Int. Adv. Otol. 2013; 9:(3) 403-416

REVIEW

Cisplatin Ototoxicity: Where We Are ?

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Objective: Ototoxicity is a dose limiting side effect of Cisplatin based chemotherapy protocols. Currently there is no approved agent against Cisplatin ototoxicity. Researches on cisplatin ototoxicity is mainly focused on effects on preventive agents as well predictive measures.

Materials and Methods: A systematic review on cisplatin ototoxicity is performed by using Pubmed and Scopus database.

Results and Conclusion: Currently there is not any approved safe protective agent which could be clinically used for cisplatin ototoxicity. Many studies on this field are still going on by using upstream and downstream protective agents. Ultrahigh frequency audilogical investigations seem to be promising in early detection of ototoxicity. Recently emerged another important field is to predict patients susceptible to cisplatin induced ototoxicity. Studies on genome analyses also pretherapeutic genetic tests especially in children will improve the quality of life and will avoid consumption of resources to treat or to rehabilitate these patients.

Submitted : 20 September 2013 *Accepted : 10 October 2013*

Introduction

Cisplatin –cis diamminedichloro platinum (CDDP) was first synthesized by Peyron in 1845 and thereafter it was named as Peyrone's chloride. In 1965, it was found that an electrical current between two platinum electrodes inhibited the proliferation of Echerichia Coli. Later it was used as a chemotherapeutic agent in some malignancies. After understanding the mysteries of the cell cycle combination chemotherapy protocols emerged and cisplatin became one of the main agents used in some protocols. In 1978 CDDP was approved as a chemotherapeutic agent by Food and Drug Administration (FDA) inspite of some dose limiting side effects [1-3].

Cisplatin is widely used in pediatric malignancies such as neuroblastoma, osteosarcoma, hepatoblastoma, germcell tumors as well in adult tumors such as metastatic testicular tumors, ovarian tumors, non-small cell lung cancer and bladder cancer but it has dose limiting side effects such as nephrotoxicity, neurotoxicity and ototoxicity. Nephrotoxicity could be prevented by forced diuresis but till today any agent able to prevent ototoxicity could not be founded. The incidence of ototoxicity is 13-96% in different studies[2-6]. Especially in pediatric oncology patients this side effect leads to important problems because it causes delay in speech, language development, education and social integration. Because of those serious problems Late Effect Surveillance System and Multidisciplinary European Pancare network has been focused on CDDP induced ototoxicity and they are investigating diagnostic tools for early detection of that toxicity as well pharmocogenetic markers for risk assesment [2].

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Apart from cisplatin second and third generation platinum derivatives such as carboplatin, oxaliplatin, nedoplatin, heptoplatin and lobaplatin were later invented. Carboplatin and oxaliplatin are less ototoxic when compared with cisplatin. But phase II trials shown that ototoxic effects of nedaplatin and heptaplatin are similar to cisplatin [2, 3].

Methods

SCI and SCI- expanded journals reviewed within Pubmed and Scopus database until to August 2013. National Instıtute of Health (NIH) clinical trial page was also searched by using ‘’Cisplatin’’, ‘’Ototoxicity’’ and “ Otoprotection’’ keywords. Full text articles were obtained and evaluated if abstracts pointed out that the study is appopriate for the review.

Mechanism of Ototoxicity

Cisplatin and the other platinum agents induce monoadducts at nucleophilic sides such as guanine or adenine and lead to intrastrand and interstrand crosslinks in nuclear DNA of tumor and/or normal cells. After this event apoptotic cascades; predominantly apoptosis via mitochondrial pathway is triggered. The mechanism of the cisplatin induced ototoxicity is the same. It acts on three major region in inner ear; organ of Corti (outer hair cells), spiral ganglion cells and lateral wall (stria vascularis and spiral ligament). After administration of cisplatin it is uptaken by stria vascularis, cochlear fluids and hair cells. It passes across the blood-endolymph barrier and enter hair cells crossing by their apical membranes [2]. Some transport proteins such as organic cation transporter protein (OCT2-SLC22A2), influx copper transporters (CTR1-SLC31A1) and megalin (LRP2) are thought to play an important role for transportation [2, 6]. The cochlea is an anatomically closed system; for that reason cisplatin and metabolites accumulate and they could not easily flush out. Those events lead to decrease in antioxydant enzymes such as glutathion peroxidase, superoxide dismutase, catalase and glutathion reductase. While those enzymes are decreasing, toxic lipid peroxides and aldehydes such as,malondialdehyde, 4-hydroxynonenal and peroxynitrite increase. Calcium influx also increases in cochlea cells and apoptosis occurs predominantly by cytohrome C release from mitochondria and caspase 9, caspase 3 activation [1-13]. (Fig. 1)

Clinical findings and audiological assesment

Cisplatin causes generally bilateral, permanent sensorineural hearing loss. Ear pain,tinnitus and vertigo could be seen with hearing loss.Tinnitus could be seen in 2%-36% of the patients treated with cisplatin [1-4]. Hearing loss mostly occurs at high frequences (≥4khz) but it can progress to lower to speech frequencies also (<4khz). Hearing loss in children leads to language development, speech disturbances, physchosocial delays and neurocognitive dysfunctions. It also affects perception of music and ambient sounds. All those findings effect quality of life and school success. Even if hearing loss is mild it leads to poor reading skills, spelling, phonological short –term memory and phonological discrimination ability[2, 14].

