Mechanisms of Cisplatin-Induced Ototoxicity and Prevention

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ABSTRACT

Cisplatin is a highly effective antineoplastic agent used to treat solid tumors. Unfortunately, the administration of this drug leads to significant side effects, including ototoxicity, nephrotoxicity, and neurotoxicity. This review addresses the mechanisms of cisplatin-induced ototoxicity and various strategies tested to prevent this distressing adverse effect. The molecular pathways underlying cisplatin ototoxicity are still being investigated. Cisplatin enters targeted cells in the cochlea through the action of several transporters. Once it enters the cochlea, cisplatin is retained for months to years. It can cause DNA damage, inhibit protein synthesis, and generate reactive oxygen species that can lead to inflammation and apoptosis of outer hair cells, resulting in permanent hearing loss. Strategies to prevent cisplatin ototoxicity have utilized antioxidants, transport inhibitors, G-protein receptor agonists, and anti-inflammatory agents. There are no FDA-approved drugs to prevent cisplatin ototoxicity. It is critical that potential protective agents do not interfere with the antitumor efficacy of cisplatin.

KEYWORDS: cisplatin, otoprotection, antioxidants, apoptosis, cochlea

Cisplatin has been widely utilized to treat various solid tumors since it was approved by the Food and Drug Administration (FDA) in 1978. Tumors treated by cisplatin include adults with head and neck cancer and testicular, ovarian and lung cancers. Cisplatin is a key chemotherapeutic agent used to treat neuroblastoma, osteosarcoma, hepatoblastoma, germ cell tumors, medulloblastoma, and other pediatric cancers.[1] In a recent study, most patients (388 of 488, 80%) had a hearing loss of at least 20 dB and 40% suffered from tinnitus.[2] Sixty-three to 77% of children[3,4] suffer permanent sensorineural hearing loss

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from cisplatin chemotherapy. Cisplatin primarily affects the high frequencies in both ears and is permanent. The loss of hearing can cause a severe impact on the quality of life, particularly in young children. Such hearing loss can result in delayed speech development and seriously affect cognitive and psychosocial development when it occurs in very young children.[5] Thus, it is critically important that effective treatments to prevent or ameliorate the ototoxicity of cisplatin be developed. Currently, there are no FDA-approved treatments available. This article reviews the mechanisms of cisplatin ototoxicity and potential protective strategies.

EFFECTS ON COCHLEAR FUNCTION

Guinea pigs treated with cisplatin demonstrated shifts in compound action potential (CAP) amplitude growth curves that were greater at the higher frequencies. They also were observed to have shifts in the cochlear microphonic (CM) amplitude growth curves that appeared to be smaller than those for the CAP.[6] Distortion product otoacoustic emissions (DPOAEs) were reported to be diminished in cisplatin-treated gerbils[7] and mice.[8] Auditory brainstem responses (ABR) in cisplatin-treated animals demonstrate increased thresholds, with greatest effects in the higher frequencies.[8,9] Rats[10] and mice[8] demonstrated reduction in the endocochlear potential (EP) following cisplatin administration.

EFFECTS ON COCHLEAR MORPHOLOGY

Cisplatin appears to target at least three major tissue areas in the cochlea: organ of Corti, spiral ganglion cells (SGCs), and lateral wall (stria vascularis and spiral ligament). Cisplatin damages both the outer hair cells (OHCs) and the SGCs in the guinea pig.[6] Type I SGCs demonstrated detachment of their myelin sheaths. Injury to both OHCs and SGCs occurred in parallel, rather than sequentially.[6] Rats treated with cisplatin showed damage to the basal turn stria vascularis: edema, bulging, rupture, and compression of the marginal cells with loss of organelles from the cytoplasm.[11] Guinea pigs evaluated for more than 4 weeks after cisplatin treatment showed diminished area of the stria, caused mostly by decrease in the areas of the intermediate and marginal cells.[12] Cells in the organ of Corti, primarily the OHCs, and SGCs in the basal turn of the gerbil cochlea demonstrated apoptosis after cisplatin administration. By contrast, the stria vascularis demonstrated TUNEL-positive staining in all three turns.[7] Type I spiral ligament cells also undergo significant apoptosis after cisplatin exposure in vitro. This was related to cisplatin blockage of BK channels.[13] Normal hearing depends on ribbon-dependent synchronous release of multiple vesicles at the hair cell afferent synapse.[14] A recent study reported that rats treated with cisplatin showed a significant reduction in the average number of synaptic ribbons on each inner hair cell (IHC) in the basal and middle, but not in the apical turn by means of the synaptic marker, C-terminal binding protein 2 (CtBP2).[15]

