Review Article

Is Nanotechnology the Answer for the Treatment of Cisplatin-Induced Ototoxicity?

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Abstract

Cisplatin (CDDP) is a highly active chemotherapeutic compound that is commonly used in the treatment of a variety of solid tumors including head and neck, lungs, ovary, bladder and testicles. However, it presents several side effects, including nephrotoxicity, neurotoxicity, and ototoxicity. CDDP produces sensorineural hearing loss by the induction of apoptosis or necrosis of hair cells of the Organ of Corti.

Several compounds have been used systemically or locally to avoid cisplatin-induced ototoxicity such as corticosteroids, antioxidants, and caspase inhibitors. However, most of these drugs present severe side effects, are highly hydrophobic and therefore, difficult to administrate. Moreover, the systemic administration of these compounds can interfere with the desired effect of CDDP altering its anti-proliferative activity.

Different kinds of nanoparticles (NPs) have been used to cross the round window membrane and increase the bioavailability of drugs in the inner ear, and therefore, can also be used to provide an appropriate dose of the otoprotective drug with minimal interference with CDDP if locally administered in the middle ear.

In this review the nanoparticulated systems that have been used for inner ear drug administration are described as promising approaches to combat CDDP-induced ototoxicity.

ABBREVIATIONS

ABR: Auditory Brainstem Response; CDDP: Cisplatin; C18: Octadecylamine; IT: Intratympanic; NPs: Nanoparticles; PEG: Polyethylene Glycol; PLA: Polylactic Acid; ROS: Reactive Oxygen Species; RWM: Round Window Membrane; SNHL: Sensorineural Hearing Loss

INTRODUCTION

The inner ear is an isolated structure with limited access due to the presence of many anatomical barriers (Figure 1). It is considered a hiper-specialized region of the peripheral nervous system form by the cochlea and vestibule which are organs of hearing and balance, respectively. The cochlea is a complex structure which transduces complex sound waves into electrical neural activity in the auditory nerve by the hair cells of the organ of Corti. There are two types of hair cells in the cochlea: inner hair cells, that perform the transduction and initiate the depolarization of the spiral ganglion neurons, and outer hair cells that enhance the sensitivity and selectivity of the cochlea. Sensorineural hearing loss (SNHL) is caused by the degeneration or degradation of these hair cells or damage to the auditory nerve [1].

Cisplatin-induced ototoxicity

One of the most important causes of SNHL is ototoxicity or the functional impairment and cellular degradation of the inner ear tissues due to the use of therapeutic agents (e.g. aminoglycoside antibiotics, loop diuretics, macrolide antibiotics, antimalarials and platinum-based chemotherapeutic agents) [2].

Cis-diaminedichloroplatinum (II) or cisplatin (CDDP) was

Figure 1 Anatomical barriers of the ear (RWM: Round Window Membrane).
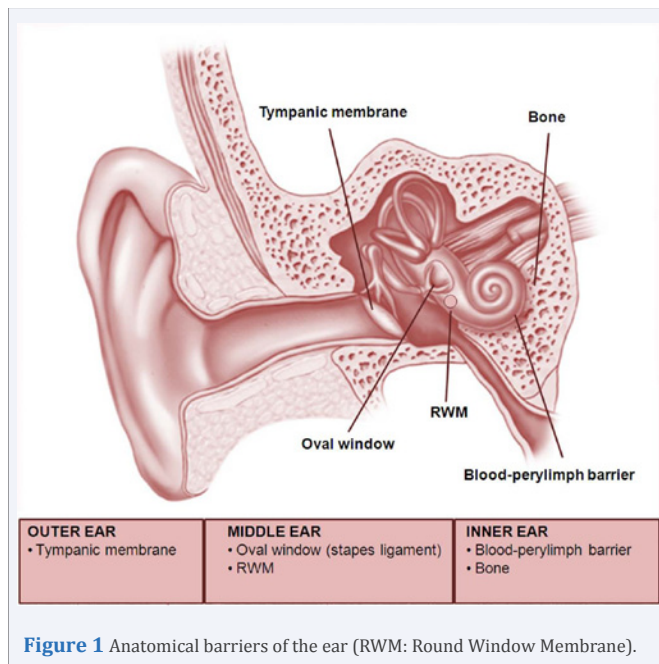
the first platinum-based drug to be used and nowadays is widely employed in the treatment of some neoplastic entities such as head and neck, ovary, bladder, lung or brain. However, it presents associated side effects such as nephrotoxicity, bone marrow toxicity, gastrointestinal toxicity, liver toxicity, peripheral nervous system toxicity and ototoxicity [3]. Most of these adverse effects can be prevented and treated. However, CDDP- induced ototoxicity, that implies irreversible hearing loss, tinnitus and dizziness due to cochlear damage, is one of the main reasons to chemotherapy discontinue.