Since cisplatin retention can prolonge up to 20 years within the body hearing loss can progress after many years of cessation of the drug [2]. Cisplatin induced ototoxicity is more severe in children than adults and generally a hearing aid needs as many as 40% of children with cisplatin induced ototoxicity [15].

If cisplatin ototoxicity is detected earlier it is necesary to cease or lower the dose of cisplatin. In this case it could even be replaced with other less ototoxic platinum derivatives such as carboplatin. Most widely used diagnostic tools to detect ototoxicity are tonal audiometry, high frequency audiometry, play audiometry, transient otoacustic emission (TOAE) and distortion product otoacustic emission (DPOAE)and rarely auditory brainstem responses (ABR). Ideally a baseline audiological evaluation should betaken before initiating chemotherapy protocols including platinum agents [16]. However in practice baseline audiogram may not be possible either because of factors related with patients age, medical status or the lack of adequate fascilities at the center. Specifically in small children and children with severe disabilities these investigations may be quite difficult and necessiates

experienced audiology team and sometimes spesific equipment. If the baseline audiological evaluation is not available it is not easy to confirm a hearing loss due to otoxicity especially if it is not severe[2]. Baseline audiogram is advised to obtain one week before the beginning of the antitumor regimen including platinum agents. Audiograms should be repeated 24 hours before of each course .Since cochlea is a closed system and retention of any molecule entering this system can remain considerably of long times audiological assesment should be repeated in every six months for up to 2 years and later yearly for 3 years [2].

