisplatin uptake or secondary effects on the stria vascularis. Both direct and indirect influences contribute to cisplatin ototoxicity, while it is still debated which path is dominant or where the primary target of cisplatin is located. Candidates for hearing protection against cisplatin ototoxicity are also discussed, with novel strategies and methods showing promise on the horizon.

Keywords: Inner ear, cochlea, cisplatin, ototoxicity, hearing protection

Introduction

Antineoplastic or chemotherapeutic drugs for cancer treatment can also harm normal tissues and cells, leading to various adverse effects [1]. Ototoxicity is a notable sequala of cancer treatment, impacting hearing and balance with symptoms including hearing loss, vertigo, dizziness, and tinnitus [2-5]. The severity of these symptoms varies and is influenced by factors including the age of the patient, the specific chemotherapeutic agent used, dosage, and administration method [1, 4, 5]. Although ototoxicity in chemotherapy is not life-threatening, its consequences, such as communication difficulties, social isolation, depression, and fatigue, can significantly impair the quality of life [6, 7]. Furthermore, hearing loss has been suggested as a significant modifiable risk factor for dementia [8]. In pediatric patients, hearing loss is even more devastating as it can delay the development of speech and language abilities, communication skills, and impede cognitive maturation [9].

To determine the ototoxic potential and mechanisms of chemotherapeutic drugs and shed light on emerging treatment compounds and approaches, we performed a comprehensive review of literature and databases on cancer

Table 1. Ototoxic chemotherapeutic drugs and their incidence of symptoms

Drug name		Cisplatin	Carboplatin	Vinblastine	Vincristine	Dasatinib	isotretinoin	Tretinoin
Drug Class		Alkylating agent		Plant alkaloid	Vinca alkaloid	Tyrosine kinase inhibitor	Retinoid	Retinoid
Antineoplastic Mechanism		DNA binding and cross-linking Protein cross-linking		microtubule inhibitor		Src kinase inhibitor	microtubule inhibitor	
Ototoxic Mechanism		ROS overload, proinflammatory cytokine production		Possible synergy with other drugs	Unclear. Possibly associated with their antitumor activity			
Incidence rate	Hearing loss	31%	12%	10-29%	No data	0.1-1%	No data	6%
	Tinnitus	31%	12%	No data	No data	1-10%	No data	No data
	Dizziness	No data	No data	Rare	No data	1-10%	No data	20%
	Vertigo	No data	No data	Rare	No data	0.1-1%	No data	No data

treatment drugs. A PubMed search revealed nearly 2,000 publications on chemotherapy-associated ototoxicity in the last decade. Among these publications, two-thirds are related to platinum-based agents used in 10-20% of the chemotherapy regimens. However, the mechanisms underlying the ototoxicity of chemotherapeutic drugs remain unclear, and effective strategies to mitigate this side effect are still in development. This review discusses the tentative mechanisms for ototoxicity and advances in hearing protection during cancer treatment, highlighting promising drugs and methodologies recently developed. Our objective is to provide clinicians who treat cancer with a foundational understanding of ototoxicity in chemotherapy. We also offer researchers insight into in-depth mechanisms and potential innovative protective strategies against it. Currently, sodium thiosulfate (STS) is the only FDA-approved drug to mitigate hearing loss during cancer treatment in pediatric patients [10].

Chemotherapeutic drugs affecting the inner ear

To identify compounds/drugs with potentially damaging effects on the inner ear during cancer therapy, we searched the online databases from the websites of the National Cancer Institute (NCI) [2], Chemocare [3], and Beaumont [4] using the search terms hearing loss, tinnitus, dizziness, and vertigo. Out of the approximately 700 compounds used in cancer treatment, only seven have listed hearing loss as a side effect (**Table 1**) [2-4].

Ninety-seven drugs reported dizziness as a side effect but not hearing loss or tinnitus (**Table 2**). While dizziness may originate from the inner ear, it can also have other origins, such as the central nervous system. Since the mechanisms underlying dizziness are multifactorial, and our primary focus is on hearing loss, drugs causing only dizziness but not any other symptoms of ototoxicity are not further explored in this review. Some other drugs, such as doxorubicin (showing ototoxic effects in animal studies [11], but clinical evidence is missing) and nitrogen mustard (strongly restricted use as a chemical weapon), are also excluded.

Platinum-based drugs. The two platinum-based alkylating agents, cisplatin (also referred to as cis-diamminedichloroplatinum (II), CDDP, and platinol) and carboplatin (paraplatin), are the most commonly reported compounds that cause hearing loss. According to the National Cancer Institute (NCI), cisplatin or similar platinum-based drugs are used in 10-20% of cancer chemotherapy regimens [12]. The most common indications include testicular, ovarian, cervical, bladder, and head and neck cancers. Multiple well-documented significant sequelae are nausea, vomiting, liver damage, kidney failure, hearing loss, tinnitus, and vertigo [13-17]. The prevalence of hearing loss during chemotherapy with cisplatin can be as high as 60-80% [14, 18]. Based on the NCI estimated cancer patients of 2018, cisplatin may cause approximately 100-300 thousand new cases of ototoxicity annually in the United States. Oxaliplatin, a third-generation platinum-based chemotherapy drug, has not been considered ototoxic based on a clinical trial with 18 patients [19]. However, case reports showed that the drug might cause hearing loss [20-23].

Vinblastine (velban, alkaban-AQ) and Vincristine are plant alkaloids listed as ototoxic drugs,

Table 2. Chemotherapeutic drugs causing only dizziness but not other symptoms of ototoxicity

Dizziness (incidence rate > 30%)		
Generic/Other Name	*Brand Name*	*Mechanism of Action*
Axicabtagene Ciloleucel	Yescarta	chimeric antigen receptor T-cell immunotherapy agent, binds CD19 B-lymphocyte antigen
Entrectinib	Rozlytrek	protein-tyrosine kinase inhibitor, inhibits tropomyosin receptor tyrosine kinases - inhibits high affinity nerve growth factor receptor, BDNF/NT-3 growth factor receptors, NT-3 growth factor receptor, proto-oncogene tyrosine-protein kinase ROS, tyrosine-protein kinase JAK2
Tisagenlecleucel	Kymriah	chimeric antigen receptor T-cell immunotherapy agent, binds CD19-expressing cells and promotes T-cell expansion, activation, target cell elimination
Larotrectinib	Vitrakvi	tropomyosin receptor kinase (TRK) inhibitor, inhibits TRKA, TRKB, TRKC preventing neurotrophin-Trk interaction and Trk activation inducing apoptosis and inhibition of cell growth
Dizziness (incidence rate 10-29%)		
Generic/Other Name	*Brand Name*	*Mechanism of Action*
13-cis-Retinoic Acid	Accutane, Isotretinoin	retinoid, acts on nuclear receptors RAR or RXR
5-Azacitidine, Azacitidine	Vidaza, Onureg	antimetabolite and demethylating agent
Abemaciclib	Verzenio	cyclin-dependent kinase inhibitor (CDK4 and CDK6), arrests G1 to S phase
Brentuximab vedotin	Adcetris	CD30-direct antibody drug conjugate (monoclonal antibody that disrupts microtubules)
Ado-Trastuzumab Emtansin	Kadcyla	Anti-HER2 monoclonal antibody combined with microtubule inhibitor DM1 (maytansine derivative)
Anagrelide	Agrylin	phospholipase A2 inhibitor (prevents maturation of megakaryocytes)
Hydrocortisone, Hydrocortone Phosphate, Ala-Cort, Cortisone, Hydrocortisone Sodium Succinate, Hydrocortisone Sodium Phosphate	Solu-Cortef, Hydrocort Acetate, Lanacort	glucocorticosteroid
Aldesleukin, Interleukin-2, IL-2	Proleukin	cytokine, increases production of T lymphocytes and NK cells and improves function of lymphokine-activated killer cells and tumor-infiltrating lymphocytes
Alemtuzumab	Campath	CD52 monoclonal antibody
All-Trans Retinoic Acid, Tretinoin	Vesanoid	Retinoid, acts on nuclear receptors RAR or RXR
Interferon Alfa, alpha interferon, IFN-alpha	Intron A, Roferon-A	cytokine and biologic response modifier
Altretamine, Hexamethylmelamine, HMM	Hexalen	alkylating agent, hydrazine and triazine
Amifostine	Ethyol	chemoprotective agent, deactivates harmful components of chemotherapy drugs; scavenger, binds free radicals produced by cisplatin or radiation therapy
Aminoglutethimide	Cytadren	adrenal cortex corticosteroid production inhibitor, decreases production of estrogens and androgens
Nilutamide	Nilandron, Anandron	antiandrogen, blocks androgen/testosterone receptors
Apalutamide	Erleada	antiandrogen, blocks androgen/testosterone receptors
Arabinosylcytosine, Cytarabine, Ara-C	Cytosar-U	antimetabolite, inhibits DNA polymerase beta; cross-linking/alkylation of DNA, blocks G1/S
Nelarabine	Arranon	antimetabolite, adenosine deaminase inhibitor, incorporates into and destabilizes DNA, inhibits DNA polymerase alpha catalytic subunit; S phase-specific arrest
Arsenic Trioxide	Trisenox	not well understood, DNA fragmentation in leukemia cells; also damages or degrades the fusion protein PML-RAR
Avapritinib	Ayvakit	tyrosine kinase inhibitor, small molecule inhibitor of platelet-derived growth factor receptor alpha (PDGFR-A), targets PDGFRA and PDGFRA D842 mutants as well as multiple KIT mutations

Bevacizumab	Avastin, Mvasi, Zirabrev	targets and inhibits VEGF preventing angiogenesis
Belinostat	Beleodaq	histone deacetylase inhibitor
Carmustine, BCNU	BiCNU, Gliadel wafer	alkylating agent, nitrosurea, cross-links DNA and RNA
Binimetinib	Mektovi	oral MEK inhibitor, targets MEK1 and MEK2 protein kinase; usually given with a BRAF kinase inhibitor
Blinatumomab	Blincyto	bispecific T-cell engager monoclonal antibody, induces T-cells to bind CD19 on surface of B-cell leukemia or lymphoma cells
Bortezomib	Velcade	proteasome inhibitor, inhibits 26S proteasome, inhibits proteasome subunit beta type-5 and type-1; cell arrest in G2-M phase; in multiple myeloma, works by blocking adhesion molecule activation
Bosutinib	Bosulif	kinase inhibitor, targets ABL and SRC kinases
Encorafenib	Braftovi	BRAF kinase inhibitor
Busulfan	Busulfex, Myleran	alkylating agent, alkylsulfonate
Cabozantinib	Cometriq, Cabometyx	oral receptor tyrosine kinase inhibitor (RET, MET, VEGF), blocks cell division pathways
Carfilzomib	Kyprolis	tetrapeptide epoxyketone proteasome inhibitor, irreversibly binds to N-terminal threonine-containing active sites of 20S proteasome
Crizotinib	Xalkori capsules	oral receptor tyrosine kinase inhibitor, inhibits ALK, hepatocyte growth factor receptor (HGFR, c-Met) and receptor d'origine natais (RON); blocks cell division pathway
Decitabine	Dacogen	antimetabolite and demethylating agent, restores function of tumor suppressor genes and cytotoxic effect on rapidly dividing cells
Daratumumab and Hyaluronidase	Darzalex Faspro	CD38 monoclonal antibody (present on myeloma cells), IgG1k human monoclonal antibody binds CD38 and induces apoptosis through mediated cross linking and immune mediated tumor cell lysis through complement dependent cytotoxicity, antibody mediated cytotoxicity, and antibody dependent cellular phagocytosis; hyaluronidase helps with absorption into blood
Daunorubicin and Cytarabine (Liposomal)	Vyxeos	Daunorubicin is an anthracycline, intercalates between DNA base pairs inhibiting DNA synthesis and DNA-dependent RNA synthesis; Cytarabine is an antimetabolite (S phase); liposome helps with drug distribution and lengthens time of effect of the drug allowing for extended treatment effect
Glasdegib	Daurismo	hedgehog pathway inhibitor, inhibits increases in tumor size and decrease the amount of CD45+/ CD33+ cells in the bone marrow; binds and inhibits Smoothened (SMO) receptor
Dexamethasone, Dexamethasone Sodium Phosphate, Dexamethasone Acetate	Decadron, Dexasone, Diodex, Hexadrol, Maxidex	glucocorticosteroid
Prednisolone	Delta-Cortef, Orapred, Pediapred, Prelone	glucocorticosteroid
Prednisone	Deltasone, Liquid Pred, Meticorten, Orasone	glucocorticosteroid
Denileukin Diftitox	Ontak	biologic response modifier agent, a fusion protein (combination of diphtheria toxin and IL-2), selectively delivers the cell-killing activity of diphtheria toxin to targeted cells; binds to lymphoma cells that express high affinity IL-2 receptor (IL-2 part of fusion protein binds cell surface) and halts protein synthesis
Dexrazoxane	Zinecard	chemoprotectant agent, binds free radicals formed by doxorubicin; extravasation antidote, binds chemotherapy drug that leaked from vein preventing damage to surrounding tissue