CDDP induces apoptosis of inner ear cells by different mechanisms: binding of cisplatin to guanine bases on DNA and the formation of inter- and intra-strand chain cross-linking, generation of reactive oxygen species (ROS) with increased lipid peroxidation and Ca^{2+} influx and, finally, inflammation events [4]. Strong variability in susceptibility to the ototoxic effects of CDDP is reported in the literature. Hearing impairment appears when the clinically used doses are higher than 100 mg/m^2 that affects firstly at high frequencies and progresses to midrange. When patients received ultrahigh doses of CDDP, hearing loss in the high and extended high frequencies occurs in 100% of cases [5]. However, testicular cancer patients treated with low cumulative doses of CDDP had an almost 50% chance of complete reversibility of the symptoms [6].

OTOPROTECTIVE APPROACHES TO PREVENT CDDP-INDUCED OTOTOXICITY

CDDP- induced ototoxicity has been treated with corticosteroids, antioxidants or caspase inhibitors. However, pharmacotherapy is not efficient enough due to the difficulty to reach the inner ear.

Corticoids

In order to avoid CDDP-associated inflammatory events in the cochlea corticosteroids like hydrocortisone,

6α-methylprednisolone, and dexamethasone were used. However corticosteroids showed poor delivery to inner ear by systemic administration, and high doses were needed to achieve otoprotective therapeutic concentrations which are associated to severe side-effects including hyperglycemia, peptic ulcers, hypertension, and osteoporosis [7]. Moreover, corticosteroids also down-regulate apoptosis genes in tumor cells, resulting in a decreased efficacy of the CDDP antitumor properties [8].

A recent randomized controlled study tested the potential effect of dexamethasone administered intratympanically in CDDP-induced hearing loss. Authors reported a significant attenuation in hearing loss at some frequencies in patients who suffered some neoplastic disease [9].

In a recent study Özel et al., [10] compared the effect of intratympanic administration of 6α-methylprednisolone and dexamethasone in middle-age rats which received a dose of 8 mg/kg of CDDP, concluding that dexamethasone have a better effect against CDDP-induced hearing loss.

Antioxidants

The administration of antioxidants to down regulate CDDP-induced ROS production may prevent apoptosis and hearing loss. Recent studies have investigated attenuating effects of molecules with antioxidant properties such as ginkolide b [11], a major component of *Ginkgo biloba* extracts, showing a significant suppression in CDDP-induced hearing loss due to a decrease in ROS generation resulting in a reduction of ototoxicity in Sprague-Dawley rats enhancing heme-oxigenase-1 antioxidant pathway. Hesperetin, a flavonoid with antioxidant properties, was tested by Kara et al., [12] in Wistar rats cloncluding that hesperetin may prevent ototoxicity by increasing antioxidant enzymes and reduced ROS. Yumusakhuylu et al., tested in a guinea pig model the systemic administration of resveratrol with promising effects 72h after a concomitantly dose of 10mg/kg CDDP administration [13].

Roldán-Fidalgo et al., [14] tested *in vitro* and *in vivo* a carotenoid with antioxidant and anti-inflammatory properties, lutein, against CDDP-induced ototoxicity obtaining protection only in HEI-OC1 cells, but not in the Wistar rat model. Authors concluded that the large molecule size, the low dose administered or restriction to diffusion in the inner ear could be related with the negative *in vivo* result.

Yoo et al., [15] evaluated the efficiency of L-*N*-acetylcysteine administered intratympanically in patients of head and neck cancer who received CDDP chemotherapy. However, only 18.2% of the treated patients showed some improvement. They concluded that better delivery methods could improve the otoprotective efficacy of the drug.

