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OTOTOXIC EFFECTS OF CARBOPLATIN IN ORGANOTYPIC CULTURES IN CHINCHILLAS AND RATS

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Abstract

Carboplatin, a second-generation platinum chemotherapeutic drug, is considerably less ototoxic than cisplatin. While common laboratory species such as mice, guinea pigs and rats are highly resistant to carboplatin ototoxicity, the chinchilla stands out as highly susceptible. Moreover, carboplatin causes an unusual gradient of cell death in chinchillas. Moderate doses selectively damage type I spiral ganglion neurons (SGN) and inner hair cells (IHC) and the lesion tends to be relatively uniform along the length of the cochlea. Higher doses eventually damage outer hair cells (OHC), but the lesion follows the traditional gradient in which damage is more severe in the base than the apex. While carboplatin ototoxicity has been well documented in adult animals in vivo, little is known about its in vitro toxicity. To elucidate the ototoxic effects of carboplatin in vitro, we prepared cochlear and vestibular organotypic cultures from postnatal day 3 rats and adult chinchillas. Chinchilla cochlear and vestibular cultures were treated with carboplatin concentrations ranging from 50 μM to 10 mM for 48 h. Consistent with in vivo data, carboplatin selectively damaged IHC at low concentrations (50-100 μM). Surprisingly, IHC loss decreased at higher doses and IHC were intact at doses exceeding 500 μM. The mechanisms underlying this nonlinear response are unclear but could be related to a decrease in carboplatin uptake via active transport mechanisms (e.g., copper). Unlike the cochlea, the carboplatin dose-response function increased with dose with the highest dose destroying all chinchilla vestibular hair cells. Cochlear hair cells and auditory nerve fibers in rat cochlear organotypic cultures were unaffected by carboplatin concentrations <10 μM; however, the damage in OHC were more severe than IHC once the dose reached 100 μM. A dose at 500 μM destroyed all the cochlear hair cells, but hair cell loss decreased at high concentrations and nearly all the cochlear hair cells were present at the highest dose, 5 mM. Unlike the nonlinear dose-response seen with cochlear hair cells, rat auditory nerve fiber and spiral ganglion losses increased with doses above 50 μM with the highest dose destroying virtually all SGN. The remarkable species differences seen in vitro suggest that chinchilla IHC and type I SGN posse some unique biological mechanism that makes them especially vulnerable to carboplatin toxicity.

Key Words: Carboplatin ; ototoxicity ; organotypic culture

Introduction

Carboplatin (diammine [1,1 cyclobutane dicarboxylato (2)-0,0’] platinum) is one of the platinum agents with enhanced anti-tumor activity and reduced side effects in comparison with cisplatin [1-6]. As a second-generation platinum compound, carboplatin expresses its anti-neoplastic effect in a similar fashion as cisplatin by forming

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inter/intra-strand DNA cross-links when activated by conversion into aquated species [7, 8]. For the reason of being highly efficient in anti-cancer actions and of low toxic side-effects, carboplatin has been used widely to treat various types of solid tumors in humans. The side effects of carboplatin were similar to all adverse effects of cisplatin, such as myelosuppression, nephrotoxicity, gastrointestinal upset, peripheral neurotoxicity, electrolyte disturbance and hepatotoxicity, as well as ototoxicity. Although the level of side effects of carboplatin is less severe than that of cisplatin, however, they have attracted increasing attention from clinical doctors and research scientists [9-22].