PHARMACOKINETICS

Guinea pig studies demonstrated rapid achievement of high levels of cisplatin in the basal turn scala tympani with delayed elimination relative to serum. This could account for the preferential damage to the basal turn of the cochlea.[16] Cisplatin demonstrates a biphasic clearance pattern in humans receiving an intravenous infusion. Plasma half lives in patients were 23 minutes and 6 hours. Excretion into the urine is approximately 17% within 24 hours. Cisplatin is strongly bound to serum proteins. Thus, the half-life of total platinum in serum is much longer than that of free cisplatin.[17] Cisplatin is retained in the cochlea for months to years in mouse and human cochlea as shown in temporal bone studies. The level of cisplatin is very high in the stria vascularis.[8]

UPTAKE MECHANISMS

The cochlea has several transport mechanisms that could influence the uptake of cisplatin.[18,19] The copper transporter (Ctr1) is strongly expressed in tissues targeted by cisplatin,

namely, OHCs, IHCs, stria vascularis, and spiral ganglion neurons.[20] The organic cation transporter (OCT2) is expressed in the organ of Corti and stria vascularis. Pharmacologic blockade of this transporter protects against cisplatin ototoxicity.[21] Mechanotransduction (MET) channels may be involved in the uptake of cisplatin. Inhibition of MET channels protects against cisplatin-induced damage to hair cells in zebrafish, and zebrafish mutants lacking these channels were shown to be resistant to cisplatin-induced cell death.[21]

MOLECULAR MECHANISMS

The molecular mechanisms that underlie cisplatin ototoxicity are reviewed by Hazlitt et al.[21] After transport into the cells of the cochlea, cisplatin undergoes hydrolysis to form aqua cisplatin complexes. These complexes are highly reactive and can damage DNA.[22] DNA damage triggers ataxia telangiectasia mutated (ATM), which activates p53.[23] Activation of p53 increases the level of Bax, a proapoptotic protein that increases mitochondrial membrane permeability, leading to cytochrome c release which activates caspase 3.[24]

Cisplatin activates the cochlear-specific NADPH oxidase, NOX-3. This results in an increase in reactive oxygen species (ROS) in the cochlea.[19] This can produce lipid peroxidation and deplete the levels of antioxidant enzymes and also cause mitochondrial cytochrome c release resulting in apoptosis.[21]

Cisplatin also increases the production of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), and nuclear factor kappa B (NF-kB).[25] NF-kB activation can induce the production of more proinflammatory cytokines, activation of caspases 3 and 9, and increase in expression of inducible nitric oxide synthase (iNOS), leading to an increase in the free radical nitric oxide (NO).[21] Cisplatin also increases the expression of signal transducer and activator of transcription-1 (STAT-1) and reduces the expression of signal transducer and activator of transcription-3 (STAT-3 in the cochlea. These changes promote inflammation apoptosis of OHCs and hearing loss.[26]

RISK FACTORS FOR OTOTOXICITY

Risk factors include young age (children under 5 years of age),[27,28] male children,[29] elderly patients, cumulative dose > 400 mg/m,[2,27] noise exposure,[30] combination with other ototoxic drugs[31] including carboplatin,[32] nutritional depletion and anemia,[33] cranial irradiation,[31] and genetic predisposition (pharmacogenomics).