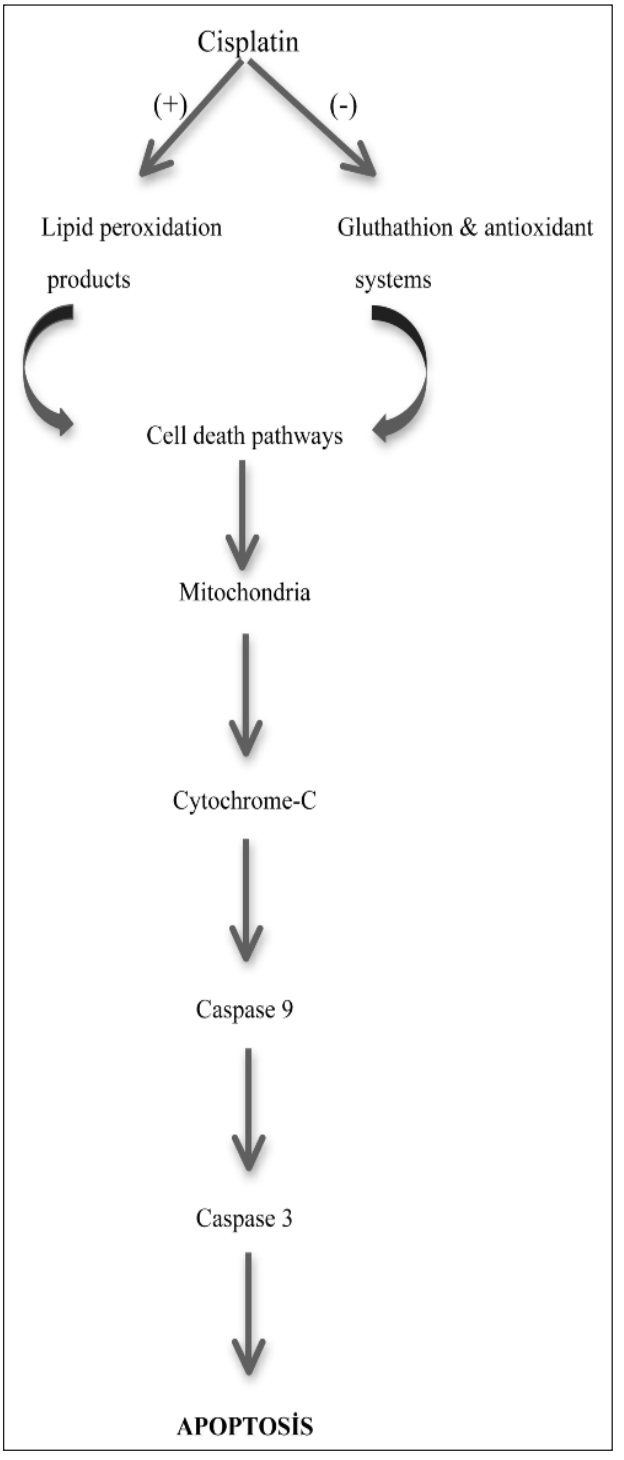

Figure 1. Apoptotic mechanism of Cisplatin toxicity

Apart from baseline audiograms, high frequency audiometry (6000-10000kHz) and extended high frequency audiometry (10000-16000kHz) is very helpful in the diagnosis of ototoxicity before detection of with conventional audiometry. Distortion product otoacustic emissions (DPOAE) at 8 kHz also found to be sensitive in early period [16-19]. Since the cumulative doses are one of the most important factors in CDDP ototoxicity early detection of ototoxicity is logic in order to lower the doses or to begin a less toxic derivative or to use otoprotective agents [19-21].

Hearing loss due to CDDP ototoxicity mostly begin at high frequencies and gradually covers to low frequencies. Adult patients can tolerate high frequency losses better than children. However children any loss within 2-4 kHz region can lead serious problems. Children are more sensitive to toxic effects of cisplatin. In patients younger than age of neural maturation speech and language delays may occur even if the hearing loss is mild. Mild to moderate hearing losses in children at school ages even can lead to learning diffuculties spesifically in foreign languages [1, 17].

Since the platinum derivatives are widely used for many different malignancies in many different ages, standardisation of the audiological investigations is a relatively complex issue. Most of the efforts come from the oncology groups and grading systems were mainly developed for children. One of the pioneering work came from Khan [22] in 1982 which divides ototoxic hearing loss to 4 grades. However this grading system was not adequate and in 1991 Brock [23] offered a different grading system which better delineates the site and degree of hearing loss. In 1997 WHO guidelines for hearing impaired children deos not

cover high frequency hearing losses and is not appropriate to report ototoxicity.[24] Although American Speech and Hearing Association 1994 ototoxicity guidelines are widely used system to report ototoxicity it does not allow early identification and does not always adress the severity of hearing loss [25]. In 2007 Schmidt [26] proposed another detailed system called Muenster Classification. In this system hearing losses were grouped with 20 dB steps at both high and low frequencies. Although by this system one can catch ototoxicity in a relatively early stage, it is rather complicated[27]. National Cancer Institute Common Terminology Criteria (CTCAE) version 4 was published in 2010 [28]. In this version chemoterapy induced hearing losses were grouped as grade 1, 2, 3 and 4. Grade 3 corresponds 20dB or more hearing loss at 3kHz or above which necessicates rehabilitation with hearing aids and or FM systems and other services for hearing disabled children. Grade 4 even corresponds a degree of hearing loss which requires cochlear implantation. Levin et al. [29] proposed a grading scale developed at Dana Farber Cancer Institute in 2009.This 0-3 scale is also seems to sensitive by adressing 20 dB hearing losses and probing 2kHz and 3kHz. But it was criticised by not catching differences between mild to moderate hearing losses. Chang et al. [16] developed a system adapted from Brock grading scale in 2011.This system is further detailed by covering losses 6-12 kHz region and accepting 20 dB hearing loss as a minimum loss. He claimed that this system by active involvement of pediatric audiologists is more sensitive in detecting early losses. He also stressed importance of co-existing conductive hearing losses and adviced to use tympanometry routinely in monitoring ototoxicity. Muenster and Chang grading scales seems to be helpful for early detection of ototoxic hearing losses. CTCAE of NCI and Boston Scale redeveloped by Societe International Pediatric Oncologie (SIOP) in 2012, seems more appropriate in reporting end-results of chemotherapy regimens rather than detecting ototoxicity in early stages. Although there are many different grading scalea none of them seems excellent. Ideally any clinically valid grading system should be simple enough to help to oncologists, should have a high sensitivity and specifity in detecting ototoxic hearing loss before occuring clinically evident. Currently protocols which probes high and ultra-high frequencies both with audiometry and/or DPOAE methods seems clinically more appropriate.

Risk factors

While ototoxicity are frequently related with dose and duration of the cisplatin, some other risk factors may also play a role on this undesirable side effect. These factors can be classified non-genetic and genetic risk factors.