The toxic effects of carboplatin were discussed in clinic reports in as early as the 80s [23-28]. Since then, the ototoxicity of carboplatin has been studied in several common laboratory species, such as rats [29-35]. guinea pigs [6, 30, 36-40], rabbits [41], monkeys [42], mice [43-48], and zebrafish [49]. The ototoxic effects of carboplatin have been observed vary significantly across these species. There is very little evidence of ototoxicity in mice. Guinea pigs are relatively resistant to carboplatin, but damage can occur when extremely high doses of carboplatin are given. High doses of carboplatin can cause high frequency hearing loss and outer hair cell lesion in guinea pigs and the damage progresses from the base of the

cochlea to the apex, much like most other ototoxic drugs, such as aminoglycoside antibiotics, or cisplatin [50-53]. In laboratory rats, moderate-doses of carboplatin do not affect the cochlea[54]. However, high doses of carboplatin result in a significant reduction in amplitude of distortion product otoacoustic emissions (DPOAEs) which suggests that the severe damage takes place at the outer hair cells level [34, 55]. In contrast to all the above mentioned animals, chinchillas treated with carboplatin develop an unusual hair cell lesion that selectively destroys the inner hair cells and type I spiral ganglion neurons in the cochlea [10,56-74]. In the chinchilla vestibular system, the ototoxic effects of carboplatin has been studied, showing that Type I hair cells and larger ganglion neurons in the vestibular system are more susceptible to carboplatin [60, 66, 71, 74, 75]. Although the ototoxic effects of carboplatin have been well documented in various experimental animal species, however, the mechanism of carboplatin ototoxicity is not quite clear. Specifically, the different targeting effects of carboplatin in the cochlea among the chinchilla and other species are unknown.

The in vitro ototoxic effects of carboplatin have been studied in very few species using as cell lines and cochlear cultures [30, 76-78]. According to the very limited discoveries from previous studies in the rat cochlear culture system, carboplatin absolutely selectively destroys outer hair cells and spiral ganglion neurons in the postnatal day 3 rat cochlear explant [76-78]. In contrast, carboplatin selectively destroys inner hair cells and type I spiral ganglion neurons in chinchilla cochlear explants as it does in vivo, from our preliminary findings reported in an abstract at the Association for Research in Otolaryngology annual meeting[77]. To determine if the striking species differences in carboplatin ototoxicity are due to cellular intrinsic response, we evaluated dose dependent cell degeneration using cochlear and vestibular organotypic cultures treated with various concentrations of carboplatin from postnatal day 3 rat pups, adult rats, and adult chinchillas respectively.

Methods

Cochlear and vestibular organotypic cultures and carboplatin treatment

The cochlear and vestibular organ culture procedures are similar to those described previously [50,79-85]. The organotypic cultures of cochlear and vestibular end-organs were prepared from postnatal day 3 SASCO Sprague-Dawley rats, adult SASCO Sprague-Dawley rats, and adult chinchillas respectively. Experiments were performed according to the rules and regulations of the Institutional Animal Care and Use Committee of the State University of New York at Buffalo and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

For culturing cochlear and vestibular explants from postnatal day 3 rats, a drop of rat tail type I collagen gel was added in Basal Medium Eagle containing 2% sodium carbonate in a 35 mm culture dish. Type I rat-tail collagen (Collaborative Research, 3.76 mg/ml in 0.02 N acetic acid) was mixed with 10X Basal Medium Eagle (BME, Sigma) and 2% sodium carbonate at a 9:1:1 ratio. A 10 µl drop of the collagen solution was placed on the surface of a 35 mm culture dish and allowed to gel for approximately 30 min. Afterwards, 1.3 ml of culture medium (0.01 g/ml bovine serum albumin, 1% Serum-Free Supplement [Sigma I-1884], 2.4% of 20% glucose, 0.2% penicillin G, 1% BSA, 2 mM glutamine, 95.4% of 1X BME) was added to the dish to level the apical of the collagen gel. The cochlear basilar membrane including spiral ganglion neurons in Rosenthal's canal and vestibular end-organs including the maculae of saccule and utricle, and cristae of ampulla were carefully micro-dissected out, and positioned on the drop surface of collagen gel and a flat surface preparation was made by gently pressing on the tissue with forceps. Surface tension from the thin layer of culture medium helped to hold the tissue against the underlying collagen. The cochlear and vestibular explants were placed in an incubator (Forma Scientific 3029, 37°, 5% CO_2) overnight. On the second day, the serum-free medium was exchanged with new medi-

um that contained a specific concentration of carboplatin (10 μM, 50 μM, 100 μM, 500 μM, 1000 μM, 5000 μM, or 10000 μM, Sigma C2538), and incubated for 48 h. Control samples containing only the serum-free medium, were run concurrently with the experimental samples.

