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Strategies to reduce the risk of platinum containing antineoplastic drug-induced ototoxicity

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Abstract

Introduction—Cisplatin is a highly effective chemotherapeutic agent against a variety of solid tumors in adults and in children. Unfortunately, a large percentage of patients suffer permanent sensorineural hearing loss. Up to 60% of children and at least 50% of adults suffer this complication that seriously compromises their quality of life. Hearing loss is due to damage to the sensory cells in the inner ear, primarily the outer hair cells and cells of the stria vascularis and spiral ganglion. The mechanisms of cochlear damage are still being investigated. However, it appears that most damage to the inner ear is triggered by reactive oxygen species (ROS) formation and inflammation.

Areas covered—In this review we discuss a number of potential therapeutic targets that can be addressed to provide hearing protection. These strategies include enhancing the endogenous antioxidant pathways, heat shock proteins, G protein coupled receptors and counteracting enzymes that produce ROS and reactive nitrogen species, and blocking pathways that produce inflammation, including TRPV1 and STAT1.

Expert opinion—A number of potential protective agents show promise in animal models by systemic or local administration by transtympanic or intracochlear injection. However, clinical trials have not shown much efficacy to date with the exception of sodium thiosulfate administration in two studies of pediatric tumors. There is an urgent need to discover safe and effective protective agents that do not interfere with the efficacy of cisplatin against tumors yet preserve hearing.

Keywords

cisplatin; ototoxicity; cochlea; clinical trials; drug targets; mechanisms; transtympanic; hearing loss

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1. Introduction

Cisplatin is a widely used chemotherapeutic agent for the treatment of solid tumors such as testicular, bladder, ovarian, advanced cervical cancer, endometrial, lung, head and neck, pancreatic, breast, esophageal, lymphomas, metastatic osteosarcomas and melanomas among others. It has gained the status of an essential medicine by World Health Organization. While it is a very efficacious chemotherapeutic agent, it has several dose-limiting side effects such as nephrotoxicity, ototoxicity and neurotoxicity. In this review, we will discuss:

1. History of cisplatin
2. Pharmacodynamics
3. Mechanisms of cisplatin uptake
4. Mechanisms of anti-neoplastic action
5. Ototoxicity
6. Mechanisms of cisplatin ototoxicity and targets for otoprotection
7. Experimental drug treatments to reduce platinum ototoxicity
8. Drug candidates in clinical trials
9. Current clinical measures
10. Conclusion
11. Expert Opinion

This review should give the reader a comprehensive look at cisplatin, mechanism of cisplatin induced ototoxicity and an updated list of all otoprotective strategies in preclinical as well as clinical testing.

1.1 Brief history of platinum drugs

Cisplatin was first synthesized by an Italian chemist Dr. Michele Peyrone in 1844 and was known as ‘Peyrone’s chloride’ ($PtCl_2(NH3)_2$) [1]. Its structure, coordination chemistry and isomerism were elucidated by Swiss chemist Dr. Alfred Werner in 1893 who received the Nobel prize for his work in 1913 (Werner’s theory of coordination compounds).

1.2 The accidental discovery

Barnett (Barney) Rosenberg was a biophysicist at Michigan State University in 1961, where he began investigating the effect of magnetic field on eukaryotic cell division. This was prompted by his fascinating observation that arrangement of iron filings in the magnetic field was very similar to that of mitotic spindle seen in cell division [2, 3]. Rosenberg’s experiments with E. coli in buffered solution of ammonium chloride in the presence of an electric field generated using inert platinum electrodes showed that the E.coli actually stopped dividing, but continued growing and formed long filamentous cells (200–300 times their normal length) [4]. Subsequent studies indicated that it was not the electric field that was responsible for inhibition of cell division, but small amounts of platinum from the

electrodes that dissolved in solution which then reacted with ammonium and chloride ions in the growth medium that led to the generation of compounds responsible for inhibition of cell division [5, 6]. These compounds were then identified as cis-dichlorodiammineplatinum (II) (cisplatin or CDDP) and cis-tetrachlorodiammine platinum(IV). Both these compounds were found to have anti-tumor activity on sarcoma and mice models of leukemia and Rosenberg published his findings in 1969 [6]. It was then identified that the cis form of platinum (II) complex cis-$[PtCl_2(NH3)_2]$ had the most potent anticancer activity [7] and that the trans isomer was ineffective and inefficient in inhibiting cell division [5, 8, 9]. Cisplatin displayed broad-spectrum activity against viral and carcinogen induced tumors, including highly advanced tumors [6].

The side effect profile of cisplatin discouraged physicians from using it in cancer patients early on. In 1977, there was a significant change in the administration of cisplatin with both pre and post treatment hydration and mannitol induced diuresis being used to minimize the nephrotoxic effects. This allowed doses as large as 120 mg/m^2 to be given without much concern about nephrotoxicity [10, 11]. This unleashed the therapeutic potential of cisplatin, and the world of oncology witnessed a marked increase in the rates for testicular cancer remission from as low as 36 % to as high as 100 percent with 70 percent of patients achieving complete remission [3]. Limited success was also achieved in ovarian and recurrent head and neck cancers. The development of potent anti-emetics like the five hydroxytryptophan-3 antagonists helped overcome the challenges of nausea and vomiting [11–13]. Cisplatin is used in the treatment and management of solid tumors such as advanced genitourinary cancers including testicular, bladder, ovarian and cervical cancers, head and neck cancers, esophageal cancers, as well as both small cell and non-small cell lung cancers, as well as pediatric age group cancers. Over time it has become one of the most widely used anti-cancer medications worldwide.

2. Pharmacodynamics of platinum drugs

A classical structure-activity-relation (SAR) for these platinum complex dictates certain rules governing their molecular structure to possess anti-cancer activity [8]. These structural properties include centrally placed platinum in square-planar geometry with coordination of two cis-amine ligands and two cis anionic ligands. They are neutrally charged molecules. The binding of the anionic moiety to the platinum determines the toxicity or the activity of the drug. These anionic ligands are loosely bound leaving groups which determines the strength of its cytotoxic activity towards cancer cells. These two amine or anionic ligands are replaced with chelating dicarboxylate or diamine which were namely *cis*-diamminecyclobutane-dicarboxylatoplatinum(II) or carboplatin and *R,R*-cyclohexane-1,2-diamineoxalatoplatinum(II) or oxaliplatin respectively which were approved by the FDA for clinical use in US [14], as shown in Figure 1. These two compounds obey the same classical SARs and were thought to operate by a mechanism of action similar to that of cisplatin. Carboplatin and oxaliplatin have reduced toxic side effects compared to cisplatin which is attributed to their lower reactivity. The stereochemical structural of these chiral complexes also determine the activity of the drug. For example, the trans isomer of cisplatin is not an effective anti-cancer agent. Transplatin forms fewer DNA adducts compared to cisplatin

[15]. Cisplatin was found to produce almost 80% of intrastrand crosslinks in DNA [16, 17], while trans isomer rarely produced intrastrand adducts [18].

The main cellular target of all three platinum drugs is nuclear DNA. The activated mono-aquated platinum drug can react with nucleophilic purine bases of DNA at N7 positions of guanosine and adenosine residues. The coordination sites of an anionic ligand permit cross-linking of adjacent guanine bases. The major DNA-cisplatin adduct formed is intrastrand dGpG cross-link, which facilitates the distortion of DNA double helix [19].

3. Mechanism of cisplatin uptake

3.1 Uptake and efflux

Cellular uptake and efflux are vital steps in the mechanism of action of these platinum drugs, with both active and passive processes being involved Cisplatin administered systemically retains its neutral charge and does not get aquated immediately. This is due to higher concentration of chloride ions in the blood plasma (~100 mM) which limits the replacement of the chloride ligand by water molecules. Cisplatin enters cells by either diffusion or through active transport by transporters and encounters lower intracellular chloride concentration (4–20nM) [20]. In the intracellular environment of lower chloride concentration, cisplatin undergoes mono-aquation wherein one chloride ligand gets replaced by water forming a positively charged reactive species which is membrane impermeant and cannot leave the cell without expending enormous amounts of energy, and hence mono-aquated cisplatin accumulates in the cells [2]. Additionally, cisplatin in the blood binds to plasma proteins, especially those containing thiol groups such as human serum albumin and the amino acid cysteine. Several studies indicate that 24 hours following cisplatin administration, the majority of the platinum (65–98%) is bound to serum proteins [21–23]. This protein binding has been implicated in not only some of the severe side effects of cisplatin, but also deactivation of the drug [24–28].