Methylprednisolone	Duralone, Medrol, Medralone, M-Prednisol, Solu-Medrol	glucocorticosteroid
Eculizumab	Soliris	monoclonal antibody, binds C5 complement protein preventing formation of MAC, prevents hemolysis and stabilizes hemoglobin
Eltrombopag	Promacta	colony stimulating factor, thrombopoietic agent, growth factor that stimulates platelet production by binding to and activating the thrombopoietin (TPO) receptor; a thrombopoietin nonpeptide agonist
Tagraxofusp-erzs	Elzonris	biologic response modulator and cytokine, combination of recombinant human IL-3 and truncated diphtheria toxin, binds CD123 (alpha chain of IL-3 receptor) and delivers diphtheria toxin to cells, blocks protein synthesis; binds ADP-ribosylation factor-like protein 2
Fam-trastuzumab deruxtecan-nxki	Enhertu	anti-HER2 monoclonal antibody (anti-HER2 IgG1) combined with a topoisomerase I inhibitor; antibody attached to chemotherapy, allows selective delivery into HER2 overexpressing cells, DNA damage
Enzalutamide	Xtandi	antiandrogen (second generation), blocks androgen/testosterone receptors
Toremifene	Fareston	anti-estrogen, estrogen receptor antagonist, blocks estrogen binding and uptake into cells
Gilteritinib	Xospata	protein-tyrosine kinase inhibitor, inhibits FLT3 receptor, serotonin receptors, TPKR UFO, and ALK tyrosine kinase receptor
Trastuzumab	Herceptin (Biosimilars: Herzuma, Kanjinti, Ogivri, Ontruzant)	HER2/neu receptor monoclonal antibody
Ibritumomab, Ibritumomab Tiuxetan	Zevalin	CD20 monoclonal antibody linked with Yttrium-90 (radioactive substance), directly delivers radiation to CD20+ cells
Ibrutinib	Imbruvica	binds to and inhibits the bruton's tyrosine kinase (BTK) signaling molecule of the B-cell receptor signaling complex
Ponatinib	Iclusig	tyrosine kinase inhibitor
IL-11, Oprelvekin, Interleukin-11	Neumega	biologic response modifier and cytokine, stimulates production, maturation and activation of platelets
Talimogene Laherparepvec, T-VEC	Imlygic	genetically modified weakened form of live HSV (oncolytic), replicates within tumors and produces GM-CSF to promote anti-tumor immune response
Interferon Alfa-2b (PEG Conjugate), PEG Interferon	PEG-Intron	biologic response modifier and cytokine, activates human type 1 interferon causing them to dimerize which activates JAK/STAT pathway
Ruxolitinib	Jakafi	oral receptor tyrosine kinase inhibitor, inhibits JAK1 and JAK2
Pembrolizumab	Keytruda	highly selective humanized monoclonal IgG4 antibody directed against the PD-1 receptor on cell surface, prevents binding and activation of PD-L1 and PD-L2 which activates T-cell mediated immune response against tumor cells
Lenalidomide	Revlimid	immunomodulatory agent and antiangiogenic agent, inhibits protein cereblon and TNF ligand super-family member 11, antagonizes cadherin-5, negative modulator of prostaglandin G/H synthase 2
Lenvatinib	Lenvima	oral receptor tyrosine kinase inhibitor, inhibits VEGF, VEGFR, FGF, PDGFR alpha, KIT and RET
Lorlatinib	Lorbrena	reversible tyrosine kinase inhibitor, blocks abnormal ALK protein
Luspatercept	Reblozyl	recombinant fusion protein, hematopoiesis agent (contains modified form of the extracellular domain of human activin receptor), binds and inhibits transforming growth factor beta super family molecules increasing expression of blood cell precursors

Olaparib	Lynparza	poly (ADP-ribose) polymerase (PARP) enzyme inhibitor (PARP1, PARP2, PARP3), induces synthetic lethality in BRCA1/2 deficient tumor cells through formation of double-strand DNA breaks
Procarbazine	Matulane	alkylating agent, hydrazine and triazine
Midostaurin	Rydapt	tyrosine kinase inhibitor, inhibits FLT3 inhibiting leukemic cell production
Niraparib	Zejula	PARP inhibitor, highly selective for PARP1 and PARP2 resulting in DNA damage and apoptosis, induces cytotoxicity in tumor cell lines w/ and w/o BRCA1/2 deficiencies
Romiplostim	Nplate	biologic response modifier, colony stimulating factor, promotes platelet production via the thrombopoietin receptor
Pertuzumab	Perjeta	HER2 monoclonal antibody (binds different area of HER2 protein than trastuzumab)
Pomalidomide	Pomalyst	thalidomide analogue, inhibits protein cereblon, TNF and prostaglandin G/H synthase 2
Sipuleucel-T	Provenge	autologous cellular immunotherapy, selectively targets prostatic acid phosphatase (PAP), a PSA
Rucaparib	Rubraca	PARP inhibitor (PARP1, PARP2, PARP3), increases formation of PARP-DNA complexes; cytotoxicity in BRCA1/2 deficient tumor cell lines and other DNA repair genes
Sacituzumab Govitecan-hziy	Trodelvy	trop-2-directed antibody-drug conjugate combined with a topoisomerase I inhibitor (SN-38) attached by a linker, binds trop-2-expressing cancer cells and is internalized, SN-38 is released in cancer cell by breaking the linker
Sunitinib, SU11248	Sutent	receptor protein-tyrosine kinase inhibitor, inhibits VEGF
Talazoparib	Talzenna	PARP inhibitor (PARP1, PARP2), strong catalytic inhibition and a PARP-trapping potential
Temozolomide	Temodar	alkylating agent, hydrazine and triazine (similar to dacarbazine, acts as a pro-drug)
Vorinostat	Zolinza	not fully understood, histone deacetylase inhibitor, inhibits HDAC2, HDAC2, HDAC3, HDAC6
Zoledronic Acid	Zometa	bisphosphonate, decreases osteoclast actions on bone

albeit with little evidence in the literature. As a microtubule inhibitor, vinblastine-induced hearing loss was observed in two case reports of patients with Hodgkin's lymphoma who underwent combined chemotherapy, including doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) [24, 25]. In one published case, vinblastine was given in a combination treatment with vincristine. It has been discussed that the observed cochlear damage [24] originated from vinblastine. However, it is also possible that the damage might have been caused by vincristine, a commonly used vinca alkaloid similar to vinblastine [26-28].

Dasatinib (Sprycel), a tyrosine kinase inhibitor targeting multiple cancer cells, is also reported to cause hearing loss in case reports but the incidence is rare (0.1-1%). Instead, more frequent side effects of dasatinib are tinnitus and dizziness.

Isotretinoin (cis-retinoic acid) and tretinoin (all-trans retinoic acid) are retinoids that can slow down the growth of cancer cells. Occasional case reports indicate that both can induce hearing loss [29, 30], while detailed studies on isotretinoin's and tretinoin's ototoxicity are still missing. Furthermore, isotretinoin is mainly used to treat severe acne. In two clinic studies with cohorts of acne patients, isotretinoin treatment leads to transient hearing improvement instead [31, 32]. These controversial results suggest their influence on the auditory system, while more studies are needed to determine the mechanisms.

Mechanisms of hearing loss in chemotherapy

The working theory for the mechanism of platinum-based drugs is related to the generation of reactive oxygen species (ROS) and DNA damage. However, their ototoxicity is mainly associated with excessive ROS generation [33, 34], while DNA damage [35, 36] likely plays a minor role. However, the mechanisms of other ototoxic chemotherapeutic drugs listed in **Table 1** remain largely unclear because the studies are rare. Some studies have speculated that it is associated with their antitumor activity [24]. Due to the availability of literature, this review focuses mainly on the ototoxicity of platinum compounds, particularly cisplatin.

Inner ear structures targeted by cisplatin

Structural and functional features of the cochlea: The cochlea comprises three fluid-filled tubes, scala tympani, scala media, and scala vestibuli (**Figure 1**). Scala vestibuli and scala media are separated by Reissner's membrane, while scala media and scala tympani by the basilar membrane and reticular lamina. The organ of Corti is located on the basilar membrane. It contains two types of hair cells: the inner hair cells (IHCs) and outer hair cells (OHCs). IHCs and OHCs have hair bundles, or stereocilia, with ion channels responsible for transforming sound-induced vibrations of soft tissue structures of the inner ear into action potentials. The channels are named mechano-electrical transduction (MET) channels, of which the core domain is recently identified as transmembrane channel-like protein 1 [37]. Opening of MET channels in OHCs results in a depolarizing current enhancing the sound-induced vibrations of soft tissue structures by changing the stiffness and length of the OHCs. In IHCs, the opening of the MET channels releases neurotransmitters and generates action potentials. The MET current is primarily carried by calcium and potassium ions [38]. Please refer to [39-41] for recent reviews on the MET channels.