Non-genetic risk factors;

Cumulative cisplatin dose is the most predictive factor. Cumulative doses greater than or equal to 400mg/m^2 increases the incidence of ototoxicity. This side effect inreases by the avarage of 5-7% in every additional 100mg/m^2 cumulative doses. Younger age at the time of exposure has been identified as an another important factor [2,4,30]. Children younger 5 years old have 20- fold increased odds of hearing loss than the patients aged 15-20 years. It has also been reported that higher age also is a risk factor [5]. Although in some studies it was shown that there was no significant effect of gender on cisplatin induced ototoxicity, in some others were found to be males are at great risk up to 4 –fold [2,30,31]. Dose and schedule are the other important factors; higher doses per course and bolus injections increase the risk [30,31]. An association between renal function and hearing loss due to cisplatin was not well studied but it it was thought that renal insufficiency, preexisting hearing looses, hypoalbinemia, anemia and nutritional status might be important factors for cisplatin induced ototoxicity [2-4, 11, 15-17, 30, 31]. As it is known head and neck radiotherapy is the part of treatment in some malignancies. Combination of high doses of cisplatin with cranial irradiation was shown to increase the risk [2, 4, 32]. Some drugs such as vancomycin, amphotericin-B, gentamycin, amikacin, furosemide can potentialise the ototoxicity of cisplatin. Cotreatment with some

chemotherapeutics such as methothrexate, vincristin and carboplatin enhances the cisplatin induced ototoxicity[2, 31]. Pre-existing hearing loss is also considered as a risk factor in cisplatin ototoxicity. (Table 1).

Table 1. Non-genetic risk factors for cisplatin induced ototoxicity

Higher doses of cisplatin
High cumulative doses (>400mg/m2)
Bolus injection
Young age (<5y)
Cotreatment with aminoglycoside, furosemide, gentamycin, amphotericin B, vancomycin
Cranial irradiation
Malnutrition, anemia, hypoproteinemia
Pre-existing hearing loss

Genetic risk factors:

Although high cumulative doses and treatment schedule are risk factors for cisplatin induced ototoxicity, hearing loss was not seen in some osteosarcoma patients treated with these cumulative cisplatin doses, but opposite of that findings, only 120mg/m^2 cumulative cisplatin dose could lead to ototoxicity in some other patients [31].Those variances showed that there are some factors other than non-genetic factors. It is very well known that pharmacological efficacy of a drug as well the side effects are due to uptake, metabolism, excretion and detoxification of the drug. Genetic factors manage these metabolic activities. Therefore they have also an important role at cisplatin induced ototoxicity.

Glutathione S transferases (GSTs) are phase II metabolic isoenzymes and they protect the cell from oxidative stress by scavenging free radicals and expressed in organ of Corti. Increased expression of GST isoenzymes in tumor cells plays a role in the resistance of chemotherapy. Also, it was shown that GSTM a member of GSTs plays a very important role in the metabolism of cisplatin. The first pharmocogenetic study about cisplatin and ototoxicity was done by Peters et al. [33] in pediatric population. They aimed to investigatethe polymorphisms of five Glutathion S-transferase (GST s) genes as the risk factor for cisplatin and found the protective effect of GSTM3*B allele against the cisplatin ototoxicity [33]. GST1, GSTT1, GSTM1 gene polymorphisms are the most frequent seen abnormalities in the cisplatin induced ototoxicity. However the results of these studies about protective effect of GSTs were contradictory because of different tumor types, ages, chemotherapy regimens, cumulative cisplatin doses, different analyses and statistic methods were used in the studies[33-38]. (Table 2)

Megalin is the one of the largest member of the lipoprotein family. In fact it is a multifunctional receptor and binds multiple ligands such as proteins carry vitamins and steroid hormones, proteases, lipoproteins and protease inhibitors. It is expressed in kidney proximal tubul cells. It was also found in the marginal cells on the apical part of stria vascularis of inner ear [35]. It was shown that aminoglycosides are uptaken by megalin in the proximal tubular cells of kidney. Also, high expression of megalin in the marginal cells of inner ear had a role in cisplatin induced ototoxicity [35]. In the recent years single nucleotide polymorphisms (SNPs) of megalin were studied. SNPs at megalin genes rs2075252 and rs 2228171 were shown to be related with ototoxicity of cisplatin [34]. Riedemann et al. [39] showed that there was a strong relationship between cisplatin ototoxicity and A allele of rs2075252 of megalin gene.

Mutations in the mitochondrial genome were found to be associated with hearing loss in the patients treated with cisplatin. While hearing loss was found frequently in European haplogroup J, but neither A74456 mutation nor 7472insC or A15556 mutation were identified in any of the patients [38]. On the other hand Knoll et al. [40] studied the mutations for GJB2 (codes for connexin) and SLC26A4 (codes for pendrin-anion transporter) genes and three mtDNA mutations such as A1555G, A3243G, A7445G from the buccal washes of 11 children and young adult

Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss; from past to present

Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss	Patient	Age	Hearing Loss Diagnosis	Genetic Test	Result	Ref. Number
Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss	39	3 y-22 y	Audiometry	GSTMI, GSTM3, GSTT1, GSTPI, GSTZI	Protective effect of GSTM3*B	33
Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss	39	3 y-22 y	Audiometry	Mitochondrial mutation as seen in the result	Neither A74456 mutation nor 7472 ins C or AI5556 mutation were identified Hearing loss more frequent in European Haplogroup J	41
Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss	11	1 y-16 y	Self – reported	Mutation screnning of GJB2&SLC26A4&mtDNA genes (A155G, A3243G, A74456) mutations	No association found.	40
Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss	50	5 y – 19 y	Audiometry	Megalin genetic polymorphism (SNPs rs 2075252&rs4668123)	No association was found with SNP rs4668123 Higher frequency of A-allele of rs 2075252 in the cisplatin induced hearing loss group.	39
Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss	238	15 y- 64 y	Self – reported tinnitus	GSTPI, GSTMI, GSTT1	Protective effect of GSTP1G GSTMI positivity is a risk factor.	36
Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss	173	18 y – 75 y	Audiometry	GSTT1, GSTMI, Codon 105 A/G (Ile/val) in GSTPI	Protective effect of 105 vol-GSTP1, GSTMP positivity is a risk factor	37
Table 2. Overview of genetic polymorphism studies on Cisplatin induced hearing loss	42	< 19 y	Audiometry	GSTMI, GSTTI	At least one null genotype of GSTM1 &GSTT1 polymorphism may predict adverse event (≥ grade 3 toxicity including ototoxicity and PFS)	38
Caronia D 2009	91	4 y – 34 y	Audiometry	SNPs ERCC2, XPC, XPA, ERCC1 , ERCC4, ERCC5	CC genotype of XPC Lys 939 Gln positivity is a risk factor (weak evidence)	42
Choeyprasert W 2012	68	< 15 y	Audiometry	GSTTI Megalin (c-alle of rs 2228172 SNPS)	GSTT 1 wild genotype C allele of megaline gene rs 2228172 SNPS are risk factors	34
Xu etal 2012	204	33 y -77 y	Self – Reported	Copper transport protein	Callele of CTR 1 rs 10981694 is a risk factor	43
Rednom S et al 2013	106	1.7 y – 23.5 y	Audiometry	GSTPI 105-G allele	GSTPI 105-G allele SPNS is a risk factor.	32
Xuetal 2012	282	34 y – 76 y	Self – Reported	22SNPS of eIF3 α	eIF3 α Arg 803 LysC polymorphism is a risk factor	44
Rose CJ etal 2009	162 (Discovery n:53 Replication n:109)	0 – 19 y	????	1949 SNPs of 220 drug-metabolism genes	Genetic variant of TPMT (rs12201199) & COMT are risk factors for cisplatin induced otoxocity.	45

x PFS: Progression Free Survival

patients treated with high cumulative dose cisplatin. Any of those genes were found to be related with ototoxicity [41].

In 2009 a paper about DNA repair genes and cisplatin ototoxicity was published and suggested that there was a weak association between CC genotype of XPC Lys939Gln and cisplatin induced ototoxicity [42]. In different two studies copper transport protein (CTR1) and eIF3 alpha polymorphism were investigated by Xu et al.[43,44]. Ross C et al. [45] investigated that 1949 SNPs of thiopurine S-methyltransferase(TPMT) gene and catechol O-methyltransferase(COMT) gene. Genetic variants in TPMT (rs12201199) and COMT (rs9332377) were found to be related with cisplatin ototoxicity.

In conclusion as seen in Table 2 some of the results about genetic studies are contradictory. This may be related with such factors; in some studies sample sizes are not adequate, study groups are not uniform for ages. Differences of cisplatin metabolism as well mechanism of ototoxicity between children and adult may also be a factor in these contradictory results. On the other hand methods used to evaluate hearing loss is not standardised, some studies relied on self reports, in others either tonal audiometry or otoacoustic emissions were used.

Prevention

Currently nearly 70% of pediatric cancer patients can be cured fort that reason Late Effects Surveillance System in USA and Multidisciplinary European Pancare Network have focused on ototoxicity of platinum derivatives particularly cisplatin. One of the major aims of of these organisations is to develop strategies for prediction of the cases who are susceptible to platinum agents induced ototoxicity, early detection and prevention [2].

The first and easy way for prevention strategies is decreasing of cumulative doses or replacing cisplatin with less ototoxic platinum drugs. Although in some protocols carboplatin, oxaliplatin could replace with cisplatin it is not easy to say whether those agents have equal anti-tumor effects with cisplatin. So usage of otoprotective agents seem to more feasible.

An ideal protective agent should be nontoxic, should reach to adequate concentrations in the inner ear, should not interfere with chemotherapetic agent and should not increase tumor cell viability[4]. Currently plenty of in vitro and invivo studies are going on on this issue.