Other drugs

Other approaches to ameliorate ototoxicity are focused in different pro-apoptotic pathways triggered by CDDP. In this sense, the use of caspase inhibitors such as caspase-3 inhibitor (z-DEVD-fmk) and caspase-9 inhibitor (z-LEHD-fmk), which play an important role in CDDP-induced apoptosis, showed to be effective reducing hearing loss and tissue damage after

chemotherapy. These results showed the otoprotective potential of drugs that were able to block a critical step in CDDP-induced apoptosis [16].

The use of oxitocin, a well-known hormone secreted during pregnancy with antioxidant and anti-inflammatory properties, was evaluated by Bilmez et al., [17] showing otoprotection against CDDP, especially by intratympanic administration. Authors emphasized importance of this way of administration to avoid the negative effect on chemotherapeutic CDDP effect.

Nanotechnology against CDDP-induced ototoxicity

Nanotechnology could palliate the current limitations of traditional treatments such as, low solubility, low stability, molecular size, related side effects, and offer a sustained and targeted drug delivery approach. Nanoparticles (NPs) with sizes of 200 nm or less have been used in drug delivery to the inner ear with good results [18]. NPs size is a limiting factor to pass through the RWM to the inner ear because the NPs need to permeate through the three-layered RWM based on two epithelial cell layers and a central connective tissue layer. The use of NP based treatments offers a more efficient solution against CDDP-induced ototoxicity than systemic or local administration of naked drugs.

Glueckert et al., [19] recently tested various types of NPs such as lipid based nanocapsules (LNCs), Quantum Dots, and silica NPs as drug carriers in HEI-OC1 with a surface modification to target TrkB receptor, mainly expressed in spiral ganglion neurons. Authors also tested LNCs loaded with rolipram (selective phosphodiesterase-4 inhibitor) used to block the degradative action of phosphodiesterase-4, to avoid CDDP-induced apoptosis. In their cultures of postnatal mouse cochlea spiral ganglion neurons and spiral ligament treated with CDDP and rolipram-loaded LNCs exhibit less cleaved caspase-3 than controls exclusively treated with CDDP. LNCs may act as an appropriate drug delivery system which needs low doses to reach sustained release suitable for inner ear application.

Our group recently developed 6α-methylprednisolone-loaded NPs based on two different families of bioactive amphiphilic pseudo-block copolymers obtained by free radical polymerization. Copolymers were based on *N*-vinyl pyrrolidone and a methacrylic derivative of α-tocopheryl succinate or α-tocopherol. Methylprednisolone was used as a model of hydrophobic drug usually administered to clinical patients trying to palliate CDDP adverse effects on hearing due to its anti-inflammatory properties. Spherical NPs were obtained, with hydrodynamic diameters around 120 nm, and good encapsulation efficiency. Low protection was achieved with unloaded-NPs. However, good results were achieved *in vitro* and *in vivo* with 6α-methylprednisolone-loaded NPs palliating CDDP-induced ototoxicity in HEI-OC1 and in a Wistar rat model which received a single dose of 10 mg/kg CDDP. Coumarin-6 loaded NPs were detected in the inner hair cells and the outer hair cells. Loaded-NPs prevent CDDP induced hearing loss in statistically significant way at high frequencies (14 and 16kHz) in the best system tested and their activity mainly depended on the amount of corticosteroid encapsulated [20].