The driving force for the MET current is the voltage difference between the endocochlear potential (EP) of about 80 mV [42, 43] and the resting potential of the hair cells. Intracochlear ion homeostasis, combined with the selective permeability for different ions across the boundaries of the scalae and active ion transport across stria vascularis, generates the EP. The perilymphatic ion concentration, high in sodium (~150 mM) and low in potassium (~5 mM), is similar to the ion concentration of the extracellular fluids. This differs from the endolymphatic ion concentration, low in sodium (~15 mM) and high in potassium (~140 mM), which is comparable with the ion composition of the cytoplasm [44]. The ion homeostasis of the endolymph is primarily controlled by the stria vascularis (SV), a structure lining the lateral wall of the scala media. The marginal cells in the SV regulate the potassium concentration [45], and the intermediate cells form tight junctions with basal cells to separate the en-

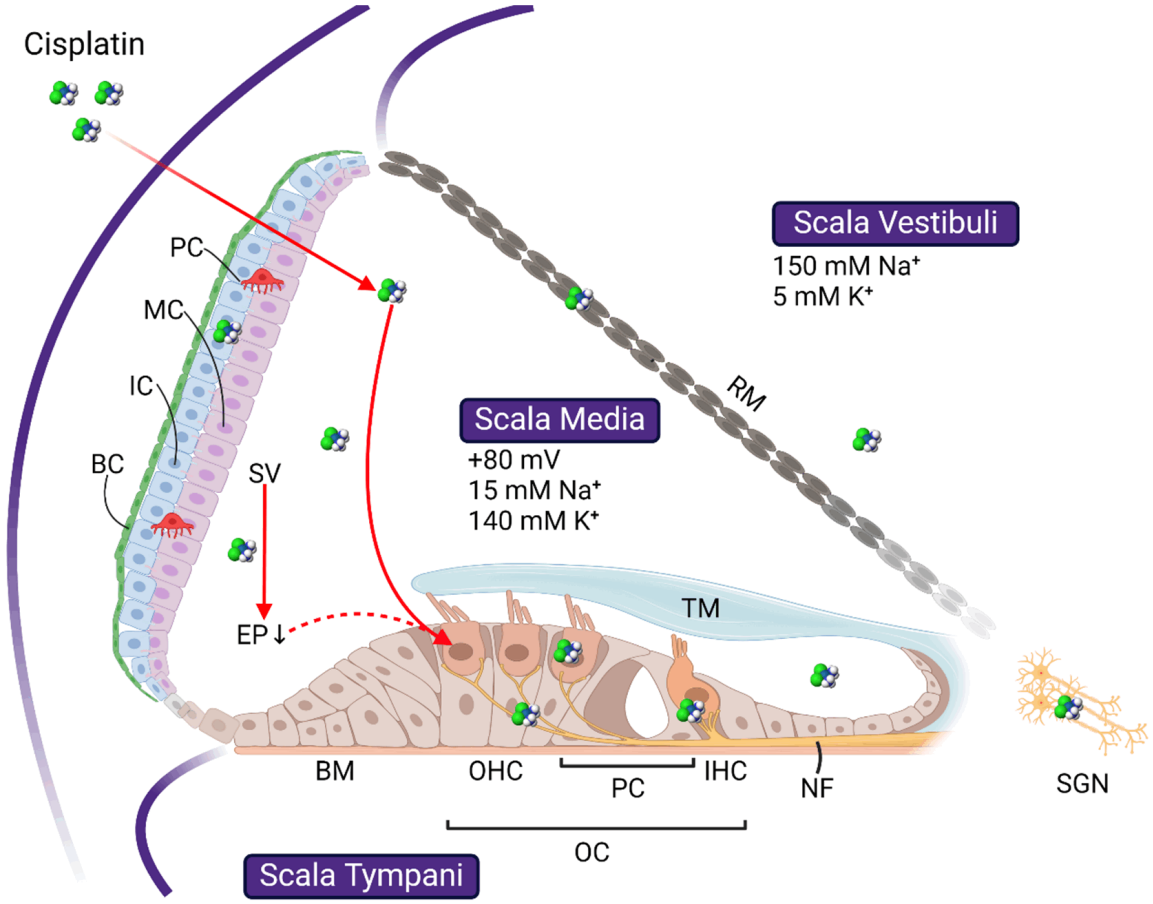

Figure 1. Schematic image showing the basic cochlear structures and the targets of cisplatin ototoxicity. Cochlear duct forms three scalae, scala vestibuli, scala media, and scala tympani, separated by the basilar membrane (BM) and Reissner's membrane (RM), respectively. Cells affected by cisplatin include the outer hair cells (OHC) and inner hair cells (IHC) in the organ of Corti (OC), basal cells (BC), intermedial cells (IC), and marginal cells (MC) in the stria vascularis (SV), and the spiral ganglion neurons (SGN) in the modiolus. Whether OHC loss is initiated directly through cisplatin uptake or indirectly through the drop of endocochlear potential (EP) following SV damage is still debating. Refer to the text for more details. TM: tectorial membrane; RM: Reissner's membrane. All images are created with BioRender.com.

dolymph from the surrounding perilymph (**Figure 1**, for recent reviews, see [46, 47]).

This blood-labyrinthine barrier (BLB) is similar to the blood-brain barrier (BBB) in terms of cellular and molecular basis [48], which is only permeable to some small molecules (up to 500 KDa) [49]. Unfortunately, this permeability to the BLB includes most ototoxic drugs, while it excludes most otoprotective agents proven effective *in vitro* [50, 51]. Furthermore, once inside the cochlea, the outflow of the ototoxic drugs is also difficult.

Path to hearing loss in chemotherapy: Cisplatin ototoxicity leads to structural changes, including shrinkage and inflammation of SV, loss of OHCs and IHCs, morphological changes of the stereocilia bundles, IHC synaptopathy, changes of supporting cells, and loss of spiral ganglion neurons (SGNs) (for reviews, see [15, 52-56]). Recent cellular and molecular biology studies also revealed additional cells and structures affected by cisplatin ototoxicity, such as the spiral ligament [57, 58], spiral limbus, spiral modiolar veins and lacunae [15, 59], and pericytes in the SV [49, 60]. Functional changes include decreased EP, threshold elevation of distortion product otoacoustic emissions (DPOAEs), auditory brainstem responses (ABRs), and compound action potentials (CAPs) [61-63]. DPOAE magnitudes and ABR wave-I amplitudes are also decreased. These changes are closely re-

lated to the cellular processes, including ROS generation, inflammation, and apoptosis [64]. Meanwhile, the inflammatory and apoptotic mechanisms of cisplatin ototoxicity are highly interconnected, which leads to a vicious cycle of inflammation, ROS production, nuclear and mitochondrial DNA damage, ER stress, and cell death, which will be discussed later.

Following cisplatin therapy, OHC loss is usually the most prominent and severe cochlear damage [46] and is likely the cause of permanent hearing loss. How OHCs are affected by cisplatin remains unclear (**Figure 1**). While early studies suggest direct damage of the OHCs following the uptake of cisplatin via different ion channels [65] or transporters [66, 67], conclusive experimental evidence of whether platinum exists in the hair cells is still missing. OHC loss might also originate from changes in the EP resulting from compromised SV function [46, 68]. This view is further supported by the following findings: (1) The highest platinum accumulation is found in the SV [20, 68, 69]. Solid evidence comes from Cunningham's group, which showed that platinum mainly accumulates in SV after chemotherapy, using the inductively coupled plasma mass spectrometry visualization technique [68]. (2) The immediate decrease of the EP after cisplatin treatment suggests the involvement of the stria vascularis in cisplatin ototoxicity [46]. (3) Platinum-DNA adduct was observed in SV marginal cells as early as 8 hours after cisplatin treatment, while ROS accumulation was not shown even after 48 hours [69]. This finding suggests that ROS accumulation might be a secondary effect of SV damage. (4) A recent study shows in cell culture that the pericytes in SV, which are also critical for EP, are the targets of cisplatin and may account for BLB breakdown in CIHL [60].

Cisplatin uptake by cochlear cells

The first step for cisplatin ototoxicity is its uptake by cochlear cells, which may occur, like in other tissues, through the organic cation transporter 2 (OCT2) [67], copper transporter 1 (CTR1) [66, 70], and LDL receptor-related protein 2 (LRP2) [71]. The high expression of CTR1 and OCT2 in IHCs, OHCs, spiral ganglion neurons (SGN), and SV [66] supports this view (for review, see [56, 66, 72]). The interaction and uptake of cisplatin via mechano-transducer (MET) channels of the hair cells have also been studied [65, 73]. One early study shows that cisplatin blocks MET channels in chicken cochlear hair cells as an acute effect [73]. However, the paper has not demonstrated whether cisplatin could pass the MET channels. A study on the zebrafish lateral line organ shows that functionally intact MET channels are required for the toxicity of cisplatin to the hair cells. Hair cell death is prevented when the MET channels are non-functional through chemical blockage or mutation [65]. Interestingly, the same publication also suggests that the roles of OCT2 and CTR1 for cisplatin uptake are insignificant in these cells [65]. A more recent study on murine cochlear hair cell explants, however, shows that both MET channel and OCT are involved in the uptake of fluorescent dye-conjugated cisplatin [74]. In addition to transporters and MET channels, cisplatin may also enter cells through passive diffusion [75], as shown in the digestive system of rats [76] and a cochlear-derived cell line OC-k3 [77]. In the *in vitro* studies on OC-k3 cells, cisplatin enters the cells via first-order kinetics without saturation before the induction of cell death. Once inside the cell, cisplatin undergoes an aquation reaction and hydrolyzes as water ligands displace chloride ligands [75]. The ability of this activated and positively charged aqua-cisplatin compound to passively diffuse back across the plasma membrane is significantly decreased. The drug is then trapped intracellularly, leading to unimpeded damage [72, 78, 79].

ROS generation and its central role in cisplatin ototoxicity

Platinum has a high affinity for sulfur ligands [80]. Once it enters the cochlea, the highly reactive aquated form of cisplatin binds to both DNA and proteins [79], which triggers a series of signaling pathways (**Figure 2**). Cisplatin may induce NADPH oxidase 3 (NOX3) directly [81] and/or via cisplatin activated transient receptor potential cation channel subfamily V member 1 (TRPV1) channel, followed by calcium influx [82]. Cisplatin-activated NOX3 leads to the generation of ROS, specifically superoxide ($O_2^{-\cdot}$) [81]. $O_2^{-\cdot}$ can induce mitochondrial translocation of the B-cell lymphoma 2 gene (Bcl2) associated X (Bax), leading to mitochondrial-

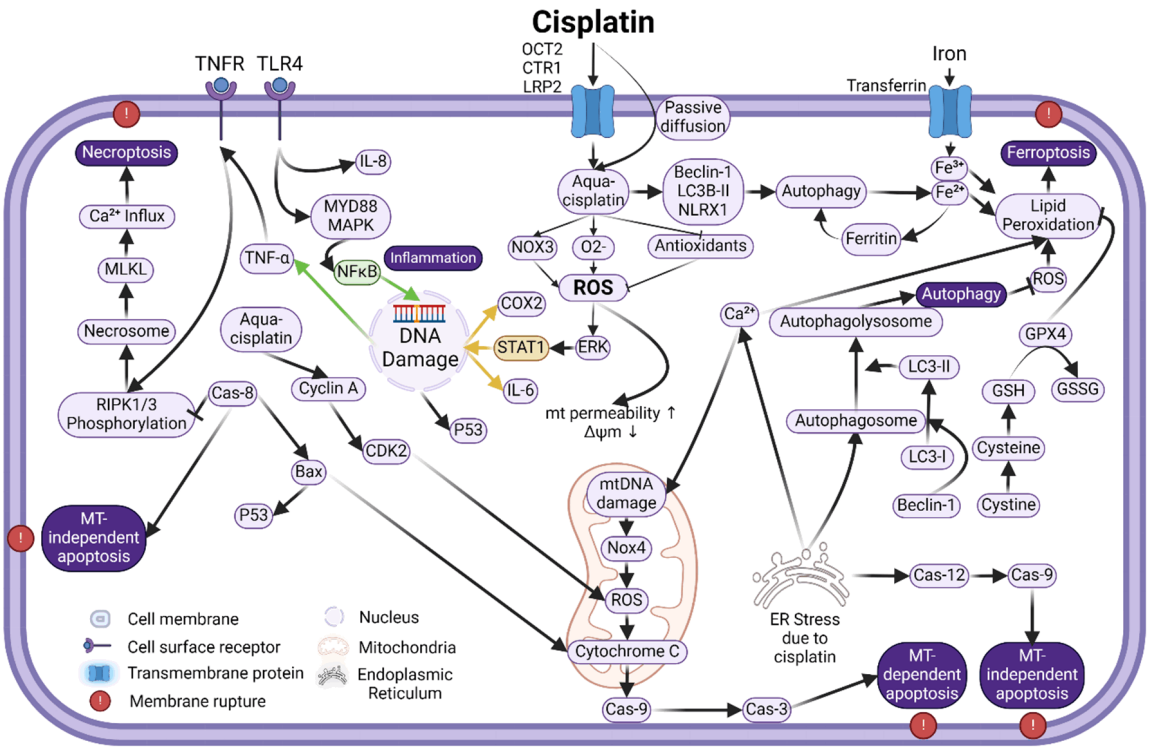

Figure 2. Schematic image showing intracellular signaling pathways associated with cisplatin ototoxicity in the cochlea. Different cell death pathways, including apoptosis (either mitochondria dependent or independent) and non-apoptotic cell death pathways are involved and interconnected. Refer to the text for details.

dependent apoptosis (further discussed below) [72, 83]. Another study demonstrates that NOX3-dependent ROS generation, rather than cisplatin itself, activates TRPV1 channels [84] (for review, see [72, 85]). Nevertheless, both NOX3 induction and TRPV1 activation by cisplatin can result in ROS generation and calcium influx. In addition, reducing TRPV1 expression [84] and knockout of NOX3 [82] both attenuate cisplatin-induced hearing loss (CIHL), during which both SGNs and OHCs are protected [82]. ROS is also a potent inducer of NOX3, which is over 50 times more abundant in the inner ear compared to other tissues [81] (for review, see [79]). It leads to a vicious cycle of ROS-induced TRPV1 and NOX3 activation, causing further calcium influx, ROS production, and TRPV1 and NOX3 activation [84].