For culturing cochlear and vestibular explants from adult rats and chinchillas, the cochlear basilar membrane and vestibular end-organs including the maculae of saccule and utricle, and crista of ampulla were micro-dissected out and embedded in the fresh-made rat-tail collagen gel. After the collagen solution became the gel, 2 ml of serum-free culture medium was added to the dish for overnight incubation (Forma Scientific 3029, 37°, 5% CO_2). The serum-free medium containing a specific concentration of carboplatin (50 μM, 100 μM, 500 μM, 1000 μM, 5000 μM, or 10000 μM, Sigma C2538) was added on the second day for another 48 h incubations.

Histology

At the end of the experiment, the cochlear and vestibular explants were fixed for 2 h with 4% formalin in 0.1 M phosphate buffer (pH 7.4). Specimens from postnatal day 3 rat pups were double-labeled with a monoclonal antibody against neurofilament 200 (Sigma N0142, clone N52) to show the auditory nerve fibers and spiral ganglion neurons plus phalloidin conjugated Alexa Fluor 488 (Invitrogen A12379) to label the cuticular plate and stereocilia bundles of the hair cells. After double labeling, specimens were rinsed in PBS, and then immersed overnight (4³C) in solution containing 20 μl of mouse anti-neurofilament 200 antibody (Sigma p1951, 1:100) dissolved in a solution containing 20 μl Triton X-100 (10%), 6 μl normal goat serum, 154 μl of 0.1 M PBS. After rinsing in 0.1 M PBS and immersed in a solution containing 20 μl of secondary goat anti-mouse IgG TRITC (Sigma T5393, 1:200) mixed in 12 μl normal goat serum, 40 μl Triton X-100 (10%) and 328 μl of 0.1 M PBS. Specimens were rinsed three times in PBS, and then stained with Alexa Fluor 488 conjugated phalloidin (1:200) for 30 min. After rinsing with PBS, specimens were mounted on glass slides as surface preparations in glycerin. Specimens from adult chinchillas were just stained with Alexa Fluor 488 conjugated phalloidin or TRITC-labeled phalloidin (Sigma P1951,1:200) for 30 min, and mounted on glass slides in glycerin. Samples were examined using a confocal microscope (Zeiss LSM-510 meta, step size 0.5 μm per slice with appropriate filters to detect the red fluorescence of TRITC labeled neurofilament 200 in nerve fibers and spiral ganglion neurons or TRITC labeled phalloidin at F-actin in chinchilla stereocilia (excitation 544 nm, emission 572 nm) and green fluorescence of Alexa 488-labeled phalloidin (excitation 488 nm, emission 520 nm) in the cuticular plate and the stereocilia of the hair cells. Confocal images from multiple layers were projected onto a single plane using the Zeiss LSM Image Examiner (version: 4, 0, 0, 91). Confocal images were further processed using adobe photoshop 5.5 software.