3.2 Transporters

Multiple copper transporters, including CTR1, CTR2, ATP7A, and ATP7B, as well as the copper chaperone ATOX1, have all been shown to be involved in the regulation of cisplatin in the mammalian cells [29].

3.2.1 Copper import transporters 1 and 2—The copper transporter proteins 1 and 2, (CTR1; SLC31A1and CTR2; SLC31A2, respectively), are surface receptors [29]. These proteins function in copper homeostasis, and have been implicated in the transport of cisplatin. There seems to be an equivalent correlation between Copper transporter-1 expression and the uptake of cisplatin within the cell and reduced expression of CTR1 has been seen in cell lines resistant to cisplatin [30]. CTR2 has a different location than CTR1 and has a lower affinity than CTR1. CTR1 is found in membranes, whereas CTR2 is located in cell organelles like the nucleus, lysosomes, and endosomes. It is speculated that it might have a different role in copper homeostasis and in interactions with cisplatin. It is also proposed that knockdown of the CTR2 causes greater platinum influx as well as the

sensitivity of the cells to platinum [31]. However, other researchers have, not seen similar results, thereby suggesting the need for further research on these transporter proteins [32]

3.2.2 P-type export transporters—The export proteins include the P-type proteins as well as the ATP7A and ATP7B. The primary function of these transporters is to regulate the amount of copper in a cell to maintain homeostasis. The ATP7A transporter protein is expressed in the choroid plexus, vascular, and cerebrovascular endothelial cells, while ATP7B is expressed in the brain and the liver. Interestingly, cells expressing ATP7B show more trafficking and extracellular efflux of the platinum and hence less cisplatin uptake, while cells expressing ATP7A showed increased cisplatin uptake and decreased efflux. This could also explain the platinum resistance seen in cells expressing more ATP7B [33]. Patients expressing more of the ATP7B also have a lower response to the platinum drugs. Latest research has focused on the use of ATP7B silencers to increase sensitivity to platinum drugs [33].

3.2.3 Organic transporters—These proteins are poly-specific as they transport multiple agents, including both endogenous and exogenous compounds, with different sizes and molecular structures. They are highly expressed in the excretory organs working to remove them from the body. They exhibit differential binding affinity, which could explain why some platinum compounds are nephrotoxic while others are not [34–36]. The organic cation transporter 2 (OCT2) is expressed in the kidney, organ of Corti (including hair cells and stria vascularis) and in dorsal root ganglia of mice.

3.3 Formation of covalent adducts with platinum agents

The platinum drugs, in their mono-aquated form in the intracellular environment, react with various cellular components, including DNA, RNA, proteins as well as phospholipids [37, 38]. Within the cell, the platinum agents complex with both nuclear as well as extranuclear DNA or mitochondrial DNA. Mitochondrial DNA is extranuclear in location and lacks histones, which causes slower repair of the intra-strand cross-links; hence a larger percentage of platinum and DNA complexes have been reported for mitochondrial DNA compared to nuclear DNA [39, 40].

4. Mechanisms of anti-neoplastic effects

Cisplatin appears to kill cancer cells by a number of different mechanisms. A detailed discussion of the mechanisms of anti-tumor cytotoxicity of platinum agents is beyond the scope of this review. However, a very brief discussion of the anti-tumor actions of cisplatin is provided here. DNA binding and adduct formation appears to be the primary target for cisplatin in the tumor cells. Binding of cisplatin to DNA inhibits transcription, DNA replication and causes cell death [41, 42]. Unsuccessful attempts to repair cisplatin-DNA adducts result in apoptosis of cancer cells [43]. Cisplatin bound to DNA generates a strong oxidative stress response in tumor cells [42, 44, 45]. Additional cytotoxic mechanisms include necroptosis, necrosis and ferroptosis [43, 46, 47].

5. Cisplatin ototoxicity

Cisplatin chemotherapy exhibits dose limiting toxicities that include ototoxicity, nephrotoxicity and neurotoxicity. Despite its wide use as an antineoplastic drug, cisplatin comes with dose limiting side-effects such as ototoxicity, neurotoxicity and nephrotoxicity [48–51]. More than 60% of pediatric patients receiving cisplatin report having renal dysfunction and over 60% are known to experience permanent hearing loss [52–54]. Children are the most vulnerable group, ototoxicity affects more than 50%, and up to 23–50% of adults treated with cisplatin respectively [53, 55–57]. There are no FDA approved drugs to prevent ototoxicity. Despite the known fact that the target of cisplatin as an anticancer drug is the nuclear DNA of the proliferating tumor cells, it is still unknown why cochlear cells are susceptible, as they are not proliferative in nature (excluding pre-natal cochlear hair cells). Nonetheless, cisplatin-induced ototoxicity includes degradation of the cochlea that compromises the functional ability to perceive sound from the surrounding environment.

6. Mechanism of cisplatin ototoxicity and targets for otoprotection

Most otoprotective drugs in pre-clinical and clinical trials belong to either the antioxidant or anti-inflammatory category and work by inhibiting one or both of these pathways, this review will discuss the drug targets under specific mechanisms. A pictorial representation of the cisplatin induced ototoxic pathway and the drugs targeting the various mechanisms is shown in Figure 2. The experimental pre-clinical drugs have been separated according to the route of administration: systemic delivery (table 1) and localized delivery by either the transtympanic route, on the round window or by myringotomy, listed in table 2. Drugs in clinical trials are listed in table 3. For more detailed review on mechanisms of cisplatin ototoxicity please refer to [49, 50, 57, 58]. Drug candidates in clinical trials are discussed below.

6.1. Targeting the endogenous antioxidant system

The cochlea is a highly metabolically active organ. The delicate balance of the anti-oxidant system in the cochlea plays an integral role in maintaining the normal physiological function and healthy hearing. The anti-oxidant systems in place in the cochlea include enzymes such as glutathione peroxidase, glutathione reductase, glutathione-s-transferase, superoxide dismutase, catalase, and gamma-glutamyl cysteine synthetase, among other enzymes constitute the endogenous anti-oxidant system. High levels of antioxidants have been found in the stria vascularis, spiral ligament and supporting cells compared to sensory hair cells, indicating vulnerability of hair cells to cisplatin toxicity [59, 60]. Taken together, these findings denote the importance of ROS detoxification by promoting antioxidant enzyme activity in ameliorating cisplatin-induced hearing loss.

The cochlea is very sensitive to hypoxia and ischemia-reperfusion events [61]. Excessive cochlear stimulation by loud noise or ototoxic agents have been shown increase oxidative stress in the cochlea. Cisplatin induced ototoxicity increases the generation of reactive oxygen species (ROS) [62, 63], increasing the activity of cochlear specific ROS generating

enzymes such as NOX3 [64], xanthine oxidase [62, 65], or by decreasing the activity of the endogenous antioxidant enzyme systems [66, 67].

6.1.1 Xanthine oxidase (XO)—XO is a ROS generating system that promotes both superoxide and hydrogen peroxide generation in the cochlea. XO catalyzes hypoxanthine and xanthine to uric acid. Hypoxanthine and xanthine are metabolic derivatives of adenosine catalyzed by adenosine deaminase. Inhibition of this enzyme by allopurinol contributes to reductions in cisplatin-induced ototoxicity [65].

6.1.2 Glutathione peroxidase (GSH-Px)—The function of GSH-Px is to reduce lipid peroxides and hydrogen peroxide in the cell to their corresponding alcohol and water respectively. Cisplatin reacts and forms conjugates with glutathione which leads to mitochondrial dysfunction by increasing oxidative stress and lipid peroxidation [68]. Ebselen, a GSH-Px mimetic has been shown to decrease cisplatin induced ototoxicity [65]. Combination of allopurinol and ebselen has also been shown to be otoprotective.

6.1.3 NOX3—Cochlear-specific NADPH oxidase (NOX3), has been shown to be the primary target for cisplatin ototoxicity [64], and subsequent silencing of NOX3 by siRNA inhibited cisplatin induced hearing loss [69]. NOX3 is localized primarily in the cochlea, thus administration of NOX3 inhibitors could effectively reduce enzyme activity and treat hearing loss. NOX1 and NOX4 were also found to generate ROS in the cochlea [70].