ROS is detoxified by an intracellular antioxidant defense system composed of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH.Px), and glutathione reductase (GR) [13, 56, 72]. This antioxidant defense system can be rapidly overwhelmed by cisplatin [80, 86]. The reactive platinum moiety of aqua-cisplatin reacts with intracellular oxygen molecules, generating $O_2^{\cdot-}$ [56]. SOD converts $O_2^{\cdot-}$ to hydrogen peroxide (H_2O_2), which CAT further catalyzes to produce water and oxygen [56, 72]. In this process, GSH.Px converts glutathione from the reduced form (GSH) to the oxidized form (GSSG) during the conversion of H_2O_2 to water. GR catalyzes the conversion of GSSG back to GSH using NADPH as a cofactor [56, 72]. Cisplatin can covalently bind to the thiol group of these antioxidant enzymes, leading to their inactivation [56]. This reactive cisplatin compound also directly binds to GSH, leading to its excretion or conversion back to GSSG [55]. NADPH depletion downregulates GR antioxidant activity, which further decreases GSH levels. Depletion of GSH downregulates GSH.Px activity [56]. Most experimental evidence shows that antioxidant enzymes are reduced by cisplatin, some to the extent of 50-70% [55, 86, 87] (for reviews, see [56, 72, 88, 89]). However, other studies indicate that SOD and CAT activities can be increased instead [55, 79, 90]. Together, antioxidant depletion and ROS over-

load trigger a series of interconnected cell death pathways involving DNA damage, lipid peroxidation, protein oxidation and enzyme inactivation, ion channel expression changes, endoplasmic reticulum (ER) stress, and inflammation [56, 59, 72, 83]. The cascade of ROS signaling involves processes in different compartments of the cells, including the cytoplasm, nucleus, and mitochondria (**Figure 2**). Different cell death processes are involved, including mitochondrial-dependent and mitochondrial-independent apoptosis, necroptosis, autophagy, ferroptosis, etc. (**Figure 2**).

Mitochondrial-dependent apoptosis associated with cisplatin ototoxicity

Mitochondrial-dependent apoptosis is characterized by releasing pro-apoptotic factors from the mitochondria to cytosol. This process can be triggered by, and is associated with, different factors and pathways during cisplatin treatment, including lipid peroxidation, DNA damage, signal transducer and activator of transcription 1 (STAT1), p53 activation, Bcl2/Bax activation, cyclin/cyclin-dependent kinase (CDK) activation, Cytochrome C, Caspases, etc. In the cochlea, mitochondrial dysfunction and apoptosis due to ROS overload and disruption of intracellular redox homeostasis result in the loss of OHCs and other cells [91, 92] (for review, see [13]) (**Figure 2**). Most of the factors and pathways related to mitochondrial-dependent apoptosis have been studied. They are shown to be involved in cisplatin ototoxicity. First, cisplatin can integrate into DNA in the nucleus via adduct formation [13, 16, 69, 93], leading to cross-linking and damage [93]. DNA damage activates p53, initiating the intrinsic mitochondrial apoptosis pathway [94]. P53 increases the expression of the pro-apoptotic molecule Bax [78], which is translocated to the mitochondria, where it permeates the outer mitochondrial membrane. Mitochondrial membrane permeabilization leads to the loss of the mitochondrial membrane potential, mitochondrial ROS (mtROS) production, and release of Cytochrome C and mtROS from the mitochondria into the cytoplasm [95, 96]. Second, both NOX3-dependent ROS production [57, 79, 85, 88, 97] and ROS-mediated activation of extracellular signal-regulated kinases 1 (ERK1) [59, 64] activate STAT1 signaling. STAT1 triggers p53 activation, leading to the above-described mitochondrial translocation of Bax and downstream mitochondrial-dependent apoptosis in the cochlea [57, 85]. STAT1 activity also links the apoptotic and inflammatory pathways involved in cisplatin ototoxicity [64], which will be discussed later. Third, multiple factors, including cisplatin itself, ROS, lipid peroxidation, calcium influx, and CDKs, can act directly on mitochondria, causing an increase in its permeabilization and deterioration of its function. Cisplatin can also form mitochondrial DNA (mtDNA) adducts [98-101], which is likely the main factor in cisplatin-induced ototoxicity [15]. The platination of mtDNA leads to mtROS production and accumulation, and in turn, the damage of mtDNA, proteins, and lipids within the mitochondrial membrane. Cyclin A is another critical player in mtROS production, which can be upregulated by cisplatin and activates CDK2 kinase, consequently facilitating mtROS production [102]. Fourth, cytochrome C release and the activation of caspase-9 and caspase-3 mark the final stage of mitochondrial-dependent apoptosis, which is also observed in the cochlea [72] (**Figure 2**). As an essential component of the mitochondrial electron transport chain, cytochrome C is one of the apoptotic protease-activating factors, which activate caspase 9. Caspase 9 activation subsequently activates caspase 3, causing the fragmentation of chromosomal DNA through the cleavage of its substrates [103].

Mitochondrial-independent apoptosis pathways in cisplatin ototoxicity

Apoptosis can also be induced in the cytoplasm through ROS overload, ER stress [104], inflammation, and lipid peroxidation [79].

ER stress: ER stress results from oxidative damage, intracellular calcium imbalance, and protein damage [83]. It is involved in cisplatin ototoxicity through caspase-12 activation, located on the ER plasma membrane, and autocleaves in response to ER stress. Caspase-12 then activates caspase-9, which activates caspase-3, leading to apoptosis in a mitochondrial-independent manner [97, 98, 105]. This ER-specific apoptosis pathway is also closely linked to the mitochondrial-dependent path due to the simultaneous activation of C/EBP homologous protein (CHOP) [104]. CHOP plays an important role in ER stress-induced apopto-

sis while regulating Bcl2 family expression. Decreased Bcl2 enables increased Bax activity, leading to the release of apoptotic active substances from mitochondria to the cytoplasm [106], i.e., mitochondrial-dependent apoptosis [79, 104] (**Figure 2**).

Inflammation: Inflammation is the body's defense mechanism in response to harmful stimuli, such as damaged cells, which also plays a vital role in inducing apoptosis. Inflammatory signaling pathways, most commonly the nuclear-factor kappa B (NF-κB), mitogen-activated protein kinases (MAPKs), and JAK-STAT pathways, are also involved in cisplatin ototoxicity. NF-κB is a transcription factor mediating inflammatory responses by regulating the expression of various pro-inflammatory genes, such as tumor necrosis factor-α (TNF-α). In cisplatin ototoxicity, NF-κB can be activated directly by ROS [107] or toll-like receptor 4 (TLR4) [108], leading to its translocation to the nucleus and production of inflammatory cytokines [80, 92]. TLR4 is a transmembrane protein that plays a fundamental role in pathogen recognition and activation of innate immunity. TLR4 can also be activated by cisplatin, which triggers the activation of proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), TNF-α, and NF-κB [108]. Translocation of NF-κB to the nucleus, mediated by TNF-α and IL-6, further induces the *de novo* synthesis of TNF-α, IL-6, IL-1β, and inducible nitric oxide synthase (iNOS), and activates caspase-3 in a mitochondrial-independent manner [78, 79]. Nevertheless, NF-κB translocation can occur much earlier (1-2 hours) than maximal ROS generation (1 day), suggesting an early involvement of the inflammatory pathways or even an upstream event of ROS formation [15, 59, 79, 109, 110]. NF-κB [111] (and also STAT1 [85]) induces an increased expression of iNOS, which produces nitric oxide (NO) (for reviews, see [78, 79]). NO reacts with $O_2^{\cdot-}$ to form peroxynitrite ($ONOO^-$), a highly reactive oxidizing molecule that damages proteins [112]. Peroxynitrite induces protein peroxidation via nitration of tyrosine residues (nitrotyrosine) [112, 113], which alters protein configuration and function (for reviews, see [13, 15, 72]). In addition, expression of TNF-α also further activate NF-κB [80, 92] as well as the extrinsic apoptosis pathway by binding to TNF receptors (TNFR). It leads to caspase-8 activation, which in turn activates caspase-3, leading to mitochondrial-independent apoptosis (**Figure 2**) [71, 93], which is also known as the death receptor pathway [42] or extrinsic apoptosis [94].

Other than the NF-κB pathways, early phosphorylation of two well-characterized MAPK families, ERKs and the c-Jun N-terminal kinases (JNKs), is activated by cisplatin treatment in two cochlear-derived cell lines - House Ear Institute-Organ of Corti 1 (HEI-OC1) cells [109] and OC-k3 cells [77] (**Figure 1**). Early activation of JNK may play a minor role in cisplatin-induced ototoxicity [109] or may assist in DNA repair in response to cisplatin-DNA adducts [114]. The activation of MAPK/ERK also facilitates secretion of the pre-existing TNF-α, IL-1β, and IL-6 [77] (**Figure 2**), which in turn activates the translocation of NF-κB to the nucleus [109]. Nevertheless, most of the studies mentioned above are performed on cell cultures, although the role of the MAPK family in cisplatin toxicity is well documented [115]. It is also worth noting that most of the research on the molecular mechanism of cisplatin ototoxicity included in this review has been conducted using *in vitro* cell cultures or *ex vivo* cochlear explants. A recent study has highlighted that the molecular pathways implicated in cisplatin ototoxicity may differ between the *in vitro/ex vivo* and the *in vivo* settings [116].

JAK-STAT pathway activation allows the transfer of signals from the receptors to the nucleus. It thus involves a repertoire of processes, such as apoptosis and tissue repair, through a cytokine-membrane receptor-JAK-STAT cascade [117]. In CIHL, the JAK-STAT pathway regulates cell death and inflammatory responses by activating the expression of inflammatory cytokines such as cyclooxygenase-2 (Cox-2) and TNF-α. Suppression of the JAK-STAT pathway with an adenosine A1 receptor (A1AR) agonist decreases cisplatin-induced apoptosis of OHCs [118]. Among STAT proteins (STAT1-6), STAT1 is likely the most relevant to CIHL because it is known to directly induce apoptosis and p53-mediated apoptosis [64, 85]. In rat models, STAT1 signaling is linked with ROS, causing cisplatin-induced cochlear cell apoptosis [85]. Pre-treatment with STAT1 siRNA (48 hours before) [85] or oral uptake of an inhibitor of STAT1 signaling (45 minutes before) [64] both

protect against CIHL. STAT3 and STAT6 are also important players in CIHL [119]. STAT6 works through inflammatory cytokines IL-4 and IL-13, and knockout of STAT6 protects against CIHL [119].