Efficacy of several agents were investigated invitro in different cell lines such as House Ear Institute Organ of Corti 1 (HEI-OC1) cell line as well organ of Corti explants of neonatal rats or zebra fish larvas. Korean red ginseng, ginko biloba extracts, cannabinoid receptor2 agonists, curculigo orchioides, apocyanin, minocycline, purple bamboo-salt, epitachin and resveratrol were studied invitro and found to be protective against cisplatin induced ototoxicity [46-54].

Invitro studies also showed that neurotrophins (neurotrophin-3 and brain derived neural growth factor) had a protective effect against cisplatin induced ototoxicity. Otoprotective effect of Brain Derived Nerve Growth Factor was also supported with an invivo study[4].

Apart from invitro studies invivo researches are the corner stones before the clinical trials against chemotherapy toxicities. Invivo cisplatin toxicity prevention studies are concentrated on two ways named upstream and downstream protection [1].

Upstream protection

Some antioxidant molecules such as thiol groups could reduce the damage of inner ear by preventing of cell death in cochlea. This protection mechanism is named as upstream protection. Thiols such as sodium thiosulphate (STS), diethyldithiocarbamate, D-or L-methionine, lipoic acid N-acetylsysteine, thiopronin, glutathione ester, methylthiobenzoic acid and amifostine could act as a free radical scavenger [1, 2, 4].

Amifostine is one of the first thiol group agent studied for cisplatin induced ototoxicity in clinical trials. It detoxifies reactive metabolites of cisplatin and scavenges reactive oxygen species but its protective effect is dose dependant. Dose which could prevent toxicity was shown to cause neurotoxicity and it was manifested with prolongations in the ABR interpeak

latency. In adult and pediatric oncology patients clinical trials, amifostine was given before and after cisplatin infusions and were shown to have no protective effect against ototoxicity. Even it has been on the market since the mid-1990s for cisplatin induced nephrotoxicity with advanced ovarian cancer patients, it is stil not recommended for ototoxicity [2, 55].

Sodium thiosulphate (STS) was studied in guinea pigs with local administration before cisplatin infusion. Wang et al. [56] used this agent via perilymphatic infusion to the cochlea and showed that it had a protective effect. However this route seems to be very invasive to apply to patients. Wimmer et al. [46] applied STS to the round window membrane with an osmotic mini pump but could not found any protective effect. There might be some questionnable points at this study such as placement of the catheter could not be proper or there could be dislodgement. Besides the dose of STS could not be sufficient. Briefly, results of invitro and invivo studies are contradictory. Since it does not interfere with tumoricidal effect of cisplatin, large randomised controlled multicentric phase III studies are going on for STS against cisplatin ototoxicity in pediatric tumor patients [2].

It was shown that D –and L-methionine had a protective effect as well they did not interfere with cisplatin cytotoxic effect. D-methionine was applied to the round window niche in chinchillas and it was found to prevent topically applied cisplatin induced ototoxicity [5, 57, 58]. Translation from bench to bed has recently started with a Phase III clinical trial about D-methionine effect against noise-induced hearing loss (NCT01345474) [2].

N-acetyl cystein had been used with intravenous route before intra arteriel cisplatin infusion in rats and was shown to be effective against cisplatin ototoxicity [1, 2]. Ototprotective efficacy of N-acetylcystein was also shown with transtympanic injection after intraperitoneal cisplatin in rats [59]. In a Phase I/II study including adult cancer patients 10% N-acetylcystein was given with transtympanic route and reduced frequency and grade of hearing loss due to cisplatin [60].

Alpha tocopherol had otoprotective effect in guinea pigs and combination of alpha tocopherol with tiopronin was found more effective. Besides tiopronin was shown to enhance tumorigenic effect of cisplatin [61]. Trolox (Oxis) a water soluble form of vitamin E applied to the round window in cisplatin administered guinea pigs and suggested that it had a protective effect against cisplatin induced ototoxicity [62].

Salycilate was tried in rats and prevented hearing loss due to cisplatin. The mechanism was explained with the antioxidant effect of the drug. It was also shown that it had no interference with antitumor effect of cisplatin but of course side effects such as gastrointestinal disturbances and bleeding with the depletion of thrombocyte functions should be kept in mind [1,63].

Intratympanic application of dexamethasone prevented cisplatin ototoxicity in rats [64]. However systemic dexamethasone did not prevent cisplatin induced ototoxicity in guinea pigs [65]. On the other hand there was no protective effect of intratympanic methylprednisone against cisplatin induced ototoxicity in guinea pigs [66]. Some clinical studies about intratympanic dexamethasone and intratympanic steroid are going on at NIH. (Table 3)

An inhibitor of Inducible nitric oxide synthase (INOS), aminoguanidine had partial protective effect on cisplatin induced ototoxicity. Aminoguanidine pretreatment reduced cisplatin induced inner ear damage in rats and it was shown to decrease malondialdehyde production in cochlea and improvement in ABR thresholds. But it did not reduce nitric oxid production and it acted as free radical scavenger rather than INOS inhibitor [67].