6.1.4 Reactive nitrogen species (RNS)—Nitrosylative stress (RNS) plays a significant role in cisplatin-induced ototoxicity. 4-hydroxynonenal (4-HNE) and nitrotyrosine are formed as byproducts of ROS and contribute to pro-inflammatory and pro-apoptotic pathways within the cochlear cells [64, 71, 72]. In addition, cisplatin activates nitric oxide synthases (NOS) in the cochlea and induces the production of nitric oxide [73, 74]. Inhibition of NOS by [NG-nitro-L-arginine methyl ester (L-NAME)] showed decreased staining for single stranded DNA in the cochlear sections suggesting amelioration of cisplatin ototoxicity [75]. Increased level of NO can cause nitration of cochlear proteins such as LIM Domain only 4 (LMO4), a transcriptional factor involved in cell survival and death [72, 76]. Thus, both ROS and RNS target cochlear cells and disrupt the delicate balance of cell survival leading to ototoxicity. The use of NOS inhibitors could prove to be an important otoprotective strategy, especially if used transtympanically to avoid unwarranted systemic effects.

6.1.5 Nrf2/HO-1 antioxidant pathway—Nuclear factor erythroid 2-related factor 2 (Nrf2) is a cytoprotective transcription factor that regulates cellular redox balance by regulating antioxidant genes like heme oxygenase −1 (HO-1). Up-regulation of Nrf2/HO-1 pathway by curcumin and ferulic acid have been shown to be otoprotective in cisplatin ototoxicity [77]. Curcumin induced otoprotection was attributed to both anti-oxidant as well as anti-inflammatory mechanisms. Curcumin also chemo-sensitized the cancer cells to cisplatin. Thus, polyphenols like curcumin would make good candidates for clinical translation.

6.1.6 NADH:Quinone Oxidoreductase1 (NAD$^+$/NQO1) pathway—

NADH:Quinone Oxidoreductase1 is an antioxidant flavoprotein that catalyzes the reduction of quinones to hydroquinones by utilizing NAD(P)Has an electron donor and increases NAD$^+$ in the cell. Maintenance of NAD$^+$ levels in the cell is important for cell survival. Kim et al., demonstrated that cisplatin attenuates intracellular NAD$^+$ levels in the cochlea and that augmenting the levels of NQO1 using β-Lapachone (a natural NQO1 substrate) can ameliorate cisplatin induced hearing loss [78]. Natural β-Lapachone and its synthetic analog ARQ 761 are in clinical trials as anti-cancer agents[79]

6.1.7 Miscellaneous antioxidants—Several antioxidants such as allicin, bucillamine, β-lapachone, salicylate, CYM-5478 (agonist for Sphingosine 1-Phosphate Receptor 2), melatonin,d-methionine, erdostiene and epicatechin among others show oto-protective effects in vivo. Most of these antioxidants act by decreasing ROS generation and thus inflammation in the cisplatin treated animal cochlea.

6.2. Targeting cochlear inflammation

Cochlear inflammation has been shown to be critical in cisplatin ototoxicity. ROS is also considered as one of the major inducers of cochlear inflammation. Thus, stimulation of cisplatin induced cochlear inflammation may be due increase in ROS production and increased expression of different proinflammatory cytokines such as IL-1β, IL-6, TNF- α. The primary site of cisplatin induced ototoxicity is OHC, while IHC, spiral ganglion and stria vascularis also show significant increases in inflammation [80, 81].

6.2.1 TNF-α—Tumor necrosis factor-α is a master regulator of systemic inflammation. Administration of TNF- α neutralizing agents (i.p., or transtympanic) prior to cisplatin treatment promoted cell viability and significantly decreased cisplatin induced hearing loss [82, 83]. Inhibition of TNF- α reduced proinflammatory cytokines and diminished ROS generation [83].

6.2.2 Transient receptor potential vanilloid 1 (TRPV1)—TRPV1 is a non-selective cationic channel involved in thermal and pain sensation, expressed abundantly in the c-fibres associated with neurogenic pain and inflammation [84, 85]. Activation of transient receptor potential vanilloid 1 channel (TRPV1) was associated with ROS generation in cochlear hair cells after cisplatin treatment [86], which suggests that Ca^{2+} influx via TRPV1 is one of the main factors in ROS production [87]. Knock down of TRPV1 expression by siRNA suppressed Ca^{2+} influx, NOX3 expression, and protected against cisplatin-induced hearing loss [86]. Paradoxically, capsaicin, a TRPV1 specific agonist has also been shown to protect from cisplatin induced hearing loss by both systemic administration (oral gavage) and by transtympanic route of delivery [88].This effect may have resulted from desensitization of TRPV1 receptors in the cochlea.

ROS is proposed as one of the major inducers of cochlear inflammation. Knockdown of TRPV1 and NOX3 via siRNA decreased ROS generation as well as inflammatory and pro-apoptotic mediators. These data suggest a close cross connection between inflammatory responses and ROS generation in inner ear pathology after cisplatin treatment.

6.2.3 Signal transducer and activator of transcription (STAT)—These transcription factors are known to induce proinflammatory cytokine synthesis. STAT1 is considered as a major mediator of apoptosis in cisplatin-induced ototoxicity. STAT1 is a pro-apoptotic, pro-inflammatory transcription factor, while STAT3 serves as pro-survival, pro-resolution of inflammation protein. Cisplatin treatment significantly increased phosphorylation and activation of STAT1 and decreased phosphorylation and activation of STAT3 [82, 88]. Knock down of STAT1 by siRNA decreased cisplatin induced ototoxicity [82]. Bhatta et al., demonstrated that the increased ratio of STAT1:STAT3 tilted the balance towards apoptosis in cochlear cells after cisplatin exposure [88]. Additionally, administration of cisplatin to rats increased STAT1 expression along with increase in inflammatory proteins such as TNF- α, cycloxygense 2 (COX2), and inducible NOS (iNOS) in the lateral wall, ganglion cells, spiral limbus and OHCs These results indicate that cisplatin promotes inflammation and induces cytokine production in the cochlea via upregulation of pro-inflammatory STATs and NF-kB activity, which leads to cisplatin ototoxicity. [80, 82]. Suppression of STAT1 activity by using EGCG, a STAT1 specific inhibitor, attenuated the induction of inflammatory mediators and prevented cisplatin-induced hearing loss [80]. These inflammatory responses to cisplatin were also found to increase the infiltration of immune cells such as CD14 and CD45 [82].

6.2.4 G-Protein Coupled Receptors (GPCR's)—GPCR's are membrane receptors that mediate response to diverse stimuli under physiological as well as pathological conditions. GPCR's that have been shown to be otoprotective in the cochlea are: 1) A_1 adenosine receptors (A1AR); 2) Cannabinoid receptor 2 (CB2) and 3) Sphingosine 1-Phosphate Receptor 2 (SIP2). The presence and function of adenosine receptors (A1AR) are well-characterized in the inner ear: organ of Corti, stria vascularis and spiral ganglion neurons [89]. Activation of A1AR by its agonist R-phenylisopropyladenosin (RPIA) has been found to increase the activities of antioxidant enzymes such as GSH.Px and SOD in the chinchilla [90]. Pre-treatment with A1AR agonist, RPIA, prior to cisplatin showed significant reduction in cisplatin-induced increase in hearing threshold shifts, mainly due to the anti-inflammatory role of A1AR in the cochlea [91], by suppression of NOX3/STAT1 pathway which also decreases oxidative stress. Ghosh et al., demonstrated the presence of CB2 receptor in the organ of Corti, stria vascularis, spiral ganglion and neurites of the rat cochlea. Activation of CB2 receptor by its agonist, JWH-015, significantly reduced cisplatin-induced ABR threshold shifts, hair cell death and synaptopathy [92]. Wang et al., have illustrated that activation of SIP2 alleviated cisplatin-induced hearing loss by reducing ROS generation and prevented cochlear degeneration [93]. Thus, targeting cochlear-specific GCPRs can be an effective clinical solution to combat hearing loss due to cisplatin treatment.

6.3 Other drug targets

6.3.1 p53 tumor suppressor—p53 is a major tumor suppressor gene, that regulates cell cycle and apoptosis. Under physiological conditions p53 is found in low levels, however, cisplatin administration has been shown to increase p53 levels in the cochlea. It is speculated that either stress signals such as massive chronic increases in ROS or possible DNA damage may trigger the increase observed in cisplatin ototoxicity. Activation of cochlear p53 after

cisplatin administration has been shown [80, 94] and systemic or transtympanic administration of p53 by pifithrin-α was otoprotective [94]. Reversible inhibition of p53 by localized administration seems to be a reasonable target for translation.

6.3.2 Heat shock proteins (HSP)—Heat Shock protein family consists of constitutively activated as well as inducible stress response proteins. Some members of this family act as molecular chaperones and help in folding, transport, localization and activity of proteins. Stress activated HSP's prevent misfolding, aggregation, and facilitate refolding and removal of damaged proteins [95]. Activation of heat shock proteins (HSP70, HSP27, HSP90) by sound preconditioning has been shown to be otoprotective against cisplatin induced hearing loss, by activating SOD and the anti-oxidant systems in the cochlea [96].