Lipid peroxidation: Downstream effects of $O_2^{\cdot-}$ can also cause lipid peroxidation. Catalyzed by SOD, $O_2^{\cdot-}$ is converted to hydrogen peroxide, which is catalyzed by iron to form hydroxyl free radicals [15]. These highly reactive ROS react with polyunsaturated fatty acids in cellular membranes, producing the highly toxic aldehydes, 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) [72]. Antioxidant enzyme depletion has been linked to increased levels of MDA, indicating high lipid peroxidation [80]. This inverse relationship between glutathione and MDA activity (low glutathione levels and high MDA levels) has been shown *in vivo* using cisplatin-treated rats [86]. Lipid peroxidation, especially the production of 4-HNE, induces calcium influx into the cells [15, 88]. ROS further enhances calcium influx. An early study found that $O_2^{\cdot-}$, but not H_2O_2, increases the intracellular calcium concentration via transmembrane influx in OHCs of guinea pig cochleae. Researchers suggested that $O_2^{\cdot-}$ stimulates voltage-sensitive calcium channels. However, they did not rule out the possibility of increased calcium permeability caused by lipid peroxidation [120]. According to a review article, ROS can open the ER calcium channel, ryanodine receptor, L-type, T-type, and the TRPV1 plasma membrane calcium channels [79]. A study by Yoshida et al. found that intracellular NO and H_2O_2 open TRPV1 channels and facilitate calcium influx [121]. In an earlier study, inhibition of T-type calcium channels did not inhibit ROS generation. Still, it significantly inhibited lipid peroxidation, mitochondrial membrane permeabilization, and cytochrome c release in HEI-OC1 cells and rat organ of Corti explants treated with cisplatin [96]. The findings suggest that calcium influx via T-type calcium channels occurs downstream of ROS and plays a major role in intrinsic apoptosis of cochlear cells under cisplatin-induced stress conditions. Nevertheless, increased cytosolic calcium causes mitochondrial membrane permeabilization and loss of membrane potential, initiating the intrinsic mitochondrial-dependent apoptosis pathway [122].

Other cell death pathways

Necroptosis: In addition to activation of the extrinsic apoptosis pathways, TNF-α induces cell death via necroptosis pathway activation in cisplatin-induced ototoxicity [116, 123] (for reviews, see [59, 124]). Studies indicate a dose-dependent effect on cisplatin-induced cell death using HEI-OC1 cells [103] and OC-k3 cells [77]. Specifically, apoptosis occurs at lower doses, while necroptosis occurs at higher doses. As opposed to the organized breakdown of cells during apoptotic cell death, necroptosis results from cellular and organelle membrane permeation, releasing intracellular substances and exacerbating inflammation [122]. Activation of caspase-8 inactivates receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and RIPK3, which induce activation of the extrinsic mitochondrial-independent apoptosis pathway as described above. Inactivation of caspase-8 leads to RIPK3 activation, shifting death receptor-mediated cell death from apoptotic to necroptotic pathways [124]. In necroptosis, TNF-α binds its receptor 1 (TNFR1) and, without caspase-8, leads to the formation of the RIPK1/RIPK3 complex, also known as necrosome [123, 124]. Downstream effects of necrosomes involve the activation of Mixed Lineage Kinase Domain-Like Pseudokinase (MLKL), which induces calcium influx via Transient Receptor Potential Cation channel, subfamily M, member 7 (TRPM7) channels. It creates pores in the plasma membrane, leading to leakage of substances, cell lysis, and cell death [125].

Autophagy: As a protective mechanism of cells, autophagy removes specific cellular structures or components, such as damaged organelles. Under stress conditions such as cisplatin treatment, autophagy can also lead to cell death [126]. The interest in the involvement of autophagy in CIHL has increased rapidly in recent years. Increased expression of three autophagic mediators has been observed in CIHL, including Beclin-1 (the initial autophagy promoter), microtubule-associated protein light chain 3 II (LC3-II), and mitochondrial-bound [127] nucleotide-binding domain and leucine-rich-repeat-containing family member X1 (NLRX1) [59]. Autophagy signaling involves specific factors and interconnections with other cell death pathways. First, intracellular organelle or

protein damage leads to the activation of AMP-activated protein kinase, which inhibits the mammalian target of rapamycin (mTOR), leading to autophagy [128] and to the activation of phosphoinositide 3-kinase (PI3K), inducing Beclin-1 formation of the phagosome [129]. Second, beclin-1 activates the conversion of unlipidated LC3-I to lipidated LC3-II, which is required for complete autophagosome formation and autophagosome-lysosome fusion [126, 129]. Third, overexpression of NLRX1 correlates with the accumulation of autophagosomes and acceleration of autophagic cell death [83, 126]. In addition, NLRX1 overexpression also accelerates mitochondrial-dependent apoptosis in cisplatin treatment, evidenced by increased Bax, caspase-3, and ROS levels [127]. The interactions between autophagy and apoptosis pathways are also shown in mitochondrial autophagy or mitophagy. Activation of mitophagy can negatively regulate cisplatin-induced apoptosis in hair cells and SGNs [130] and vice versa [131], suggesting a protective role of mitophagy in CIHL. Nevertheless, questions still exist about whether autophagy plays a protective role or induces cell death in CIHL, and the arguments are far from settled (for reviews, see [59, 132, 133]).

Ferroptosis: Ferroptosis is another form of non-apoptotic programmed cell death, which requires irons generated by processes such as lipid peroxidation and autophagy in response to stress [134, 135]. Hallmarks of ferroptosis, such as lipid peroxidation and impaired antioxidant capacity, have been observed in cochleae in mice after cisplatin treatment, suggesting its potential roles in CIHL [135]. A recent study showed that transferrin 1, a marker of ferroptosis, is increased in OHCs but not IHCs, SV, or supporting cells after cisplatin treatment. RNA sequencing completed in the same study showed that the expression of the ferroptosis-related gene is upregulated in CIHL [136]. Suppressing ferroptosis using ferrostatin-1 reduces CIHL by protecting cochlear hair cells [135, 137, 138] while facilitating ferroptosis by blocking lipid repair function leads to an exacerbated breakdown of mitochondrial membrane potential in cultured HEI-OC1 cells. Ferrostatin-1 also protects hearing against CIHL in mice and rescues OHCs in a knockout mouse model lacking a key regulator for ferroptosis [136]. However, ferroptosis is highly dependent on mitochondrial function and interconnected with other signaling pathways, including lipid peroxidation and autophagy. Further studies are needed to address the potential interference of ferroptosis with the antitumor effects in chemotherapy involving cisplatin. More discussion regarding the roles of ferroptosis in CIHL and the underlying mechanism can be found in some recent studies [139, 140] and reviews [89].

Hearing protection in chemotherapy

Current studies on hearing protection in chemotherapy are mainly focused on cisplatin ototoxicity. Among the four ototoxic chemotherapeutic drugs listed in **Table 1**, vinblastine and dasatinib have rarely been studied in hearing protection, while the studies on carboplatin are almost all associated with cisplatin [141, 142]. Since the damage to the cochlea is largely irreversible and regeneration has not been successful yet, intervention before and during chemotherapy to protect hearing is critical. However, preventing cisplatin ototoxicity using drug therapies is facing severe challenges, including interference with the therapeutic effects of cisplatin, bioavailability, and side effects. Concerns regarding interference with the antitumor effects of cisplatin lie in the fact that interventions with antioxidants that reduce cisplatin ototoxicity may also affect the outcomes of the cancer therapy by deactivating cisplatin [78, 143, 144] and by protecting tumor cells [145] (for review, see [78, 146]). Challenges to bioavailability include the permeability of otoprotective drugs through BLB [50, 51] and cell membranes. Side effects are manifested by the toxicity of hearing protective drugs to other tissues. An example is amifostine [147], for which high drug doses are required for treatment because of the drug's impermeability of the BLB *in vivo*.

Drug delivery strategies

Local drug administration: The challenges mentioned above can be addressed through transtympanic delivery [148], which has proven to be successful for some candidate drugs for hearing protection. Local drug administration through trans-tympanic injection has been developed in early studies to treat inner ear disorders such as Ménière's disease and sudden

hearing loss using steroids (for review, see [149]). It is also adopted to treat cisplatin ototoxicity with antioxidants to avoid interfering with the antitumor effects [143, 148, 150, 151]. Depending on the site, two types of local drug administration are usually used: intratympanic (middle ear and round window) and intra-cochlear/labyrinthine (perilymph). Intratympanic injection, sometimes combined with a small tube through tympanic annulus for multiple doses, allows localized and high-dose treatment, as shown in some clinical trials treating cisplatin ototoxicity [152]. Intra-cochlear/labyrinthine delivery further renders the benefits of passing through the BLB, sustained treatment, and (semi-) dose control when combined with chronic implantation of an osmotic pump [153]. Local administration is shown to be effective for many drugs without interfering with the antitumor effect of cisplatin [154-156]. Concerns for local drug administration include potential pain during myringotomy and damage to the tympanic membrane, middle ear structures, and round window. Intra-cochlear/labyrinthine injection may even cause permanent damage to the inner ear, which may prevent its potential application in clinics. A more detailed introduction of the local delivery techniques and their application in cisplatin ototoxicity [79] can be found in research papers and reviews [157-161].

Other drug administration: Some other strategies have been developed to increase the efficiency of hearing protection and reduce the side effects and risk of counteracting the antitumor effect of cisplatin. Hydrogel has been used for intra-tympanic drug delivery to improve drug sustainability on the round window by reducing drainage through the eustachian tube [162-164]. However, subsequent conductive hearing loss posed by the residue on the round window and middle ear ossicles might be a concern. Nanoparticles, with a diameter of less than 1 μm, are another strategy to increase the efficacy of local drug delivery for treating cisplatin ototoxicity [165]. A few groups have studied the influence of particle size and various transportation vehicles [166]. The paper by Yu et al. details various nanoparticles and hydrogel preparations for improved drug delivery to the inner ear and cochlea manipulations [79]. As stated in the paper, inadequate cellular uptake of nanoparticles limits its use. However, it can be reduced with various endogenous (pH, redox, and enzymes) and exogenous (heat, ultrasound, light, and magnetic field) measures. Delayed or staggered drug delivery is also used to reduce the possibility of interference with the antitumor effect of cisplatin. Nevertheless, showing accurate drug concentration in the cochlea will help to determine the mechanism and effective doses of the drugs. In this regard, proof of the existence of the drugs in the cochlea by measuring the concentration from the extraction of cochlear fluids is a gold standard for candidates claiming hearing protection [167-169].

Antioxidants as traditional candidates for hearing protection against cisplatin ototoxicity

Since ROS plays a central role in cisplatin ototoxicity, various exogenous antioxidants that work as free radical scavengers have been tested for hearing protection in chemotherapy in early studies [51, 170-172]. A common feature of antioxidants is the presence of a thiol, which can act as a substrate for redox reactions. Some antioxidants have been tested in clinical practice, such as N-acetyl-L-cysteine (NAC) [172], D-methionine [173, 174], STS [155, 163], and amifostine [147, 175] (for reviews, see [13, 72, 78, 142, 147, 176]). A list of antioxidants tested for their hearing protective effects against cisplatin ototoxicity is presented in **Table 3**. Some well-known and promising candidates and their roles are shown in **Figure 3** and described below.