Results

Damage in cochlear explants from postnatal day 3 rat pups

In the cochlear explants from postnatal day 3 rat pups, carboplatin treatment for 48 h at various concentrations (10 μM, 50 μM, 100 μM, 500 μM, 1000 μM, or 5000 μM) resulted in two distinct patterns of damage of the cochlear hair cells and the spiral ganglion neurons (Fig. 1). Cochlear hair cells were heavily labeled with Alexa 488 phalloidin (green) and the auditory nerve fibers and spiral ganglion neurons were stained with neurofilament 200 antibody (red) respectively in normal cochlear explants after 48 h of culturing in standard serum-free medium without carboplatin (Fig. 1A). After treatment with 50 μM carboplatin for the same duration, cochlear hair cells were intact, while the density of auditory nerve fibers started to decline (Fig. 1B). When carboplatin concentration increased to 100 μM, the damage to hair cell and auditory nerve fibers were more evident (Fig. 1C). Carboplatin treatment at 500 μM destroyed all hair cells and heavily damaged auditory nerve fibers and spiral ganglion neurons (Fig. 1D). It is worthwhile to note that when carboplatin concentration was increased to 1000 μM, the damage to cochlear hair cells actually decreased, although degeneration of auditory nerve fibers and spiral ganglion neurons became worse (Fig. 1E). One interesting phenomenon is that 5000 μM carboplatin resulted in a complete destruction to auditory nerve fibers and spiral ganglion neurons, but most cochlear hair cells survived (Fig. 1F).

Hair cell damage in vestibular explants from postnatal day 3 rat pups

The photomicrographs in figure 2 are representatives of macula of utricle 48 h after culturing with various concentrations of carboplatin. Figure 2A shows normal surface structures of vestibular hair cells from a normal, untreated macula of utricle. Exposing vestibular explants to 10 μM carboplatin for 48 h resulted in a reduction of hair cell density (Fig. 2B). When the concentration of carboplatin increased to 50 μM or 100 μM, most hair cells were completely destroyed (Fig. 2C, 2D). However, as can be seen in figure 2E and 2F, the hair cell survival was distinctly increased when the concentration of carboplatin exceeded 500 μM.

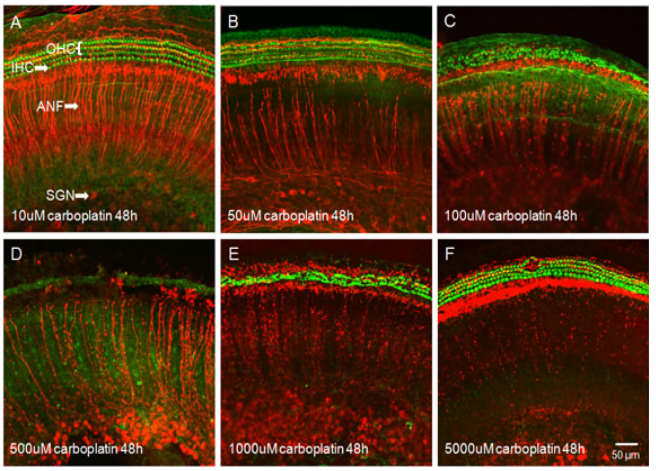

Figure 1. Photomicrographs of representative cochlear organotypic cultures double labeled with a monoclonal antibody against neurofilament 200 to show the auditory nervous system (red) and phalloidin to show the stereocilia bundles of the hair cell (green). (A) A cochlear explant treated with 10 μM carboplatin for 48 h showing no obvious damage of either hair cells or auditory nervous system. (B) A specimen treated with 50 μM carboplatin showing substantial damage of auditory nerve fibers. (C) A specimen treated with 100 μM carboplatin showing massive loss of both auditory nerve fibers and cochlear hair cells. (D) A specimen treated with 500 μM carboplatin showing destruction of most hair cells and severe damage to the auditory nervous system. (E) Even greater auditory nervous degeneration after 1000 μM carboplatin treatment, although with less missing hair cells. (F) Complete destruction of auditory nerve fibers and spiral ganglion neurons following 5000 μM carboplatin treatment. Unexpectedly, most cochlear hair cells have survived.