Acetyl-L-carnitine was shown to have protective effect against cisplatin ototoxicty in cisplatin administered rats. It was used before cisplatin infusion and was shown the efficacy with ABR and transmission electron microscopy [68].

Resveratrol was also studied for otoprotective effect against cisplatin. In some studies showed that intraperitoneal usage of resveratrol was effective against cisplatin ototoxicity in rats [69-71]. However different oral doses of resveratrol were not effective

Table 3. Ongoing clinical trials at NIH web page.

Agent/ Mechanism	CI Trial Identifier	Title	Study Design	Study Phase	Population	Estimated Enrollment	Treatment Protocol	Recruited	Publication
Lactated Ringers Prevantion of acidosis Antioxidant and freeradical scavenger	NCT 00584155	Protection from cisplatin ototoxicity by lactated ringers	Randomized, single blind placebe-control	Phase I	Head&neck cancer (ages ≥18y)	----	Lactaced ringers 0.03% oflaxacin ear drop at start time 30 minutes after CT hourly for 4 hours after infusion	Completed	Not provided
Lactated Ringers Prevantion of acidosis Antioxidant and freeradical scavenger	NCT 01108601	Transtympanic Ringer's lactate for the prevention of Cisplation ototoxicity	Randomized open label	Phase I / II	Patients over age of 14 undergoing of platinum-based CT (CDDP, carboplatin)	20	Lactated Ringers with %0,03 ciproflaxin ear drops twice a day.	Recruting	On publication
Dexamethasone Attenuation of inflammation created by ROS	NCT 01372904	Prevention of cisplatin indu cear hearing loss by intratympanic Dexamethasone treatment	Randomized open label	Phase IV	≥18y patients treated with cisplatin based CT	30	Before CDDP CT, 0,7 ml of dexamathasone phosphate (10mg/ml)injected unilateraly to the middle ear.	Recruting	Not provided
Methylprednisolone Attenuation of inflammation created by ROS	NCT 01285694	intratympanic steroid treatment for the prevention of ineer ear toxicity associated with systemic treatment with cisplatin	Open Label	Not provided	≥18 years old adult patients treeated with cisplatin	20	Before CDDP treatment 0,5cc Methylprednisolone (62,5 mg/cc) intratympanic injection	Not yet recruting	Not provided
Alpha-Lipoic Acid Antioxidant	NCT 00477607	Alpha-Lipoic acid in preventing hcaring loss in cancer patients undergoing treatment with cisplatin	Randomized, Double blind placebe-control	II/III	≥18y patients treated with cisplatin	200	Alpha-lipoic acid once a day beginning 1 week before cisplatin and continuing for up 1 month after cisplatin	Completed	Not provided
Ginkgo biloba extract (GBE761) Antioxident ROS scavenger	NCT 01139281	The protective effect of ginkgo biloba extract on cisplatin-induced ototoxicity in humans	Randomized placebo controlled double blind	II	≥18y patients treated with cisplatin	15	GBE761 120mg twice a day before cisplatin	Completed	Multipublic-ation
Pantaprazole Inhibiting organic cation transporter 2	NCT 01848457	Preventing nephrotoxicity and ototoxicity from osteosarcoma therapy	Randomized crossover assignment	II	1-30 years patients with osteosarcoma treated with cisplatin	24	0,3 mg / kg IV pantaprazole 15 minutes prior to cisplatin on day 1,2	Recruiting	Not provided
Sodium Thiosulfate Binding and inactivating platinum&free radical scavenger	NCT 00716979	A randomized phase III study of Na-Thiosulfate fort he prevention of cisplatin-induced ototoxicity in children	Randomized, open label, multi center	III	1-18 years pediatric oncology patients treated with cisplatin.	135	Sodium thiosulfate IV over 15 minutes beginning 6 hours after the completion of cisplatin infusion	Recruiting	Not provided
Sodium Thiosulfate Binding and in activating platinum&free radical scavenger	NCT 00652132	A multicentre open label randomised phase III trial of the efficacy of Sodium Thiosulfate in reducing ototoxicity in patients receiving cisplatin chemotherapy for standard risk hepatoblastoma.	Randomized, open label, multi center	III	Pediatric hepatoblastom a patients	115	Sodium thiosulfate IV over 15 minutes, 6 hours after the completion of cisplatin	Unknown	Not provided
ASA Antioxidant, attenuation of ROS-generated inflammation	NCT 005578760	Does aspirin have a protective role against chemotherapeutically induced ototoxicity?	Randomized, double blind, placebo controlled	---	≥18y patients diagnosed with germ-cell, bladder head&neeck tumors	110	325 mg ASH OD for the duration of cisplatin	Unknown	Not provided
SPI -1005 (oral ebselen) formulation mimics of glutahione peroxidase , reduces ROS	NCT 01451853	Safety and efficacy study of SPI-1005 for prevention of chemotherapy induced hearing loss	Randomized placebo controlled double blind	II	19y-80y patients diagnosed with head&neck cancer, advanced lung cancer	80	200 mg, 400 mg, 600 mg oral ebselen (SPI-1005) 3 days of eachcycle of chemotherapy	Not yet open	----
N-acetyl cysteine Antiapoptatic & Prevention of production of free oxygen radicals	NCT 01138137	N-acetycysteine given IV cisplatin and paclitaxel in patients with ovarian cancer	Single group assignment, open label	I	18y-75y patients diagnosis with ovary cancer or primary peritoneal carcinoma	33	Onday 2 NAC was given with escalating doses (150mg/kg, 300mg/kg, 600mg/kg, 800mg/kg, 1000 mg/kg, 1200 mg/kg) 60 minutes before Intraperitoneal cisplatin infusion	Suspended	Not provided
Amifostine	NCT 00003269	A phase II open label, trial evaluating the efficacy of Amifostine in patients with cancers receiving outpatient dose-intensive cyclophosphamide etoposide and cisplatin (DICEP) chemotherapy.	Single group assignment, open label	II	18y-70y patients treated with DICEP chemotherapy protocol.	20	Amifostine IV over 15 minutes on days 0-2, 30 minutes prior to high-dose chemotherapy	Completed (No Results available)	Not provided