6.3.3 Cyclin dependent kinase 2 (CDK2)—CDK2 belongs to the family of serine/threonine protein kinases, regulates cell cycle and has a role in the G1/S transition. Thus, CDK's have been implicated in tumors and several CDK inhibitors are being tested as anti-cancer therapy. Interestingly, inhibition of CDK2 by kenpaullone when administered transtympanically shows robust otoprotection from cisplatin induced hearing loss [97].

6.3.4 Copper transporter—More et al., successfully demonstrated the use of copper sulfate (CTR1 inhibitor) to be otoprotective in cisplatin ototoxicity. Significant hearing protection were seen at all three frequencies (8, 16, and 32 kHz) [98]. However, due to the known toxicity of copper sulfate newer and less toxic inhibitors need to be developed and tested.

6.3.5 Organic transporter—Ciarimboli et al., successfully showed that co-administration of cimetidine, a substrate for OCT2, with cisplatin reduced otoxicity in mice [99, 100].

6.4 Miscellaneous

Drug coated nanoparticles—Many drugs administered systemically may not have favorable penetration of the blood-labyrinth barrier to achieve therapeutic concentrations in the cochlear tissues. To overcome this challenge, protective agents have been administered locally by transtympanic or intracochlear injection. In addition, drugs incorporated into nanoparticles have been applied to obtain sufficient concentrations in the inner ear to provide protection against cisplatin ototoxicity in preclinical trials (see below).

7. Experimental drug treatments to reduce platinum ototoxicity

7.1 Pre-clinical drug candidates for otoprotection

These experimental drugs broadly belong to either the antioxidant or anti-inflammatory category and work by inhibiting one or both of these pathways. The experimental drugs have been separated according to the route of administration: systemic delivery (table 1) and localized delivery by either the transtympanic, round window or by myringotomy as listed in table 2.

Ease of administration makes systemic delivery a good choice, however otoprotectants delivered by this method may interact with cisplatin, thereby compromising its chemotherapeutic efficacy. Localized transtympanic delivery limits the potential interaction of the otoprotectant with systemic cisplatin and minimizes potential toxicity of systemic administration. The transtympanic delivery method, is a minimally invasive technique that is used extensively in the clinic [101].

7.2 Shortcomings of pre-clinical candidates that prevent clinical translation

There are several reasons that exclude a pre-clinical drug candidate for clinical translations, mainly: **1)** inactivation of cisplatin, as seen with anti-oxidants with thiol groups (NAC, STS, Amifostine) [102–106] or sulfur-containing antioxidant such as d-methionine [107] that directly bind to cisplatin and decrease the availability of cisplatin, **2)** not reaching the inner ear in a high enough concentration in humans to provide protection, such as trans-tympanic administration of dexamethasone [108, 109], **3)** patient compliance is a huge factor, when prescribing oral vitamin E and C [110].

7.3 Pre-clinical drug candidates that show no inhibition of cisplatin's chemotherapeutic ability

Studies of pre-clinical oto-protective drugs showing no effect on cisplatin's tumor killing ability are essential, especially when administered systemically. Thus far, very few studies have shown the co-administration of otoprotective pre-clinical drug with cisplatin in a SCID mouse xenograft study (EGCG, capsaicin, pifithrin-α) [80, 88, 94].

Interestingly, the trend now is towards transtympanic administration of drugs for otoprotection to prevent systemic interactions and side effects. However, the feasibility of repeated trans-tympanic administrations prior to every chemotherapy session in the extremely vulnerable pediatric population or the older adults seems like a daunting task, especially since binaural administration will be required.

The urgent need is to find drug candidates that are able to restore hearing and synapses that can be administered systemically without interfering with the chemotherapeutic ability of cisplatin.

8. Drug candidates in clinical trials

8.1 **Dexamethasone** is a glucocorticoid that has been shown to be otoprotective against cisplatin ototoxicity. Dexamethasone is anti-inflammatory, anti-apoptotic and anti-oxidant. Several preclinical studies showed positive results indicating significant otoprotection in several models of cisplatin ototoxicity by both systemic as well as local route of administration [111–119]. These have been listed in tables 1 and 2. A review of the clinical trials reveal that transtympanic delivery of dexamethasone is the preferred method of delivery. This circumvents the adverse systemic actions of dexamethasone which may protect cancer cells from the chemotherapeutic effect of cisplatin and produce other undesirable systemic effects. However, none of the clinical trials have shown any positive data [108, 109].

8.2 **Sodium thiosulfate (STS),** is a thiol containing compound that has been shown to ameliorate oxidative stress and by supporting antioxidant enzymes such as SOD [103]. STS also forms biologically inactive complexes with cisplatin [104, 105], which not only inactivates cisplatin's chemotherapeutic ability, but also decreases cisplatin-induced toxicity. Thus, it is imperative to administer systemic STS in a time delayed manner after cisplatin treatment. STS administration at 4 hr post cisplatin has been shown to decrease the chemotherapeutic potential modestly, with significant otoprotection seen. This effect was time dependent, with no otoprotection seen when STS was administered at 12h post cisplatin treatment [120]. A randomized Phase 3 clinical trial indicated that STS treatment in young patients receiving cisplatin showed significantly lower likelihood of hearing loss, however, in patients with disseminated disease showed lowered survival rate [121]. In another randomized phase 3 study in patients with localized cancers, STS therapy when administered 6 h post cisplatin compared to cisplatin treatment alone showed lowered incidence of cisplatin ototoxicity without any significant difference in survival rate compared to cisplatin alone. However, the greatest difference between the two groups (those treated with STS and those not receiving STS) was in Brock Grade 0. This grade includes patients with hearing losses less than 40 dB. As discussed in the supplement for this publication, Grade 0 does not mean that hearing was normal. More details on the precise hearing threshold difference between the two groups would have been helpful [122]. Another randomized phase 3 trial, in which cisplatin was administered directly into the nutrient artery of the tumor and STS was given intravenously simultaneously to neutralize systemic cisplatin, showed 10% decrease in hearing loss at 8, 10 and 12.5kHz (speech perception frequencies) [123]. The transtympanic administration of STS gel for the prevention of cisplatin ototoxicity did not show any statistically significant protection [124].

8.3 **N-Acetylcysteine (NAC),** is an acetylated form of cysteine and protects from cisplatin induced ototoxicity by acting as an anti-oxidant, supporting the anti-oxidant system by acting as the precursor for GSH system [125], and finally by forming a complex with cisplatin [102] [105]. Transtympanic administrations of NAC showed protection from cisplatin induced ototoxicity at 8 kHz in two double blind randomized clinical trials [126, 127], but no significant protection in another trial [128]. However oral administration did not provide any significant protection from cisplatin ototoxicity [129].

8.4 **Amifostine:** Amifostine is a prodrug that is dephosphorylated by alkaline phosphatase in cells to a pharmacologically active free thiol metabolite, believed to be responsible for free-radical scavenging, DNA protection and repair acceleration. Amifostine pre-treatments have shown inconsistent results in attenuation of cisplatin induced hearing loss pre-clinical in animal models [66, 130], and showed neurotoxic side effects. Clinical trials have provided inconsistent results. Metastatic melanoma patients treated with amifostine showed no significant otoprotection [131], similar lack of efficacy of amifostine

in otoprotection was seen in the study with children with neuroblastoma or germ cell tumors and were on a chemotherapeutic regimen including cisplatin [132, 133]. Later studies showed that higher doses of amifostine were able to provide significant otoprotection in patients with average-risk (AR) medulloblastoma treated with craniospinal radiotherapy and cisplatin [134]. However, meta-analysis of several studies indicate a trend towards otoprotection but statistical significance was not observed [106]. Thus, large randomized controlled trials are needed.

8.5 **Vitamins and micronutrients:** Vitamin E and Vitamin C are part of the cochlear endogenous antioxidant system. Both these vitamins with either curcumin or selenium have been used in preclinical studies *in vivo* and in clinical trials. In the animal models these antioxidant vitamins have been shown to be strongly otoprotective [135–137], while in clinical trials there was no significant oto-protection seen [110], however, patients with highest plasma concentrations of all three antioxidant micronutrients showed significant amelioration of high-tone hearing. The investigators indicate that lack of oto-protection in the interventional arm of the study was probably due to poor compliance and suggest using higher dose and combination with other antioxidants should be tried.