Pharmacogenomics

Various genes that code for enzymes involved in the transport or metabolism of cisplatin or DNA repair may be altered in patients and could either enhance or reduce susceptibility to cisplatin ototoxicity. Lanvers-Kaminsky and Ciarimboli[34] recently summarized articles that reported genes and single nucleotide polymorphisms (SNPs) associated with either increased or decreased susceptibility of patients to cisplatin ototoxicity. Contradictory reports have shown that some of these genetic factors have negative effects, some have protective effects, and others have no effect. Japanese patients may be more likely to suffer cisplatin ototoxicity.[35] Genes and SNPs that have been shown to be associated with increased susceptibility to cisplatin ototoxicity include *XPC* (rs228001); *LRP2* (rs2075252), (rs2228171); SLC31A1 (rs10981694); *SOD2* (rs4880); *TMPT* (rs12201199); *COMT* (rs9332377); *ABCC3* (rs1051640); and *ACYP2* (rs1872328).[34] A more recent study showed increased risk of hearing loss in cisplatin-treated pediatric patients with the null genotype for *GSTT1*, the A/A genotype at rs1695, and the C/C genotype at rs1799793.[36] However, this report contradicts a previous study demonstrating that the null phenotype for *GSTT1* was protective against cisplatin ototoxicity.[37] *MSH3* GG or GA and GT haplotype of *EXO1* rs1047840 and rs9350 SNPs predisposed patients to significantly greater probability of pronounced ototoxicity from cisplatin than *MSH3* AA genotype and other *EXO1* haplotypes, respectively.[32]

Genetic changes associated with better hearing outcomes have also been reported. Genes and SNPs related to protection against cisplatin ototoxicity include *GSTM3* (rs1799735), *GSTM1 null*, *GSTP* (rs1695), *SLC16A5* (rs4788863), *OTOS* (rs77124181; rs2291767),

and *OCT2* (rs316019).[34] The NFE2L2 promotor variant rs6721961 also appeared to protect against hearing loss in patients treated with cisplatin.[38] Additional more comprehensive genetic studies need to be performed to clarify genetic predispositions to resistance or susceptibility to cisplatin ototoxicity in patients.

PROTECTIVE AGENTS—PRECLINICAL STUDIES

Preclinical studies have explored a variety of potential protective agents against cisplatin ototoxicity in animal models.[19,21] Several different antioxidants have provided amelioration of cisplatin-induced hearing loss. Some of these drugs contain thiol groups which have a high affinity for cisplatin. The latter property provides a risk for interference with the therapeutic effects of cisplatin if these drugs are administered systemically.[21]

N-acetyl cysteine, sodium thiosulfate, D-methionine, lipoic acid, and others each contain thiol groups.[19]

Ebselen is a selenium-containing compound. Ebselen combined with allopurinol did not interfere with antitumor effects of cisplatin but actually enhanced the antitumor activity against breast and ovarian cancer in animal models.[39]

Sodium thiosulfate can be safely administered with minimal antitumor interference systemically if given 4 to 6 hours after cisplatin.[40]

A calcium channel blocking agent, flunarizine, reduced cell death from cisplatin by activation of antioxidant protective mechanisms in the cochlea Nrf2 and heme oxygenase-1 (HO-1).[41]

Flunarizine also was able to inhibit inflammatory pathways by reducing the activity of NF-kB.[25]

Several G-protein–coupled receptors have been characterized in the cochlea, and these appear to have a protective effect against ototoxicity from cisplatin when acted upon by agonists. The adenosine A1 receptor agonist R-PIA was found to protect against cisplatin ototoxicity in the rat.[26] The activation of A1 adenosine receptors in the cochlea exerts an anti-inflammatory effect by preventing ROS from being generated by the NOX3 enzyme and by downregulation of the STAT-1 inflammatory pathway.[26] Adenosine amine congener administered systemically provided protection against cisplatin ototoxicity.[42]

An inhibitor against TNF-α, etanercept, a drug widely used against rheumatoid arthritis, was found to be effective in preventing cochlear damage and hearing loss in rats treated with cisplatin when administered intratympanically.[19]

The cannabinoid 2 (CB2) receptor is present in the rat cochlea and is also a G-coupled receptor. A CB2 agonist protected against cisplatin ototoxicity. This effect was found to be mediated in part by inhibition of STAT1, thereby preventing cell death in the cochlea.[43] An extract from green tea epigallocatechin-3-gallate (EGCG) was shown to protect against cisplatin ototoxicity in the rat and tumor-bearing mouse without interference with the tumor killing efficacy of cisplatin.[15] EGCG protected against cisplatin-induced hair cell damage, ABR threshold shifts, and prevented a decrease in the strial Na/K-ATPase activity.[15] Intratympanic application of siRNAs against TRPV1, NOX3, and STAT1 provided protection against cisplatin ototoxicity in rat model by decreasing ROS generation and preventing inflammation in the cochlea.[19] A novel compound was recently reported to ameliorate cisplatin ototoxicity in rodents.