against cisplatin ototoxicity even it enhanced the apoptotic effect of cisplatin in high doses [72].

In cochlea adenosine receptors (ARs) were shown to be related with antioxidant defense mechanisms. A1AR and possibly A3AR may have cytoprotective effect in inner ear. Preapplication of R-phenylisopropyladenosine(R-PIA) to the round window in chinchillas had a protective effect against cisplatin induced ototoxicity. Receptors were upregulated with this agent and cochlea was protected from cisplatin induced ototoxicity [5].

Despite many agents have been tried in invitro and invivo studies only few clinical trials have been completed. Currently there is not any protective agent against cisplatin induced ototoxicity approved by Food&Drug Administration(FDA) and/or European Medicines Agency. Only Sodium thiosulphate and N-acetyl cysteine have received FDA orphan status for this purpose[2]. Summary of the clinical trials going on at National Institute of Health (NIH) was shown in Table 3 [2,73].

Downstream protection

Downstream protection means prevention of ototoxicity by blocking proapoptotic pathways. Caspase 3 and caspase 9 are corner stones in cisplatin induced apoptosis. Intracochlear perfusion of inhibitor of caspase 3 (z-DEVD fmk) and inhibitor of caspase 9 (z-LEHD fmk) were shown to reduce hair cell loss –apoptosis after cisplatin administration in guinea pigs[1, 2, 5, 74].

JNK-1 is a cell permeable peptide. Intracochlear perfusion of D-JNK-1 had a protective effect. This otoprotective effect occured by inhibiting JNK-mediated activation of c-Jun. This blocking leads to preventing of mitochondrial release of cytochrom-c and apoptosis was stopped [1, 2, 5, 74].

Pifithrin-alpha is a p53 inhibitor which prevents cisplatin induced apoptosis. It was also shown to have protective effect in organotypic cultures of outer hair cells. However it should be remembered that it could have positive effect on tumor growth [1, 2, 5, 74].

Conclusion

Currently there is not any approved safe protective agent which could be clinically used for cisplatin ototoxicity. Clinical trials are going on by using upstream and downstream protective agents. Timing and the route are very important points for the efficacy and safety of the candidate agent. Upstream protection agents can interfere with cisplatin tumoricidal effect and also they themselves may increase tumor cell viability. Some of them may be effective by local application or delayed systemic administration by oral or intravenous route. Majority of downstream protection studies were focused on local administrations such as round window application. By this route interferation of cisplatin with candidate agent can be avoided, systemic side effects of the protective agent can be prevented and also protective agent can reach sufficient concentrations in the inner ear. However application by this route is difficult and time consuming for the patients especially for the children. Also for p53 inhibitors there is a danger of prevention of tumor apoptosis. One of the promising venues in otoprotection is gene therapy. Cochlear penetration of antiapoptotic genes necessicates novel noninvasive delivery systems such as nanotechnology techniques.

Ultra high frequecy audilogical investigations seems to be promising in early detection of ototoxicity. Recently emerged another important field is to predict patients susceptible to cisplatin induced ototoxicity. Studies on genome analyses also pretherapeutic genetic tests especially in children will improve the quality of life and will avoid consumption of resources to treat or to rehabilitate these patients.

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