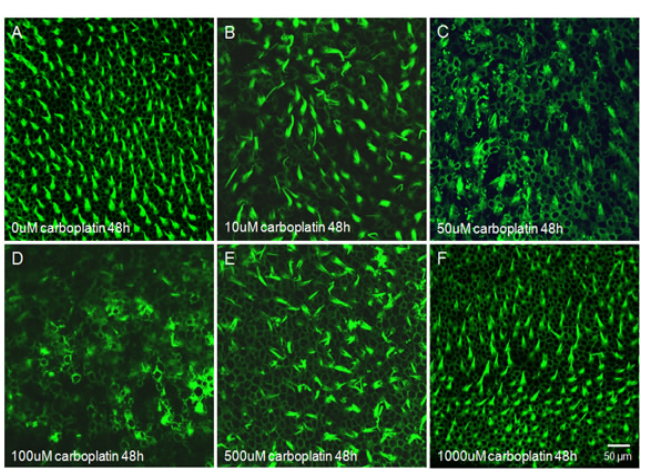

Figure 2. Photomicrographs of rat vestibular hair cells in the macula of utricles labeled with Alexa Fluor 488 conjugated phalloidin. (A) Untreated vestibular explants after 48 h culturing, showing well-organized vestibular hair cells as in normal controls. (B) Visible hair cell loss after treatment with 10 μM carboplatin. (C) Massive loss of vestibular hair cells after 50 μM carboplatin treatment. (D) Destruction of most vestibular hair cells after treatment with 100 μM carboplatin. (E) Increased hair cells survival after culturing with 500 μM carboplatin. (F) Mostly intact hair cells after 1000 μM carboplatin treatment.

Hair cell damage in cochlear explants from adult rats

The dose effects of carboplatin on adult rat cochlear explants were somewhat similar to those on postnatal day 3 rat pups explants: i.e., low dose carboplatin damaged hair cells while high concentration of carboplatin did not. In addition, outer hair cells were clearly damaged more than inner hair cells (Fig. 3), consistent with previous in vivo and in vitro studies in rats [31, 34, 76-78].

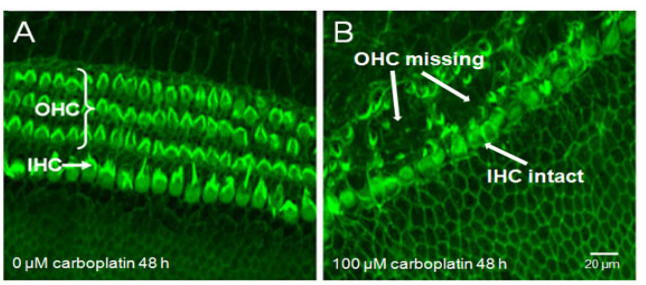

Figure 3. Carboplatin-induced structural damage on the organ of Corti in adult rat cochlear explants. (A) normal control rat cochlear orgnotypic culture in standard serum-free medium after 48 h without carboplatin treatment. (B) Rat cochlear explants treated with 100 μM carboplatin for 48 h. Note massive loss of outer hair cells, with essentially intact inner hair cells.

Hair cell damage in vastibular explants from adult rats

A similar destructive trend to vestibular explants from postnatal day 3 rat pups by carboplatin was found in adult rat vestibular culture system. Examination of the vestibular hair cells following 48 h of carboplatin exposure at various concentrations (10 μM, 50 μM, 100 μM, 500 μM, 1000 μM, or 5000 μM) revealed that vestibular hair cells were destroyed at lower concentrations (10 μM, 50 μM, 100 μM, and 500 μM) (Fig. 4A, 4B, 4C, and 4D), but remained intact at the higher concentrations (1000 μM, and 5000 μM) (Fig. 4E, and 4F).

Hair cell damage in cochlear explants from adult chinchillas

The photomicrograph in Fig. 5A shows the orderly structure of three rows of outer hair cells and a single row of inner hair cells in a normal cochlear organotypic culture from an adult chinchilla. carboplatin treatment at 10 μM for 48 h resulted in the loss of inner hair cells, while sparing the outer hair cells (Fig. 5B). When the dose of carboplatin was increased to 50 μM, besides the IHC loss, most outer hair cells were also destroyed (Fig.