8.6 **Statins,** are drugs used to treat hypercholesterolemia, and have shown effectiveness in an animal model [138], though the mechanism is thought to be multifaceted and not determined as yet. Statins have been shown to have anti-oxidant and anti-inflammatory effects [139, 140]. The study listed in Table 3 is a purely observational study that has been completed, but no results have been posted as of writing this manuscript.

8.7 **Aspirin,** also known as acetylsalicylic acid (ASA) is widely used to help alleviate pain, fever and inflammation. In a phase II double-blind, randomized controlled trial to determine whether aspirin can protect from cisplatin induced hearing loss, no protection was observed [141].

8.8 **Other antioxidant anti-inflammatory compounds studies in clinical trials that showed no significant protection from cisplatin ototoxicity:** Alpha lipoic acid, ebselen, amifostine, Vitamin E, Vitamin C and selenium have all showed positive results in preclinical studies as listed in tables 1 and 2. However in clinical trials these compounds failed to show any protection from cisplatin induced hearing loss.

9. Current clinical measures

9.1 Subjects at risk

Risk Factors for Cisplatin Related Hearing Loss: There are a number of risk factors that can contribute to increased incidence of ototoxicity of cisplatin. These have been reviewed by Blakley et al., [142] and Paken et al., [143]. A list of confounding factors for cisplatin associate hearing loss are:

- Dosing
  - Method of Administration
  - Number of Cycles
    - Duration
    - Cumulative dose
- Age
  - Younger Children
  - Adults >46 years old
- Renal Insufficiency
- Decreased Serum Albumin Level
- Anemia
  - Decreased Hemoglobin Level
  - Low Red Blood Cell Count
  - Low Hematocrit
- Genetic Factors
- Pre-exposure Hearing Loss
- Other Ototoxic Medications
- Radiation to the Cochlea

9.2 Replacement by less ototoxic analogues

Several lesser toxic analogues of cisplatin have been developed. Those that have been more widely tested for efficacy and ototoxicity are discussed below.

9.2.1 Carboplatin—The development of less toxic platinum analogs include diamine (1, 1-cycloutanedicaroxylato) platinum (II) (JM-8, carboplatin) [11], which has lower nephrotoxicity and neurotoxicity as well as lower emetic potential compared to cisplatin. It has been well documented that the metabolites formed from carboplatin were similar to cisplatin, but the rate of formation was about ten times slower, which meant that carboplatin concentrations needed to be 20 to 40 times higher to produce the same number of compounds and hence lower toxicity [144]. The primary dose-limiting side effect was bone marrow toxicity leading to leukopenia and thrombocytopenia, both of which were reversible in 4 – 6 weeks after the treatment [11]. Carboplatin is used for the treatment of ovarian cancer and acute leukemia, lymphomas, breast cancers, melanomas, gastro-intestinal cancers, including gastric and esophageal, as well as colorectal cancers. Lower neurotoxic and nephrotoxic potential also makes it a great agent for palliative management in oncology [11]. Carboplatin is less ototoxic than cisplatin [145].

9.2.2 Oxaliplatin—The search for agents with a better anti-cancer spectrum and lower side effect potential led to the development of third-generation platinum drugs with oxaliplatin (trans-L-diaminocyclohexane oxalate platinum (II) being the most prominent of them [11, 146]. Oxaliplatin was initially discovered in 1976 by Professor Kidani at Nagoya City University, Japan and was granted U.S.patent in 1979 [147]. Interestingly, prior to the discovery and approval of oxaliplatin, the use of platinum drugs for colorectal cancer was not widely accepted [148, 149]. The response rates for cisplatin were around 19–20%, but these response rates markedly improved to around 50% with oxaliplatin [150]. Ototoxicity of oxaliplatin appears to be very rare. Only a few isolated case reports have been published [151–153].

9.2.3 Nedaplatin—Nedaplatin (cis-diammine-glycolatoplatinum), is a second generation cisplatin analog with two ammine ligands. It was developed in 1983 by Shionogi Pharmaceutical Company, Japan, and was designed to offer chemotherapeutic efficacy comparable to cisplatin, yet cause a lower incidence of gastrotintestinal and renal toxicity. Phase II studies suggested that nedaplatin might be effective in treatment of patients with esophageal cancer [154] head and neck cancer, non-small cell lung cancer, malignant neoplasms of the uterine cervix or urothelial cancer [155]. A randomized phase III clinical trial of nedaplatin vs. cisplatin showed that patients with nasopharyngeal carcinoma treated with nedaplatin had only half the incidence of grade 3 or 4 ototoxicity of that found in patients treated with cisplatin [156].

9.2.4 Satraplatin—Satraplatin (JM216) is a platinum derivative that produces cytotoxicity in tumor cells by forming DNA crosslinks leading to apoptosis. The advantage of this agent is that it is given orally. Satraplatin is under investigation for treatment of patients with advanced prostate cancer who have failed previous chemotherapy. It has not yet received approval from the U.S. Food and Drug Administration. No hearing loss was observed in a phase I clinical trial of satraplatin in children and young adults with refractory solid tumors [157].

The continuing development of less ototoxic platinum agents has the potential to reveal novel agents with a broad spectrum of antineoplastic activity. Also, novel formulations of cisplatin such as liposomal encapsulation could produce less toxic methods of delivery of higher doses of cisplatin to tumor targets.

9.3 Ototoxic interactions between cisplatin and other agents

9.3.1 Aminoglycosides—Aminoglycosides are broad-spectrum antibiotics used to treat tuberculosis and gram-negative infections, including life-threatening sepsis in cancer patients [158]. Some of these patients will be undergoing treatment with cisplatin chemotherapy. Pre-clinical studies in guinea pigs indicate that combination of kanamycin or gentamicin with cisplatin appeared to cause additive damage to the cochlea [159, 160]. However,clinical studies indicate that the risk of ototoxicity was not significantly increased in patients with ovarian cancer receiving cisplatin plus aminoglycosides compared with those who received cisplatin alone [161]. Survivors of cancer in children treated with

cisplatin developed hearing loss. However, the risk of hearing loss was not increased in those children treated with aminoglycosides in addition to cisplatin [162].

9.3.2 Loop diuretics—Loop diuretics act on the loop of Henle in the kidney to increase the clearance of electrolytes and water and help to alleviate pulmonary edema. Loop diuretics may be administered to patients receiving cisplatin chemotherapy to alleviate congestive heart failure and pulmonary edema and in low doses may be used to protect the kidneys against cisplatin induced acute kidney injury [163, 164]. Several preclinical studies have shown that cisplatin ototoxicity is greatly increased when combined with a loop diuretic [165–168]. Clinical studies revealed that the risk of increased ototoxicity in patients treated with a combination of furosemide and cisplatin seem to depend on patient age. A significant increased risk of ototoxicity in survivors of pediatric cancers treated with furosemide and cisplatin was reported [162]. However, in adult patients treated with furosemide combined with cisplatin with higher doses, did not exhibit increased risk of ototoxicity compared to patients treated with cisplatin alone [169]. Ovarian cancer as well as testicular cancer patients treated with cisplatin with or without furosemide did not differ significantly in the incidence of hearing loss, even those treated with higher doses of cisplatin compared to patients receiving cisplatin alone. Furosemide did not appear to potentiate cisplatin ototoxicity in the adult population [161]. Thus, the effects of furosemide in pediatric patients appear to differ from those reported in adults.

9.3.3 Other chemotherapeutic drugs—1) Patients with testicular cancer treated with high doses of **vincristine** combined with cisplatin demonstrated reversible ototoxic symptoms. Final hearing was similar in patients treated with both drugs to hearing in patients treated with cisplatin alone. High cumulative doses of cisplatin were significantly associated with greater hearing loss [169]. **2)** An increased incidence of ototoxicity was reported in children with cancer who survived after treatment with cisplatin in combination with **carboplatin**. For carboplatin alone, the frequency of hearing loss was 17%. For cisplatin alone, 45% were found to have hearing loss. But among patients who received both cisplatin and carboplatin, the incidence of hearing loss was 75%. Thus, there appears to be a synergistic ototoxicity among children treated with both drugs [162].

9.3.4 Caffeine—Caffeine has been shown to increase the incidence of ototoxicity in cisplatin treated rats in a dose dependent manner. Caffeine treatment alone (single or multiple doses) did not show any ototoxicity. Caffeine administered orally (15mg/kg) as a single dose was found to exacerbate cisplatin-induced hearing loss while multiple doses of caffeine not only potentiated cisplatin induced hearing loss, but also potentiated the associated synaptopathy [170]. Thus, a possible drug-drug interaction appears to occur between caffeine and cisplatin, indicating sensitization of the cochlea to cisplatin. Dose conversion shows that 15mg/kg in the rat model is equivalent to ~145 mg of caffeine in a grown adult (60kg). A typical cup of coffee contains 80–120 mg of caffeine. It may serve to caution patients receiving cisplatin chemotherapy against excessive caffeine intake on infusion days.