Sun et al., [21,22] tested the controlled release properties and hearing protection effect of polyethylene glycol-polylactic acid (PEG-PLA) dexamethasone loaded NPs intratympanically (10 mg/ml of NPs) and by intraperitoneal systemic administration (10 mg/kg) against 12 mg/kg dose of CDDP. Authors prepared dexamethasone loaded PEG-PLA NPs by emulsion and evaporation technique and obtained NPs with hydrodynamic diameter of 130 nm, that were appropriate to reach the inner ear. Sustained dexamethasone release over 24 days was achieved in phosphate buffered saline (pH 7.4), over 5 days in artificial perilymph (pH 7.4), and 1 day in rat plasma. NPs loaded with coumarin-6 were detected in the spiral ganglion, stria vascularis and the Organ of Corti, 1 hour after NPs administration intratympanically. Authors also reported an enhancement in dexamethasone bioavailability in the rat inner ear with dexamethasone loaded NPs with respect to free drug. Auditory brainstem response (ABR) threshold shifts were measured at four frequencies (4, 8, 16, and 24 kHz) 1 day before and three days after CDDP treatment and dexamethasone loaded NPs were able to protect cochlear cells and preserved hearing partially at 4 kHz and 8 kHz frequencies after CDDP treatment in guinea pigs [21]. The same nanocarrier system was also tested by authors by intraperitoneally administration 1 hour after CDDP administration. Authors reported that dexamethasone loaded NPs significantly preserved functional and structural properties of the cochlea, at histological and functional levels showing potential as therapeutic treatment to enhance corticosteroid delivery to the inner ear to palliate CDDP-induced hearing loss [22].

Drug administration strategies to treat CDDP-induced SNHL

The administration ways to reach this part of the ear are systemic administration by the labyrinthine artery that supplies the cochlea and vestibule, and local administration by the round window membrane (RWM), which is the barrier between middle and inner ear. All these structures are an anatomic challenge to drug delivery to the inner ear (Figure 2).

Inner ear has been traditionally treated by systemic drug administration, but there is variable penetration into the inner ear due to the blood–cochlea barrier. Systemic therapies are usually based in the use of corticosteroids, antioxidants or caspase inhibitors, and therefore, associated side effects and
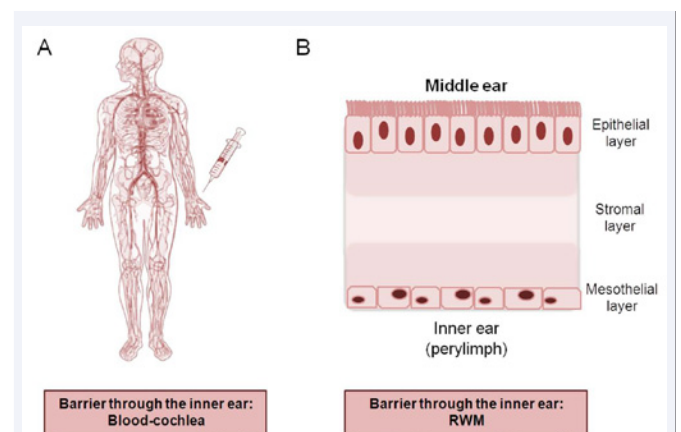
Figure 2 Administration ways to reach the inner ear. (A) Systemic administration. (B) Local administration in the middle ear.

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systemic interaction between the otoprotective agent and CDDP may occur, reducing effectiveness of the chemotherapy.

Therefore, intratympanic administration (IT) represents a better option than systemic administration to avoid systemic interaction between potential otoprotective drugs and CDDP, as it offers the possibility to concentrate the drug in the inner ear minimizing systemic related side effects.

The main challenge to this way of administration is to achieve enough concentration of the drug in the cochlea passing through the RWM. The permeability of the drug through this three-layered semi permeable membrane depends on the size, concentration, solubility, structure and charge of the drug [23].

Future perspective and challenges

Local administration of nano-particulated systems bearing oto-protective drugs offers a more efficient and specific treatment to CDDP-induced hearing loss avoiding interaction with the chemotherapeutic effect of CDDP in undesired tissues. Nanoparticulated systems have demonstrated an improvement in the solubility and bioavailability of many drugs in the cochlea. Indeed, NPs improved the capability of the therapeutics to reach the inner ear by crossing the biological barriers which protect it. However, only a few NPs have been used to reach the inner ear and deliver drugs to treat CDDP-induced ototoxicity. An intense research in biomaterials science and nanotechnology is needed to rationally optimize the physico-chemical properties of the systems in order to cross the multiple biological barriers that naturally protect the inner ear. Moreover, the incorporation of targeting moieties on the surface of the NPs will provide active targeting systems that specifically exert its action in the desired kind of cell, almost completely avoiding related effects in undesired tissues. However, long-term effects of multidose administration of NPs based systems, such as NPs accumulation or metabolism alterations should be studied before clinical approval.

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