Kenpaullone, an inhibitor of cyclin-dependent kinase 2 (CDK2), protected mice and rats against cisplatin-induced hearing loss after intratympanic injection. CDK2 has proapoptotic effects in the cochlea. Kenpaullone promoted cell survival in the cochlea and prevented hearing loss by reducing cisplatin-induced mitochondrial production of ROS.[44]

PROTECTIVE AGENTS—CLINICAL STUDIES

Sodium Thiosulfate

The most promising agent for protection against cisplatin-induced hearing loss appears to be sodium thiosulfate. Two phase 3 clinical trials reported efficacy of sodium thiosulfate against cisplatin ototoxicity. An open-label, phase 3 trial comparing sodium thiosulfate versus observation in pediatric cancer patients receiving cisplatin demonstrated a reduced incidence of hearing

loss in patients administered sodium thiosulfate. However, high-risk patients with disseminated cancer who were given sodium thiosulfate had reduced survival.[45,46]

In a randomized, phase 3 trial, children treated with cisplatin for hepatoblastoma who received intravenous sodium thiosulfate 6 hours later were found to have a lower incidence of hearing loss compared with those receiving cisplatin alone. No apparent interference with antitumor efficacy was demonstrated.[3]

N-acetylcysteine

Transtympanic injections of N-acetylcysteine appeared to protect against cisplatin-induced hearing loss in cisplatin-treated patients in a double-blinded comparison with dexamethasone injections. The latter drug was less effective than N-acetylcysteine.[47] One previous study showed protection only at 8 kHz,[48] while another study failed to show significant protection.[49]

Amifostine

Clinical studies using amifostine as a putative protective agent have shown mixed results. Amifostine has FDA-approved labeling for use in reducing cumulative renal toxicity in patients receiving repeat doses of cisplatin for advanced ovarian cancer and non-small-cell lung cancer.[50]

Two clinical studies showed efficacy for amifostine in reducing cisplatin-induced ototoxicity in pediatric patients with medulloblastoma. One year after treatment initiation, 13 patients (37.1%) in the control group versus nine (14.5%; $p = 0.005$) of the amifostine-treated patients had at least grade 3 ototoxicity, requiring hearing aid in at least one ear. These authors concluded that amifostine can reduce the risk of severe ototoxicity in patients with medulloblastoma.[51]

A second clinical study demonstrated efficacy for amifostine as a protective agent against cisplatin-induced severe hearing loss only in average-risk patients with medulloblastoma, but failed to show significant protection against hearing loss in high-risk tumor patients.[52]

Another study showed efficacy for amifostine protection against cisplatin ototoxicity in ovarian cancer patients.[53]

Other studies have failed to demonstrate significant protection against cisplatin ototoxicity in patients with medulloblastoma,[54] pediatric germ cell tumors,[55] head and neck cancer,[56] melanoma[57] and in patients with hepatoblastoma.[58] Future studies may be indicated to evaluate the potential protective effects of amifostine against cisplatin-induced ototoxicity.[21]

Dexamethasone

A phase 2 clinical trial to investigate the efficacy of intratympanic dexamethasone against cisplatin ototoxicity found that the treatment provided statistically significant protection only at 6 kHz.[59]

Vitamin E

A randomized, placebo-controlled trial of oral vitamin E demonstrated significant hearing protection at 2 and 8 kHz in cisplatin-treated patients compared with placebo-treated subjects.[60]

CONCLUSION

This article has discussed the targets and molecular and functional effects of cisplatin on cochlear function. Numerous preclinical investigations have been performed to ameliorate cisplatin ototoxicity. Clinical trials have yielded mixed results in some cases. However, promising phase 3 clinical trials with sodium thiosulfate administration have been reported. It is critically important to avoid interference with the chemotherapeutic efficacy of cisplatin when attempting to preserve hearing. The local treatment with intratympanic administration of protective agents is likely to avoid neutralizing the antitumor effectiveness of cisplatin and would avoid potential systemic toxicity of protective agents. Future innovations in drug delivery to the cochlea could provide novel methods to prevent cisplatin ototoxicity. This is an exciting area of clinical research and further breakthroughs are likely to appear in the near future.

CONFLICT OF INTEREST
None.

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