STS: STS has been traditionally used to treat metal poisoning. It can form strong complexes with metal ions, including platinum, thus deactivating cisplatin. Its antioxidant property comes from a reduced sulfur residue. The hearing protective effect of STS has been proven in both animal studies [172, 177] and clinical trials [178, 179]. Local delivery through intracochlear injection [148, 152] or delayed administration [177] is required to treat cisplatin ototoxicity. Although the effect of local delivery is controversial [148, 152], delayed administration of STS by 4-8 hours is proven to be protective to hearing [178, 179], while it does not interfere with the therapeutic effect of cisplatin [180]. In these clinical studies, the incidence of cisplatin ototoxicity decreased by about half, and no significant difference in overall or event-

Table 3. Antioxidant drug candidates for protection against CIHL

Drug	Main mechanism of oto-protection	Experiment model	Drug dose	Route	Outcome
STS (sodium thiosulfate) Branded as Nithiodote & PedMark	By forming complexes with platinum & antioxidant activity (PMID: 25994788)	Phase III clinical trial on hepatoblastoma in children (n = 109) (PMID: 29924955)	Cisplatin (80 mg/m^2) per dose over 6 doses	IV	Reduction in hearing loss grade 1-4 by 48%.
			STS (20 g/m^2) 6 hours after each cisplatin treatment	IV	
		Phase III Clinical trial on children (n = 104) with cancers (PMID: 27914822)	Cisplatin (totaling 200 mg/m^2)	IV	Reduction in hearing loss grade 1-4 (OR = 0.31; 95% CI 0.13-0.73; P = 0.0036).
			STS (16 g/m^2) 6 hours after each cisplatin treatment	IV	
NAC (N-acetyl-L-cysteine) Branded as Acetadote, Fluimucil & Mucomyst	Antioxidant with a thiol donating hydrogen (PMID: 29429900)	Rat cochlea cell culture treated with NAC (PMID: 35346799)	Cisplatin (50 uM) treatment to cell cultures in the background of NAC at 37°C for 48 hours	Cell culture	Hair cell loss prevented, seen through Ab staining.
			NAC (20 mM)		
		Rat treated with NAC (PMID: 15219317)	Cisplatin (6 mg/kg)	IA	Hearing protected at 4 kHz (~10 dB), 8 kHz (~20 dB), 12 kHz (~22 dB), 16 kHz (~18 dB) by change in threshold.
			NAC (400 mg/kg) 15 minutes before cisplatin	IV	
		Phase I clinical trial on children (n = 52) with cancers (PMID: 37134194)	Cisplatin (totaling 200 mg/m^2)	IV	Reduction in the risk of SIOP ≥ 2 hearing loss post-chemotherapy (OR = 0.13, CI 0.021-0.847, P = 0.033).
			NAC (6 g) 4 hours after each cisplatin treatment	IV	
		Double blind clinical trial on cancer patients (n = 114) (PMID:29993216)	Cisplatin (not stated)	Not stated	No significant changes in auditory thresholds.
			0.4-0.8 ml NAC (10%)	Intratympanic	
D-methionine (D-met)	D-methionine can donate a cysteine which can act as an antioxidant (PMID: 16366723)	Mice treated with D-methionine (PMID: 8951454)	Cisplatin (16 mg/kg)	IP	Hearing protected at 1, 4, 8, 14 kHz.
			D-met (300 mg/kg)	IP	
		Chinchilla treated with D-met (PMID: 12087338)	Cisplatin (125 μg)	Intracochlear	Hearing protected at 8, 16 kHz.
			D-met (4 μg) 30 minutes before cisplatin treatment	Intracochlear	
		Rats with MTLn3 breast cancer cells (PMID: 11405249)	Cisplatin (5 mg/kg) per dose for 3 doses with 72 hours interval	IP	Hearing protected at 1, 2, 4, 8, 16, 18 kHz. OHC protected.
			L-met (300 mg/kg) 30 minutes before each cisplatin injection	IP	
		Phase II clinical trial in cancer patients (n = 27) (PMID: 34622731)	Cisplatin totaling (264 mg/m^2)	IV	Hearing protected in the left ear only at 11.2 kHz by a mean difference of 22.97 dB.
			D-met (100 mg/kg) oral dose 1 hour before cisplatin injection	Oral	

Amifostine (WR-2771) Branded as Ethyol	Antioxidant activity through WR-1065 (PMID: 11201306)	Scid mice model of human ovarian cancer with implanted tumor cells (PMID: 9389935)	Paclitaxel (27 mg/kg) over 5 doses	IP	Amifostine improved animal survival. In cell cultures, amifostine protected normal cells from paclitaxel, while cytotoxicity was increased in malignant cells.
			Amifostine (200 mg/kg) over 5 doses	IP	
		Hamsters treated with amifostine (PMID: 15185124)	Cisplatin (15 mg/kg) over 5 doses	IP	Protected hearing at 8 kHz (~15 db), 16 kHz (~20 db), 20 kHz (~18 db) by ABR threshold shift.
			Amifostine (40 mg/kg) over 5 doses 30 minutes before cisplatin injection	IP	
		Non-randomized clinical study with cancer patients (n = 62) (PMID: 18669462)	Cisplatin (300 mg/m^2) over 4 doses	IV	Amifostine caused ~22% reduction in the probability of requiring hearing aid. Amifostine related adverse reaction in 19% patients.
			Amifostine (600 mg/m^2) 3 hours before and immediately before cisplatin injection	IV	
		A randomized clinical study (n = 25) investigating medulloblastoma in children (PMID: 15999362)	Cisplatin (40 mg/m^2) per day for 5 days	IV	Amifostine did not offer otoprotection against cisplatin combined with etoposide and bleomycin.
			Amifostine (825 mg/m^2) per day for 5 days 30 minutes before cisplatin	IV	
			Etoposide (100 mg/m^2) per day for 5 days	IV	
			Bleomycin (15 IU/m^2) per day for 5 days	IV	
Taurourso-deoxycholic acid (TUDCA)	H01 and SOD2 mediated antioxidant activity (PMID: 32061715)	Rats treated with TUDCA (PMID: 32061715)	Cisplatin (5 mg/kg) per day for 3 days	IP	Protected hearing at 4, 8, 16, 32, 40 kHz.
			TUDCA (100 mg/mL) 1 hour before cisplatin treatment	IP	
TUDCA	H01 and SOD2 mediated antioxidant activity of TUDCA (PMID: 32061715)	Rats treated with TUDCA (PMID: 33631298)	Cisplatin (4.6 mg/kg) per day for 3 days	IP	Protected hearing at 8, 16, 24, 32, 40 kHz.
			TUDCA (500 mg/kg) 1 day before cisplatin treatment	Subcutaneous	
Ebselen	Antioxidant activity acting as a GPx mimic	Mice treated with ebselen (PMID: 19286452)	Cisplatin (16 mg/kg)	IP	Protected hearing at 4, 8, 16, 32 kHz.
			Ebselen (16 mg/kg)	IP	
		Rat treated with ebselen (PMID: 21804453)	Cisplatin (14 mg/kg)	IV	Did not protect hearing.
			Ebselen (12 mg/kg) 1 hour before cisplatin treatment	IP	
		Rat treated with ebselen and allopurinol (PMID: 15721563)	Cisplatin (16 mg/kg)	IP	IP ebselen + allopurinol protected hearing at 8, 16, 24 kHz.
			Ebselen (8 mg/kg) 1 hour before cisplatin treatment	IP/oral gavage	
			Allopurinol (8 mg/kg) 1 hour before cisplatin treatment	IP/oral gavage	

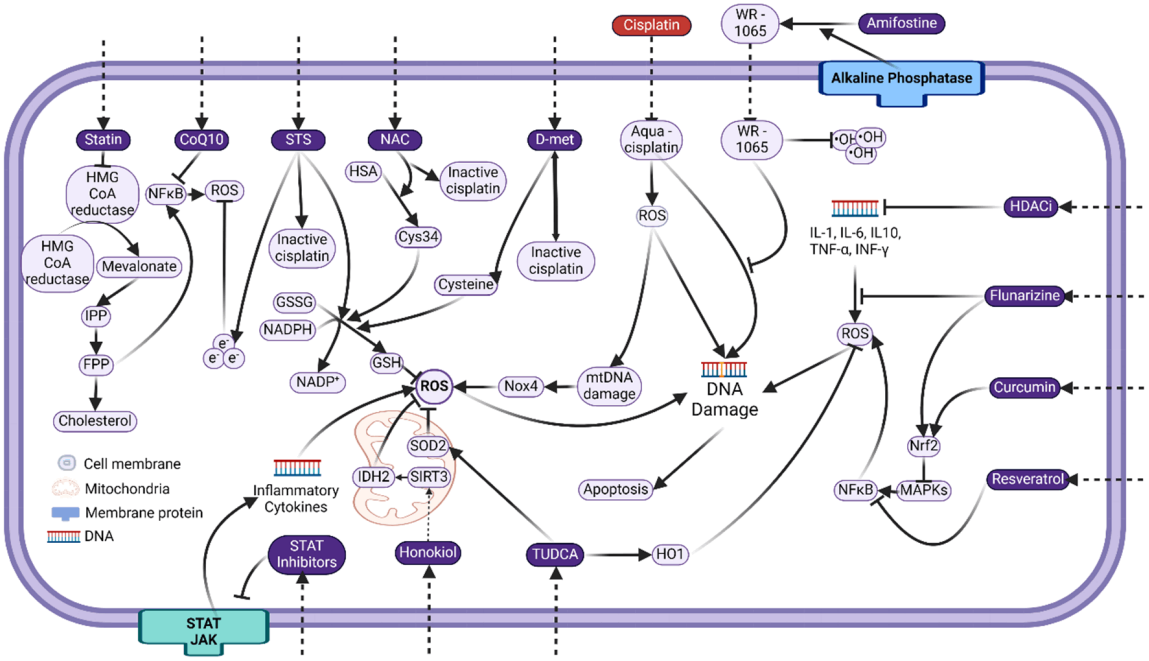

Figure 3. Schematic image showing key targeting cellular factors and signaling steps for preventative strategies against cisplatin ototoxicity. Cisplatin induces DNA damage and ROS generation. Multiple processes and cytokines also contribute to ROS generation along the apoptosis and other (such as inflammation) signaling pathways. Proposed agents (in purple) target different sites in these processes. Refer to the text for details and abbreviations.

free survival was observed between the treated group and the cisplatin-alone group [178, 179]. In 2022, the FDA approved STS for protective treatment against cisplatin ototoxicity in pediatric patients with localized, non-metastatic solid tumors. It is the first and the only approved drug to treat cisplatin ototoxicity in clinics.

NAC: NAC is a precursor to GSH, a ubiquitous thiol-containing antioxidant and a widely used cytoprotectant with a promising hearing protective effect [181-184]. As a weak free radical scavenger, NAC may protect against cisplatin ototoxicity by induction of endogenous GSH and blocking apoptosis through the caspase signaling pathway [181, 185]. Pre-treatment of NAC (400 mg/kg) improves cisplatin-induced hearing threshold shift in rats [186], although the results are slightly inconsistent with an organo-culture study [185]. Nevertheless, since NAC also protects against cisplatin cytotoxicity [181], a local or staggered administration is required for its otoprotection [186, 187]. In clinical studies using intra-tympanic injection, NAC failed to prevent hearing loss during cisplatin treatment, although it was suggested for some patients [154, 184]. In a recent phase I clinical study with children and adolescents newly diagnosed with nonmetastatic tumors delayed NAC application through intravenous (i.v.) injection (4 hours after cisplatin injection, peak concentration of NAC up to 450 mg/kg) was effective for preventing CIHL without adverse events [188]. These studies indicate that NAC might be another promising drug for treating cisplatin ototoxicity in clinics.