9.3.5 Effects of concomitant radiation therapy in different cancers—Cisplatin ototoxicity has been shown to be increased in patients treated with concomitant radiation therapy. Ionizing radiation induces oxidative stress in cancer cells and exerts cytotoxic effects through reactive oxygen species [171]. The additional ROS from radiation to the cochlea could add to oxidative stress in the cochlea induced by cisplatin chemotherapy, exacerbating its ototoxicity. This has been confirmed in patients with head and neck cancer, including nasopharyngeal cancer and in children treated for medulloblastoma.

Radiation therapy that exposes the cochlea to doses greater than 45 Gy increases the risk of hearing loss in patients receiving cisplatin chemotherapy. Schuette et al., recently reported an accurate prediction model for post-treatment hearing in head and neck cancer patients treated with cisplatin and radiation therapy. They determined that age, baseline audiometric pure tone average for 1, 2 and 4 kHz, cisplatin dose and mean radiotherapy dose to the cochlea were associated with hearing after treatment. This model predicted a greater role for cisplatin dose than cochlear radiation dose in post-treatment hearing. For every increase in cisplatin dose of 100 mg/m2 the model predicted an increase of hearing threshold of 3 dB at 1, 2 and 4 kHz. For every increase in radiation dose of 10 Gy, hearing threshold at these frequencies increased 1 dB [172]. The risks of radiation injury to the cochlea appear to be diminished by the implementation of intensity modulated radiation therapy (IMRT). IMRT leads to a low rate of severe ototoxicity. Keeping the median radiation dose to auditory structures below 42 Gy helps to reduce ototoxicity from combined cisplatin and radiation therapy [173].

9.3.6 Concomitant exposure to noise—Moderate levels of noise exposure have been shown to exacerbate cisplatin induced hearing loss in the chinchilla model with significantly higher loss of hearing and hair cells at higher frequencies. This deleterious interaction of noise with cisplatin was observed at 85dB or higher exposure but not at 70dB exposure [174, 175]. Noise exposure preceding cisplatin treatment has been shown to augmented hearing loss in the guinea pig model [176]. Similar effects were seen in testicular cancer patients treated with cisplatin chemotherapy, wherein previous noise exposure increased the risk of cisplatin induced hearing loss by three fold [169]. Thus, prolonged exposure of moderate to higher levels of noise should be avoided prior to cisplatin chemotherapy.

9.4 Otoprotective measures that are currently suitable for clinical trials

A critical review of clinical trials of protective agents in pediatric patients was recently published in the Cochrane Review in 2019 [177]. A list of drugs in clinical trials have been shown in Table 3.

9.4.1 Sodium thiosulfate—The most promising protective agent against cisplatin ototoxicity is sodium thiosulfate. Clinical trials in pediatric cancer patients, the IV administration of sodium thiosulfate 6h after cisplatin injection showed a significant reduction in hearing loss compared to children receiving cisplatin alone [121, 122]. Promising initial results in adults receiving intra-arterial cisplatin combined with IV STS led to decreased hearing loss These findings should be extended to additional clinical trials in both pediatric and adult patients.

9.4.2 N-acetyl-cysteine (NAC)—Intra-tympanic administration of NAC provided some protection against cisplatin induced hearing loss in a preliminary clinical study. Additional trials would be helpful to confirm or refute these findings.

9.4.3 Amifostine—Amifostine trials have provided conflicting data. Otoprotection has been reported in some clinical trials but not in others. More large scale randomized controlled clinical trials would help to clarify whether amifostine would be helpful to prevent cisplatin ototoxicity without adversely affecting patient survival.

There is an urgent need to confirm promising results with STS, NAC and amifostine. New agents should be tested to find more robust and convincing otoprotection without adversely impacting on patient survival.

10. Conclusion

There are several very elegant preclinical candidates that have been discussed above. It will be important to establish that the systemically administered preclinical otoprotective drugs do not interfere with cisplatin's chemotherapeutic efficacy. The drugs that have been shown to be otoprotective when administered transtympanically, are challenging to dissolve and deliver in a high enough concentration to be effective in humans as evidenced by the dexamethasone clinical trials. In addition, there are no standardized universally accepted guidelines for hearing evaluation and ototoxicity grading methods, thus making the interpretations of the clinical studies difficult. Thus, guidance on the uniform study design and evaluation of ototoxicity will enable future clinical trials to deliver robust data. The current drugs in clinical trials belong to the thiol containing compounds that neutralize cisplatin in varying degrees and have been partially successful. Perhaps, the use of different compounds that inhibit upstream targets of the ototoxicity pathway will yield better results, especially when there are several preclinical studies showing effectiveness by local delivery. Another approach of using combination drugs that will inhibit the ototoxic pathway at several points seems to be a viable option. Finally, there is an urgent unmet need to find safe effective treatments for cisplatin induced hearing loss, which can be particularly devastating in the pediatric population and have debilitating consequences on patients' quality of life.

11. Expert opinion

Cisplatin remains a highly effective and frequently used chemotherapeutic agent for a variety of solid tumors. Unfortunately, its use is associated with a high incidence of irreversible sensorineural hearing loss. It is critical that protective agents to prevent or ameliorate this side effect that has such a great impact on the quality of life. This is particularly true of children who have survived following a treatment with cisplatin. Significant increase in the incidence of hearing loss occurs when radiation therapy is combined with cisplatin, particularly when the inner ear receives a significant dose of irradiation. Damage to the cochlea by cisplatin is characterized by loss of sensory cells, especially outer hair cells. Mechanisms that are involved in this process include DNA damage, oxidative stress and inflammation that lead to cell death. Preclinical studies have targeted ROS by the administration of anti-oxidants. Other strategies have sought to reduce

cochlear inflammation. The caveat for systemic administration of otoprotective agents is the risk of interference with cisplatin's therapeutic effect. Therefore, a number of preclinical investigations have used local therapy with putative protective agents to target the cochlea and to avoid systemic effects and to provide greater drug concentrations in the cochlea. Clinical trials to date have been mostly inconclusive. Delayed systemic administration of sodium thiosulfate shows some promise in reducing cisplatin ototoxicity in children in two trials, but in the first trial increased mortality was shown in children with disseminated cancer. Future clinical trials are urgently needed to effectively reduce the ototoxicity of cisplatin and thus to improve the quality of life of cancer survivors.

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Declaration of interest

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Abbreviations

CDDP	Cis-dichlorodiammineplatinum
GI	Gastrointestinal
SAR	Structure-activity-relation
FDA	Food and Drug Administration
OCT	Organic Cation Transporter
CTR	Copper Transporter
ATOX1	Antioxidant Protein 1
SLC	Solute Carrier
PARP-1	Poly ADP-ribose Polymerase 1
TNFR	Tumor Necrosis Factor Receptor
RIP1	Receptor Interacting Protein
RIPK1	Receptor Interacting Serine/Threonine Protein Kinase 1
MLKL	Mixed Lineage Kinase Domain-like Protein
ROS	Reactive Oxygen Species
DRG	Dorsal Root Ganglion

CNS	Central Nervous System
BBB	Blood Brain Barrier
ABR	Auditory Brainstem Recording
OHC	Outer Hair Cells
DPOAE	Distortion Product Oto-Acoustic Emissions
EP	Evoked Potential
IHC	Inner Hair cell
SGN	Spiral Ganglion Neurons
SNHL	Sensorineural Hearing Loss
IMRT	Intensity Modulated Radiation Therapy
PNET	Primitive Neuro-ectodermal Tumor
GSTP1	Glutathione S-transferase P 1
Bax	BCL-2-associated X protein
BCL2	B-cell lymphoma 2
MAPK	Mitogen-activated Protein Kinase
ERK	Extracellular signal-regulated Kinase
NOX	NADPH Oxidase
NADPH	Nicotinamide Adenine Dinucleotide Phosphate H
XO	Xanthine Oxidase
GSH-Px	Glutathione Peroxidase
RNS	Reactive nitrogen species
4-HNE	4-hydroxynonenal
NOS	Nitric Oxide Synthases
L-NAME	L-arginine methyl ester
LMO4	LIM Domain only 4
Nrf2	Nuclear factor erythroid 2-related factor 2
HO-1	Heme oxygenase −1
NQO1	NADH:Quinone Oxidoreductase1
IL	Interleukin