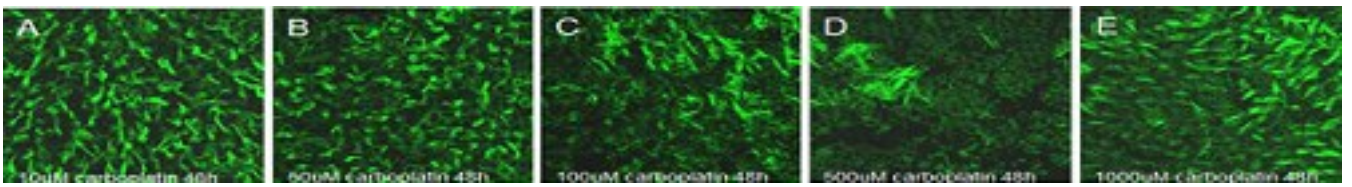

Figure 4. Photomicrographs of rat vestibular hair cells in the macula of utricles labeled with Alexa Fluor 488 conjugated phalloidin. (A) Most hair cells were visible 48 h after 10 μM carboplatin treatment. (B) Tangling and breaking cilia were detected 48 h post-50 μM carboplatin. (C) Visible damage on the cuticular plate of hair cells was found 48 h after 100 μM carboplatin cultures. (D) 50 μM carboplatin treatment for 48 h resulted in massive destruction of hair cells. (E) Most hair cells were intact 48 h after 1000 μM carboplatin treatment.

5C). However, exposure to 100 μM carboplatin for 48 h only destroyed the inner hair cells, but not the outer hair cells (Fig. 5D). Surprisingly, when the concentration of carboplatin amounted to over 500 μM, most inner hair cells and outer hair cells were intact (Fig. 5E, 5F). Severe hair cell loss was detected following 48 h exposure to 50 μM carboplatin, whereas no striking damage to hair cells were seen after exposure to 500 μM or higher concentrations of carboplatin. Figure 6 shows the selective damage to inner hair cells in chinchilla cochlear explants, concordant with in vivo observation of carboplatin-induced inner hair cell degeneration in chinchillas[12, 56-59, 61-64, 69, 73, 86, 87].

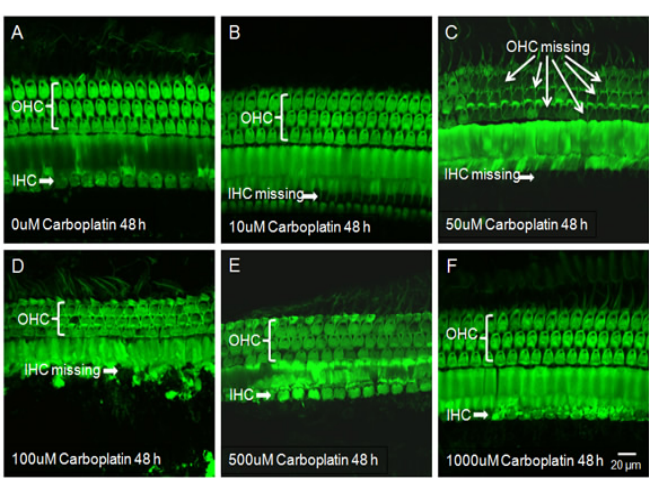

Figure 5. Surface preparations showing the dose response of carboplatin in cochlear organotypic cultures from adult chinchillas. (A) All inner and outer hair cells are intact 48 h after culturing with standard culture medium without carboplatin. (B) Massive inner hair cell loss after 10 μM carboplatin treatment for 48 h while outer hair cells are spared. (C) Destruction of both inner and outer hair cells after treatment with 50 μM carboplatin for 48 h (D), Missing inner hair cells with obvious damage in their surrounding supporting cells after 48 h culturing with 100 μM carboplatin. However, most outer hair cells have survived. (E) With the concentration of carboplatin increased to 500 μM, most inner and outer hair cells remain essentially normal. (F) Carboplatin treatment at 1000 μM for 48 h results in no damage in hair cells.