D-methionine: D-methionine is the enantiomeric counterpart of L-methionine, a naturally occurring L-alpha-amino acid. The antioxidant property of methionine is due to methionine being oxidized into methionine sulfoxide, which inhibits ROS. It also deactivates cisplatin by forming an inactive complex with it [78]. D-methionine is one of the earliest antioxidants tested in the protection against CIHL [189] and has proven to be effective both in animal studies [152, 189, 190] and in humans [174]. Since it is known that D-methionine interferes with the therapeutic effects of cisplatin, local, delayed, or staggered delivery should be considered in clinical trials. The in-human study, completed in India in 2022, used oral

uptake of D-methionine (100 mg/kg) one hour before cisplatin treatment. The study did not show adverse events significantly different from the placebo group [174]. However, the study analyzed data from only 27 of the 50 enrolled participants. Furthermore, the study did not gather data on whether D-methionine affected cisplatin cytotoxicity in tumors. Overall, more careful examinations of the research and further larger scale clinical trials might be necessary to assess the influence of D-methionine on the antitumor efficacy of cisplatin.

Amifostine: Amifostine is used in chemotherapy and radiotherapy as a cytoprotective adjuvant for reducing renal toxicity [191]. Alkaline phosphatase can dephosphorylate it into an active free thiol metabolite that functions as an antioxidant [192]. Its cytoprotective action is believed to be achieved through scavenging free radicals, stabilizing DNA, and upregulating p53 [176], thereby selectively protecting normal tissues due to their high alkaline phosphatase levels [193]. Moreover, amifostine does not affect the antitumor activity of platinum drugs [192]. In animal research, multiple high doses of amifostine, given before cisplatin, protect hearing in hamsters (40 mg/kg/day) [194] and guinea pigs (100 mg/kg/day) [195]. However, lower doses (18 mg/kg/day) fail to achieve this effect [196]. Clinical studies on amifostine's ability to protect against cisplatin ototoxicity have yielded mixed results. For instance, one study with 97 pediatric patients with medulloblastoma indicates a reduced need for hearing aids following amifostine treatment (600 mg/m^2) [147]. In contrast, another study with 11 patients shows no protective effect (1000 mg/m^2) [197]. A follow-up clinical trial with 379 patients reveals that amifostine protects from cisplatin ototoxicity only in average-risk but not in high-risk medulloblastoma patients [198]. In another study with 25 cases of pediatric germ cell tumor, amifostine treatment (825 mg/m^2) does not confer otoprotection compared to historical controls [175]. These varying outcomes may be attributed to differences in treatment regimens, sample sizes, age groups, and cancer types across studies. As a note, two retrospective evaluations of the efficacy of amifostine in preventing cisplatin ototoxicity have been either inconclusive [199] or insignificant [200], possibly due to similar complexities. While high doses of amifostine are crucial for the treatment, they are associated with adverse events, including neurotoxicity (in animal studies) [194], hypocalcemia, hypotension, and nausea and vomiting (in clinical settings) [147]. Complex administration protocols, careful medical attention, and sustained monitoring of vital signs are required to ensure patient safety during amifostine treatment [201]. A more detailed review can be found in [78].

Coenzyme Q10: Coenzyme Q10 is a crucial enzyme for electron transport in the mitochondrial respiratory chain. It also works as an anti-inflammatory and antioxidant agent in dietary supplements [202]. Co-application of coenzyme Q10 and multivitamins was tested for protection against CO in both an animal model [203] and a pilot case-control clinical trial [204]. In the animal study, Q10 terclatrate (500 mg/kg) and vitamin supplements (vitamin E and B12) given before cisplatin (4.6 mg/kg/day for three days) treatment protected against CIHL in rats. In the pilot study on cancer patients treated with a coenzyme Q10 plus dietary multivitamin, the incidence of hearing disorders and tinnitus induced by cisplatin was reduced significantly. A significant difference in threshold shift only occurred at 8 kHz [204]. However, a fully powered clinical study has not been completed, even six years after the preclinical animal study.

Candidates/approaches targeting specific pathways/factors

Candidates targeting more specific cell signaling pathways in cisplatin ototoxicity are also often proposed and tested for their hearing protective effects. An early attempt is dexamethasone, a glucocorticosteroid that can downregulate proinflammatory cytokines, inhibit apoptosis, and upregulate antioxidant enzymes [78]. While animal studies yielded promising results [150, 151, 162], dexamethasone failed in most clinical trials [154, 156], although attempts were made using nanoparticles [205] or poloxamer hydrogels [162] to increase local drug concentration in the cochlea. A detailed review regarding the protective effect of dexamethasone against cisplatin ototoxicity can be found in [78], with no further discussion in this review. As the understanding of cisplatin ototoxicity signaling pathways grows, more spe-

cific targets on factors/cytokines in metabolic [203, 204], inflammatory [206, 207], or apoptosis signaling pathways [102, 208, 209] have been identified with promising results. Some novel candidates that have been tested are listed in **Table 4**, and their potential mechanism is shown in **Figure 3**. These candidates include neurotrophins [210], hormones [154], molecules involved in endogenous metabolism [204], modulators of cell signaling pathways [102], etc.

Epigenetic modulators: The significance of epigenetic modulation in inner ear development, damage, and protection was noticed over a decade ago [211-213], while it has garnered more attention only in recent years [214-216]. Histone deacetylases (HDACs) and their inhibitors (HDACis) regulate transcription and control cell cycle through modulation of histone acetylation. HDACis, such as sodium butyrate [212] and sulforaphane [215], are protective against cisplatin ototoxicity in animal models (guinea pigs and rats). The mechanism might be related to the pro-survival pathway activation, as shown in the study on HDAC inhibition against kanamycin and furosemide-induced hearing loss [214]. In addition, both sodium butyrate [217] and sulforaphane [218] can induce the expression of an antioxidant-responsive gene, Nrf2, which may also account for their hearing protective effects [219]. HDACs have antitumor activity and are well tolerated. They are ideal candidates for treating cisplatin ototoxicity. However, their mechanisms of action are not fully understood, and explanations rely on indirect evidence only. More studies regarding their specificity are needed before testing them in clinical trials.

Other promising epigenetic modifications have been reported recently. They are still conducted in cell cultures and animals. A recent study showed that a DNA methyltransferase inhibitor, RG108, alleviates CIHL in a mouse model [216]. Inhibition of DNA methylation by RG108 upregulates BCL-2 and downregulates mitochondrial-dependent apoptosis pathway factors BAX and BCL2 Associated Agonist of Cell Death (BAD) in HEI-OC1 cell cultures. Another histone methyltransferase inhibitor, BIX01294, has been shown to protect against CIHL in a mouse model established through the combination of i.p. furosemide and subcutaneous cisplatin delivery [220]. The mechanism, verified primarily on cell cultures, is associated with activating the autophagy pathway through the Forkhead box G1 gene, a critical regulator for morphogenesis of the mammalian inner ear during development. Nevertheless, further studies to verify these results and mechanisms are necessary, and their interference with the antitumor effects of cisplatin and other adverse effects are still unclear.

Statins: Statins are widely used as anti-inflammatory agents to control lipid peroxidation. They are proposed to protect hearing against various insults (noise [221], drug [222], and age [223]. For a review, see [224]). Statins are also used in an ongoing clinical trial against sudden hearing loss (ClinicalTrials.gov ID: NCT04826237). Two recent studies showed that statins protect against CIHL in animals (lovastatin [206]) and humans (atorvastatin [207]). The non-randomized, retrospective clinical study executed by Cunningham's group showed that atorvastatin (with unknown doses) decreased the incidence of CIHL by 53% without affecting the three-year survival rates in head and neck cancer patients [207]. The same group will perform a randomized phase III clinical study (NCT04915183) on atorvastatin (20 mg) against CIHL. In this clinical trial, 186 patients with squamous cell carcinoma of the head and neck will be involved. The study aims to assess hearing using audiograms before and 2-4 months after cisplatin treatment and compare outcomes between atorvastatin users and controls. CTCAE criteria will be used for data analysis, and reduced bias will be expected. Although it might not be a major concern, sequelae of statins include muscle pain, liver damage, increased blood sugar, and memory loss.

Protein kinase inhibitors: Protein kinase phosphorylation cascades regulate multiple signal transduction pathways, including inflammation, apoptosis, and proliferation. Cytokine inhibitors can reduce cytokine synthesis and concentration and interfere with the interaction between cytokines and their receptors. Certain kinase inhibitors have been studied for their potential to protect against CIHL. Flunarizine, known initially as a calcium channel blocker, could reduce cisplatin-induced inflammatory cytokines (TNF-α, IL-1β, NF-κB seen through IHC) in

Table 4. Non-antioxidant drug candidates for protection against CIHL

Drug	Main mechanism of oto-protection	Experiment model	Drug dose	Route	Outcome
Dexa-methasone Branded as DexPak & Decadron	Inhibiting inflammatory cytokines (PMID: 9486420)	Mice treated with dexamethasone (PMC2720789)	Cisplatin (14 mg/kg) treatment after the first dexamethasone injection	IP	Protected hearing at 16 kHz.
			Dexamethasone (24 mg/ml) per day over 5 days	Intratympanic	
		Guinea pigs treated with dexamethasone (PMID: 21521888)	Cisplatin (12 mg/kg)	IP	Not protective.
			Dexamethasone (6 mg) treatment on the day before and on the day of cisplatin treatment	Intratympanic	
		Clinical trial on cancer patients (n = 26) on dexamethasone (PMID: 24618499)	Cisplatin (total 400 mg)	IP	Protected hearing at 4-8 kHz.
			Dexamethasone (~8.5 mg)	Intratympanic	
Coenzyme Q10 Branded as ubiquinone & CoQ10	Anti-inflammatory dietary supplements (PMID: 35326965)	Rats treated with Coenzyme Q10 and vitamins (PMID: 27632426)	Cisplatin (4.6 mg/kg) per day for 3 days	IP	Protected hearing at 2, 4, 8, 16, 32 kHz.
			Q10 terclatrate (500 mg/kg)	Oral	
			Acuval 400 (100 mg/kg)	Oral	
		Clinical trial on cancer patients (n = 26) being treated with Coenzyme Q10 and vitamins (PMID: 28239674)	Cisplatin (100 mg/m^2) every 21 days	IV	Protected hearing at 8 kHz.
			Acuval Audio (1.8 g) per day starting 7 days before the first cisplatin treatment	Oral	
Statins Branded as Lipitor & Mevacor	Inhibiting inflammatory cytokines (PMID: 35125240).	Mice treated with lovastatin (PMID: 32062294)	Cisplatin (3 mg/kg) per day for 4 days, followed by 10 days of recovery. 3 cycles (total 36 mg/kg)	IP	Protected hearing at 20, 40 kHz.
			Lovastatin (40 mg/kg) daily from 3 days before cisplatin treatment	Oral gavage	
		Clinical trial on head and neck cancer patients (n = 277) on atorvastatin (PMID: 33393488)	Cisplatin (~80 mg/m^2) every 3 weeks. Median cumulative cisplatin was 200 mg/m^2.	Not stated	Protected hearing at 4, 6, 8, 12.5 kHz.
			Atorvastatin (10~80 mg)	Oral	
HDACi Branded as Buphenyl & Avmacol	Epigenetically downregulating genes linked to apoptosis (PMID: 23558232)	Guinea pigs treated with sodium butyrate (PMID: 16467722)	Cisplatin (14 mg/kg)	IP	Protected hearing at 3.5~20 kHz, statistical significance not shown.
			Sodium butyrate (1.2 mg/kg) per day for 7 days before and 5 days after cisplatin treatment	IP	
		Rats treated with sulforaphane (PMID: 34344210)	Cisplatin (7 mg/kg) twice a day for 7 days	IP	Protected hearing at 4, 8, 16, 24, 32 kHz. OHC partially protected.
			SFN (30 mg/kg) per day for 7 days	IP	
		Mice treated with RG108 (PMID: 35530135)	Cisplatin (30 umol/L)	IP	Protected hearing at 8, 16, 24, 32 kHz. OHC protected seen through IHC.
			RG108 (100 umol/L) 2 hours before cisplatin treatment	IP	
STAT inhibitors Branded as Nifuroxazide etc	Anti-inflammatory and anti-apoptosis	Rats treated with STAT1 siRNA (PMID: 21776018)	Cisplatin (11 mg/kg)	IP	Protected hearing at 8, 16, 32 kHz. OHC protected seen through electron microscopy.
			STAT1 siRNA (0.g μg) 48 hours before cisplatin treatment	Intratympanic	
		Rats treated with STAT1 inhibitor EGCG (PMID: 28703809)	Cisplatin (11 mg/kg)	IP	Protected hearing at 8, 16, 32 kHz. OHC protected.
			EGCG (100 mg/kg) 45 minutes before cisplatin + 3 more post-cisplatin treatments	Oral	
		STAT6 -/- mice (PMID: 21321603)	Cisplatin (4 mg/kg) per day for 4 days	IP	Hearing protection at 4, 16, and 32 kHz.