TRPV1	Transient receptor potential vanilloid 1
STAT	Signal transducer and activator of transcription
COX2	Cycloxygense 2
EGCG	Epigallocatechin Gallate
CD	Cluster Differentiation
GPCR's	G-Protein Coupled Receptors
A1AR	A_1 adenosine receptors
CB2	Cannabinoid receptor 2
SIP2	Sphingosine 1-Phosphate Receptor 2
RPIA	R-phenylisopropyladenosine
SOD	Superoxide Dismutase
HSP	Heat shock proteins
STS	Sodium thiosulphate
NAC	N-Acetylcysteine
CDK	Cyclin-dependent Kinase

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Article highlights

- Cisplatin is an “essential”, chemotherapeutic drug that is widely used to treat solid tumors, with severe side effects including dose limiting ototoxicity and neurotoxicity.
- This manuscript discusses a brief history, pharmacology, toxicities, drug interactions, mechanisms of ototoxicity and molecular targets in pre-clinical and clinical trials.
- Cisplatin induced chronic ROS generation and inflammation are cross connected and seem to be one of the major pathways for related ototoxicity.
- Most pre-clinical and clinical experimental drugs that are otoprotective belong to two broad categories of anti-oxidants and anti-inflammatory agents.
- The pre-clinical use of new drugs such as CDK2 inhibitors, GPCR agonists and lovastatin provide exciting new areas of focus in prevention of cisplatin induced hearing loss.
- An intriguing trend in treatment of cisplatin induced hearing loss seems to be the localized route of drug delivery by transtympanic injections.

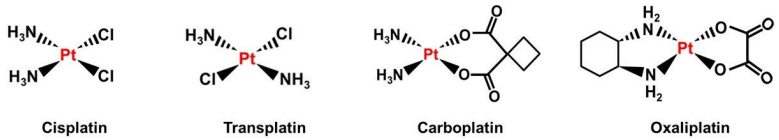

Figure 1:
Structure of different platinum analogs.

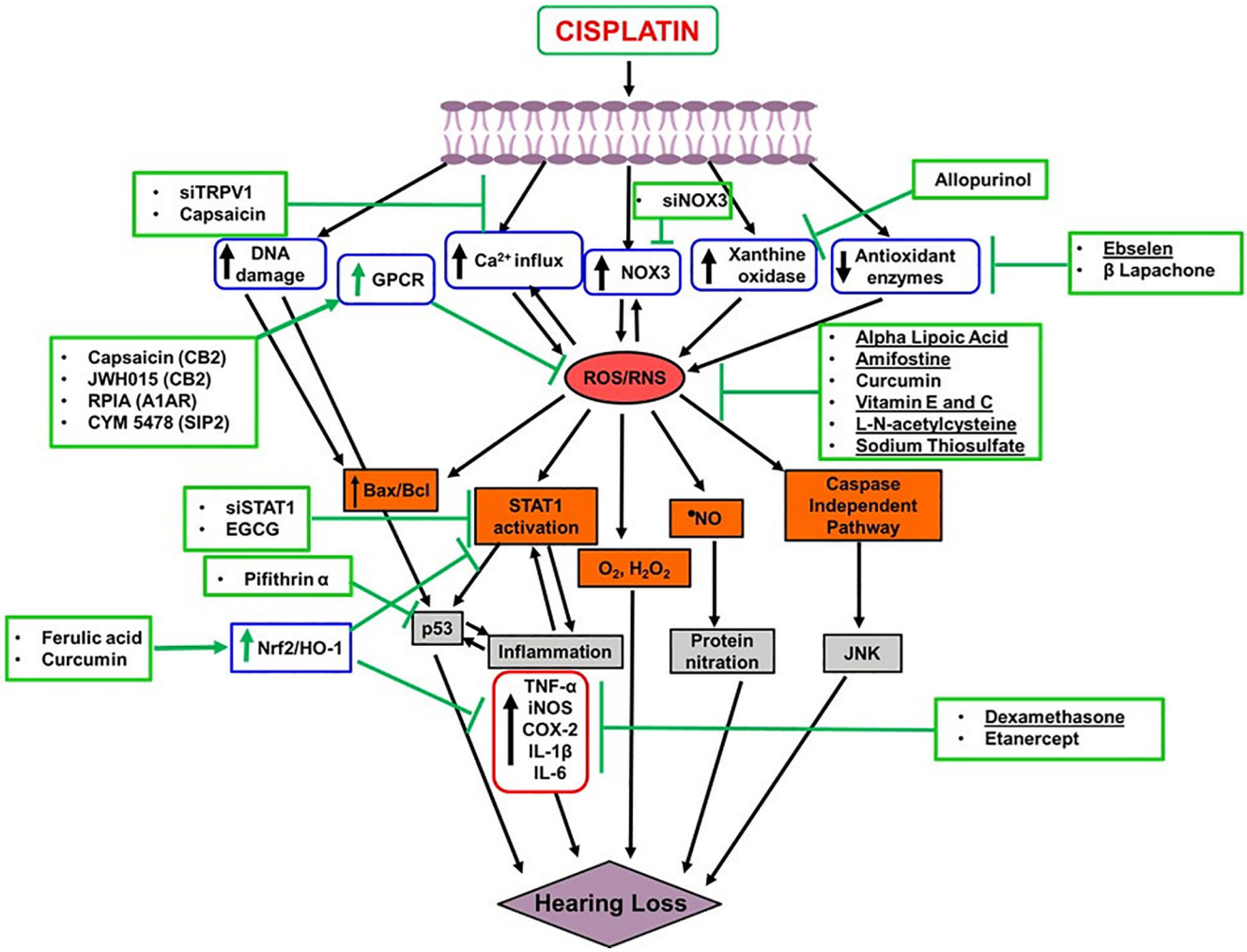

Figure 2:

Cisplatin induced hearing loss: mechanisms and drug targets. Drugs in clinical trials are underlined.

Table 1:

Systemic Administration: Potential experimental drugs and their targets for treatment of platinum induced ototoxicity.

Experimental Drug		Animal Model	Route of Administration	Mechanism of Action	Reference
1	**Allicin**	Rat	Intraperitoneal	Anti-oxidant	[178]
2	**Alpha-Lipoic Acid**	Mouse	Intraperitoneal	Anti-oxidant	[179]
3	**Allopurinol**	Rat	Oral	Xanthine oxidase inhibitor	[65]
4	**Ebselen**	Rat	Oral	Glutathione peroxidase mimic	[65, 180]
5	**Allopurinol and Ebselen combination**	Rat	Oral	Combination of inhibition of Xanthine oxidase and glutathione peroxidase mimic	[65]
6	**Erdosteine**	Rat Guinea pig	Intraperitoneal	Anti-oxidant and free radical scavenger	[181] [182]
7	**Eugenol**	Rat	Intraperitoneal	Anti-oxidant, anti-inflammatory	[183]
8	***Amifostine**	Hamster	Intraperitoneal	Free radical scavenger	[130]
9	**Bucillamine**	Mouse	Intraperitoneal	Anti-oxidant	[184]
10	**β-Lapachone (NAD+)**	Rat	Oral	Anti-oxidant	[78]
11	**Capsaicin**	Rat	Oral	TRPV1 agonist that desensitizes CB2R agonist	[88]
12	**Curcumin**	Rat	Intraperitoneal	Upregulation of Nrf2/HO-1 pathway and modulating the p53, STAT3 and NF-Kappa B activation	[77]
13	**Nano-encapsulated curcumin and dexamethasone**	Guinea pig	Intraperitoneal	Anti-oxidant, anti-inflammatory	[185]
14	**EGCG**	Rat	Oral	STAT1 inhibition	[80]
15	**Ferulic Acid**	Rat	Intraperitoneal	Upregulation of Nrf2/HO-1 pathway and modulating the p53, STAT3 and NF-Kappa B activation	[77]
16	**D-Methionine (D-Met)**	Rat	Intraperitoneal	Anti-oxidant molecule	[186]
17	**Vitamin E (α-tocopheryl succinate)**	Guinea pig, Rats	Intraperitoneal	Free radical scavenger	[137] [135]
18	**Curcumin and Vitamin E combination**	Rat	Intraperitoneal	Free radical scavenger	[136]
19	**CYM-5478, (Sphingosine 1-Phosphate Receptor 2 agonist)**	Rat	Intraperitoneal	Anti-oxidant and SIP2 agonist	[93]
20	**Lovastatin**	Mice	Oral Gavage	Commonly used drug for management of hypercholesterolemia by inhibiting 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) enzyme	[138]
21	**Pifithrin-α**	Mice	Intraperitoneal	p53 inhibitor	[94]
22	**Salicylate**	Rat	Intraperitoneal, subcutaneous	Anti-oxidant	[187] [188]
22	**Dexamethasone loaded nanoparticles (PEG-PLA)**	Guinea pigs	Intraperitoneal	Anti-inflammatory	[111, 119]

*side effect: neurotoxicity

Table 2:

Local administration: Potential experimental drugs and their targets for treatment of platinum induced ototoxicity.