Cytokine inhibitors Branded as Basiliximab, daclizumab	Inhibiting inflammatory cytokines	Mice treated with flunarizine (PMID: 18584244)	Cisplatin (4 mg/kg)	IP	Reduction of cochlear TNF-a and IL-1b.No ABR recording.
			Sibelium (143 ug/kg)	Oral	
		Rats treated with etanercept (PMID: 28730299)	Cisplatin (16 mg/kg)	IP	Protected hearing at 4.5, 6, 8, 10 kHz on day 3.
			Etanercept (6 mg/kg) 24 hours before cisplatin treatment	IP	
Honokiol Branded as Honobsolute	SIRT3-mediated antioxidant activity (PMID: 21172655)	Mice treated with honokiol (PMID: 33415008)	Cisplatin (20 mg/kg)	IP	Protected hearing at 4-32 kHz. Cisplatin cytotoxicity not affected.
			Honokiol (20 mg/kg) treatment 1 hour before cisplatin treatment		

mouse cochleae [110]. The reduction of cytokines was through the activation of Nrf2/HO-1 signaling. However, no results on ABR threshold changes were shown in the paper. Multiple inhibitors for CDK2 (kenpaullone and two unrevealed compounds) protect hearing against CIHL and noise-induced hearing loss [102, 208, 225], suggesting that CDK2 is an important target for hearing protection. Dabrafenib, a BRAF (a member of Raf family kinases) inhibitor, is also protective against CIHL and noise-induced hearing loss when combined with the CDK2 inhibitor AZD5438 [209]. However, the influence of cisplatin on the antitumor effect must be verified in tumor-bearing mice before its potential application in clinical practice.

Polyphenols: Polyphenols are a diverse group of naturally occurring compounds abundant in plants. Many polyphenols are natural antioxidants and anti-inflammatory agents that protect against various stress conditions such as UV light radiation and microbial infections. The roles of polyphenols in hearing protection against different insults are recognized, and the mechanisms studied most recently (for review, see [47, 226]). Epicatechin protected hearing in rats against CIHL by inhibiting ERK signaling in an early study [227, 228]. However, the protective effect of epicatechin needs further verification with cochlear whole-mount samples and frequency-specific ABR data. Resveratrol, a polyphenol abundant in fruits and red wine, was also shown to protect against CIHL in guinea pigs [229] and rats [230] at 10 and 0.1-0.5 mg/kg/day, respectively. In contrast, high doses (50, 10, and 1 mg/kg/day) were toxic to the cochlea in rats [230, 231]. The hearing protection mechanism is associated with activating the anti-oxidative response and reducing inflammatory responses mediated by NF-kB, IL6, and IL1β [231]. Curcumin, a bright yellow pigment extracted from the plant turmeric, is also shown to protect against CIHL in rats at 200 mg/kg [232, 233]. The mechanism is associated with decreased lipid peroxidation, potentially through the Nrf2 pathway [232]. Although arguably [234], curcumin is reactive, unstable, and non-bioavailable, which has shown to be unsuccessful in any clinical trial [235]. In addition, the interaction with the antitumor function of cisplatin is yet to be investigated. Luteolin is a flavone derived from celery, green pepper, and chamomile with anti-inflammatory, antioxidant, and anticarcinogenic properties. In a recent study, luteolin showed promising protection against CIHL by inhibiting ferroptosis [136]. The authors comprehensively studied the involvement of Gpx4, the key regulator for ferroptosis in CIHL. Luteolin protection through alleviation of ferroptosis was proven in cell cultures, cochlear explants cultures, and *in vivo* Gpx4 knockout mice levels [136]. Honokiol, a lignan extracted from the plant Magnolia, is a multifunctional small polyphenol with both antitumor effects that synergize with cisplatin [236-238] and protective effect to various tissues and organs against oxidative stress, including the brain [239-241], heart [242-244], kidney [245], liver [246], etc. In a recent study from our lab, honokiol has shown strong hearing protective effects against cisplatin ototoxicity in tumor-bearing mice undergoing chemotherapy [247]. Honokiol also increases animal survival without interfering with cisplatin's antitumor effects. The mechanism is associated with activating sirtuin 3 (SIRT3), a member of the sirtuin NAD^+-dependent deacetylase family, critical regulators of the intrinsic anti-ROS systems.

Sirtuins comprise a highly conserved NAD^+-dependent deacetylase family, all key regulators of the intrinsic anti-ROS systems [248-251]. Seven members (SIRT1-7) of the sirtuin family are expressed in the cytoplasm (SIRT1&2), mitochondria (SIRT3-5), and the nucleus (SIRT1, 2, 6, 7). The involvement of sirtuins (mostly SIRT3) in hearing protection has been shown in noise-induced [252, 253], drug-induced [254], and age-related hearing loss [255, 256]. Activation of sirtuins (mostly SIRT1) by polyphenols has also been reported in resveratrol [257], curcumin [258], and epicatechin [259]. Sirtuins might represent novel targets for hearing protection against cisplatin ototoxicity, and polyphenols are promising candidates. For example, honokiol does not interfere with the therapeutic effects of cisplatin, as shown in our study [247] and other publications [237, 238, 260, 261]. It can pass the BBB [262, 263] and diffuse across plasma and mitochondrial membranes *in vivo* [243, 263]. Although bioavailability is still a concern for most polyphenols because of their insolubility and instability, different ways for assisting the delivery of polyphenols, such as liposomal [264-266], amorphous solid dispersion [267, 268], and bio-

degradable microsphere [269], have made significant progress in recent years.

Summary and outlook

This paper sought to comprehensively review the current understanding and research on ototoxicity in chemotherapy (particularly with cisplatin) and explore strategies and compounds for hearing protection. Using symptoms including hearing loss, tinnitus, vertigo, and dizziness, we identified potential ototoxic drugs, among which four are specifically causing hearing loss. While the ototoxic mechanisms of cisplatin are extensively studied, other agents like isotretinoin, vinblastine, and dasatinib remain less understood.

The primary target of cisplatin in the cochlea is still debatable, although OHC loss is the most commonly observed change in cisplatin ototoxicity. Damage of the SV might be the leading cause, which affects the EP and may result in cisplatin influx into the scala media. These changes induce a series cascade of cellular processes, eventually leading to cell death. ROS generation is the predominant factor in this process, and its subsequent signaling pathways are discussed.

The review emphasizes the urgent need for effective otoprotective strategies that can mitigate the adverse effects of chemotherapy on hearing without compromising its anticancer efficacy. Various antioxidants, such as STS, NAC, and D-methionine, have been explored for their potential to counteract the ROS-mediated ototoxicity of cisplatin. STS, in particular, has gained FDA approval for reducing hearing loss in pediatric cancer patients receiving cisplatin. However, the challenge of ensuring that these agents do not interfere with the chemotherapeutic efficacy of cisplatin remains a critical consideration in their clinical application.

Emerging research has focused on more specific molecular targets and pathways involved in cisplatin-induced hearing loss. The review highlights the exploration of various compounds, including statins, protein kinase inhibitors, and polyphenols like epicatechin, resveratrol, curcumin, luteolin, and honokiol, for their protective effects against CIHL. These agents, often possessing antioxidant and anti-inflammatory properties, offer new avenues for otoprotection, with some showing promise in preclinical and clinical studies. Particularly, honokiol has been noted for its synergistic antitumor effects with cisplatin and its ability to protect against ototoxicity, potentially through activating the sirtuin pathway.

The review also discusses innovative drug delivery methods, such as local administration via trans-tympanic injection and advanced formulations like nanoparticles and hydrogels, to enhance the efficacy of otoprotective agents and minimize their systemic side effects. These strategies aim to improve the bioavailability of protective agents in the cochlea and reduce the risk of interfering with the systemic anticancer activity of chemotherapeutic drugs.

In conclusion, while significant advances have been made in understanding the mechanisms of chemotherapy-induced ototoxicity and identifying potential otoprotective strategies, further research is needed to develop safe and effective treatments that can be integrated into standard oncology care. The balance between preserving hearing and maintaining the antineoplastic effectiveness of chemotherapy presents a complex challenge that future studies must address to improve the quality of life for cancer patients undergoing treatment.

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Disclosure of conflict of interest

None.

Abbreviations

ABR, Auditory Brainstem Response; ABVD, Doxorubicin, Bleomycin, Vinblastine, Dacarbazine; AMPK, AMP-activated Protein Kinase; ARHL, Age-Related Hearing Loss; Bax, Bcl-2-Associated X Protein; Bcl2, B-cell Lymphoma 2; BBB, Blood-Brain Barrier; BLB, Blood-Labyrinthine Barrier; CAP, Compound Action Potential; CAT, Catalase; CDK, Cyclin-Dependent Kinase; CHOP, C/EBP Homologous Protein; CIHL, Cisplatin-Induced Hearing Loss; CTR1, Copper Transporter 1; DPOAE, Distortion product Oto-

Acoustic Emission; EP, Endocochlear Potential; ER, Endoplasmic Reticulum; FDA, Food and Drug Administration; Gpx4, Glutathione Peroxidase 4; GR, Glutathione Reductase; GSH.Px, Glutathione Peroxidase; HDAC, Histone Deacetylase; HDACi, Histone Deacetylase Inhibitor; HEI-OC1, House Ear Institute-Organ of Corti 1; IG, Immunoglobin; IHCs, Inner Hair Cells; IL, Interleukin; iNOS, Inducible Nitric Oxide Synthase; i.p., Intraperitoneal; i.v., Intravenous; JAK-STAT, Janus Kinase-Signal Transducer and Activator of Transcription; LC3-II, Microtubule-associated Protein 1A/1B-light Chain 3; LRP2, LDL Receptor-related Protein 2; MAPK, Mitogen-Activated Protein Kinase; MET, Mechano-Electrical Transduction; MLKL, Mixed Lineage Kinase Domain-Like Pseudokinase; mtDNA, mitochondrial DNA; mTOR, Mammalian Target of Rapamycin; mtROS, mitochondrial ROS; NAC, N-acetyl-L-cysteine; NCI, National Cancer Institute; NF-κB, Nuclear Factor Kappa-light-chain-enhancer of activated B cells; NLRX1, Nucleotide-binding Domain and Leucine-rich-repeat-containing Family Member X1; NOX3, NADPH Oxidase 3; Nrf2, Nuclear Factor Erythroid 2-Related Factor 2; $O_2^{\cdot-}$, Superoxide; OCT2, Organic Cation Transporter 2; OHCs, Outer Hair Cells; ONOO-, Peroxynitrite; RIPK, Receptor-Interacting Protein Kinase; ROS, Reactive Oxygen Species; SGN, Spiral Ganglion Neuron; SIRT, Sirtuin; SOD, Superoxide Dismutase; STAT1, Signal Transducer and Activator of Transcription 1; STS, Sodium Thiosulfate; SV, Stria Vascularis; TNF-α, Tumor Necrosis Factor Alpha; TRPV1, Transient Receptor Potential Vanilloid 1.

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