	Experimental Drug	Animal Model	Mechanism of Action	Reference
1	**Epicatechin**	Rat	Anti-oxidant, anti-inflammatory	[189]
2	**Capsaicin**	Rat	TRPV1 agonist that desensitizes, CB2R agonist	[88]
3	**Copper sulfate**	Mouse	CTR1 inhibitor	[98]
4	*D-Methionine (D-Met)	Chinchilla, Guinea pig	Anti-oxidant	[190, 191]
5	**Vitamin E**	Rat	Anti-oxidant	[112]
6	**Vitamin C**	Rat	Anti-oxidant	[192]
7	**Dexamethasone**	Rat	Anti-inflammatory	[112–114]
		Mouse		[115]
		Aged mouse		[116]
		Guinea pig		[117–118]
8	**Dexamethasone OTO-104**	Guinea pig	Anti-oxidant	[108]
9	**Etanercept**	Rat	TNF-alpha inhibitor	[82]
10	**JWH-015, (2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone)**	Rat	CB2R agonist	[92]
11	**Kenpaullone**	Mouse, Rat	Cyclin-dependent kinase-2 inhibitor	[97]
12	**KR-22332, (3-amino-3-(4-fluoro-phenyl)-1H-quinoline-2,4-dione)**	Rat	Suppresses ROS	[193]
13	*L-methionine	Rat	Anti-oxidant	[194]
14	**L-N-acetylcysteine**	Guinea pig (myringotomy)	Anti-oxidant	[195]
15	**Lactated Ringer's**	Guinea pig	----	[195]
16	**Magnetic Nanoparticle Mediated Steroid Delivery**	Mouse	Anti-inflammatory	[196]
17	**Melatonin**	Rat	Anti-oxidant	[197]
18	**Pifithrin-α**	Mouse	p53 inhibitor	[94]
19	**Resveratrol**	Rat	Anti-oxidant	[113]
20	**TRPV1 siRNA**	Rat	Decrease ROS	[86]
21	**NOX3 siRNA**	Rat	Decrease ROS	[81]
22	**STAT1 siRNA**	Rat	Anti-inflammatory	[82]
23	#Sodium thiosulphate	Guinea pig	Anti-oxidant	[198]
24	**Polymeric nanoparticles loaded with dexamethasone or α-tocopheryl succinate**	Rat	Anti-inflammatory, antioxidant	[199]
25	**pH-sensitive polymeric nanoparticles**	Mouse	Anti-oxidant and anti-inflammatory	[200]
26	**Dexamethasone-loaded chitosan-based genipin-cross-linked hydrogel (CBGCH)**	Guinea pigs	Anti-inflammatory	[201]
27	*A666-conjugated nanoparticles targeting prestin	Guinea pig	Anti-inflammatory	[202]
28	**Thiosulfate-hyaluronan gel**	Guinea pig	Platinum chelator	[203]

Experimental Drug		Animal Model	Mechanism of Action	Reference
29	**Trolox**	Guinea pig	Anti-oxidant	[204]
30	**R-PIA**	Rat	Adenosine A1R agonist	[91]
31	** **Non-invasive cool water**	Guinea pig		[205]

* round window administration

** external ear irrigation

cochlear perfusion

Table 3:

Drugs in clinical trials for treatment of platinum induced ototoxicity.

Experimental Drug		Study Title/Clinical Trial #	Route of administration	Study Results	Status	Reference
1	**OTO-104**	Study of OTO-104 in Subjects at Risk from Cisplatin-Induced Hearing Loss, NCT02997189	Transtympanic	(Negative Efficacy Results from the recently completed Phase 3 study 104–201506)	Terminated	[108]
2	**Dexamethasone Phosphate**	Prevention of Cisplatin-Induced Hearing Loss by Transtympanic Dexamethasone Treatment, NCT01372904	Transtympanic	Significant attenuation of hearing loss at 6kHz.	Completed	[109]
3	**Sodium Thiosulfate (STS)**	Sodium Thiosulfate in Preventing Hearing Loss in Young Patients Receiving Cisplatin for Newly Diagnosed Germ Cell Tumor, Hepatoblastoma, Medulloblastoma, Neuroblastoma, Osteosarcoma, or Other Malignancy, NCT00716976	Intravenous	Likelihood of hearing loss was significantly lower in the STS group, **patients with disseminated disease showed lower survival rate with STS **	Completed	[121]
		Cisplatin with or without Sodium Thiosulfate in Treating Young Patients with Stage I, II, or III Childhood Liver Cancer (SIOPEL6), NCT00652132	Intravenous	Lowered incidence of cisplatin induced hearing loss in children with hepatoblastoma	Completed	[122]
		Ototoxicity in a Randomized Phase III Trial of Intra-Arterial Compared with Intravenous Cisplatin Chemoradiation in Patients With Locally Advanced Head and Neck Cancer	Intravenous	10% less hearing loss	Completed	[123]
		Efficacy of Transtympanic Injections of a Sodium Thiosulfate Gel to Prevent Cisplatin-induced Ototoxicity (STS001)	Transtympanic	No statistically significant protection seen	Terminated due to poor accrual	[124]
4	**N-Acetylcysteine**	Transtympanic Injections of N-acetylcysteine for the Prevention of Cisplatin-induced Ototoxicity A Feasible Method with Promising Efficacy	Transtympanic	Statistically significant protection with NAC at 8kHz	Completed	[127]
		Protective Role of N-acetylcysteine From Cisplatin-induced Ototoxicity in Patients with Head and Neck Cancer, NCT03400709	Oral Tablet	No Results Available	Completed	No peer reviewed publication
		Transtympanic Injections of N-acetylcysteine and Dexamethasone for Prevention of Cisplatin-Induced Ototoxicity: Double Blind Randomized Clinical Trial	Transtympanic	Statistically significant protection with NAC at 8kHz	Completed	[126]
		The use of A-Acetylcysteine attenuating cisplatin-induced toxicities by oxidative stress, NCT 02241876	Oral	No significant protection observed at low doses of NAC	Completed	[129]
5	**Statins**	Hearing Loss and the Effects of **Statin Drugs** in People with Head and Neck Squamous Cell Carcinoma Treated with	**Observational**	No results Available	Completed	No peer reviewed publication

Experimental Drug		Study Title/Clinical Trial #	Route of administration	Study Results	Status	Reference
		Cisplatin Chemoradiation, NCT03225157				
6	**Aspirin**	COAST (Cisplatin ototoxicity attenuated by aspirin trial): A phase II double-blind, randomized controlled trial to establish if aspirin reduces cisplatin induced hearing-loss.	Oral	No protection observed	Completed	[141]
7	**Alpha-lipoic acid**	Alpha-Lipoic Acid in Preventing Hearing Loss in Cancer Patients Undergoing Treatment with Cisplatin, NCT00477607	Oral	No protection observed	Completed	No peer reviewed publication
8	**Ebselen (SPI-1005)**	SPI-1005 for Prevention and Treatment of Chemotherapy Induced Hearing Loss, NCT01451853	Oral	No Results Available	Unknown status	No peer reviewed publication
9	**Vitamin E**	Vitamin E neuroprotection against cisplatin ototoxicity: Preliminary results from a randomized, placebo-controlled trial.	Oral	Significant hearing protection at 2 and 8 kHz	Completed	[206]
10	**Vitamin E, Vitamin C and Selenium**	Supplementation with antioxidant micronutrients and chemotherapy-induced toxicity in cancer patients treated with cisplatin-based chemotherapy: a randomised, double-blind, placebo-controlled study.	Oral	No significant protection observed	Completed	[110]
11	**Amifostine**	Meta-analysis of the efficacy of amifostine in the prevention of cisplatin ototoxicity	Intravenous	This meta-analysis reveals a trend toward decreased ototoxicity in patients receiving amifostine infusion prior to receiving cisplatin chemotherapy. However, the results did not reach statistical significance. Further large randomized, controlled trials of amifostine use to prevent cisplatin-induced ototoxicity are needed.		[106]