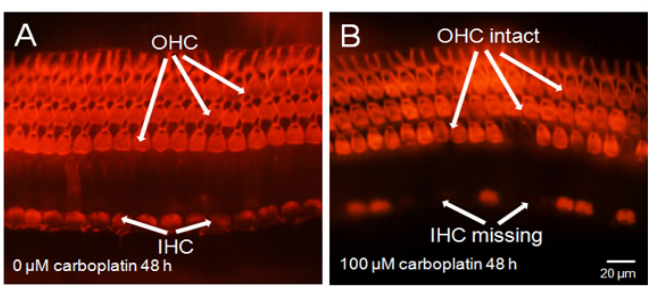

Figure 6. Carboplatin-induced structural damage on the organ of Corti in chinchilla cochlear explants. (A) Normal cochlear hair cells after 48 h culturing without carboplatin. (B) Chinchilla cochlear explants treated with 100 μM carboplatin for 48 h. Note many missing inner hair cells, but most outer hair cells remain normal.

Hair cell damage in vestibular explants from adult chinchillas

Exposing vestibular explants from adult chinchillas to carboplatin with various concentrations (10 μM, 50 μM, 100 μM, 500 μM, 1000 μM, or 10000 μM) for 48 h resulted in the destruction of hair cells in macular of utricle in a dose-dependent fashion (Fig. 7). The photomicrograph in Figure 7A shows the normal vestibular culture labeled with Alexa Fluor 488-conjugated phalloidin that intensely labels actin in the stereocilia and, to a lesser extent, the cuticular plate of hair cells. As can be seen in figure 7B and figure 7C, the low doses of carboplatin used (10 μM and 50 μM) did not cause evident damage to vestibular hair cells. However, carboplatin at 100 μM was sufficient to bring about the disarray of stereocilia bundles on some vestibular hair cells (Fig. 7D). When the concentration of carboplatin was increased to 500 μM, cilia missing was seen in many vestibular hair cells leaving remnants of the actin ring surrounding the cuticular plate (Fig. 7E). Increasing the carboplatin dose to 1000, 5000, or 10000 μM destroyed most vestibular hair cells (Fig. 7F, 7G, 7H). These results indicate that carbo-

platin-induced vestibular hair cell degeneration is in a dose-dependent manner in vestibular culture system from adult chinchilla.

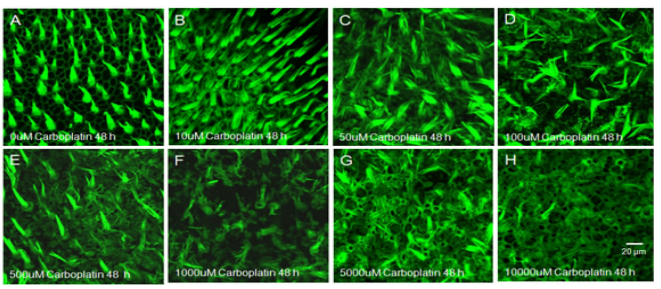

Figure 7. Photomicrographs showing vestibular organotypic cultures from the middle area of macula of utricle after 48 h treatment with carboplatin in an adult chinchilla culture system. Hair cells are labeled with Alexa Fluor 488-phalloidin. Carboplatin concentrations are shown in each panel. (A) A normal control showing normal arrangement of vestibular hair cells. Vestibular culture treated with carboplatin at a concentration of 10 μM shows no damage of hair cells (B). However, carboplatin treatment at 50 μM and 100 μM for 48 h results in abnormal arrangement of vestibular hair cells in the macula of utricle (C, D). Cilia in some vestibular hair cells missing after 48 h of carboplatin treatment at 500 μM. (E). Massive damage in the macula of utricle when the concentrations of carboplatin exceeds 1000 μM (F, G, H).

Discussion

As a second-generation platinum antineoplastic agent, carboplatin is about the same as cisplatin in biological effects due to formation of the same interstrand/intrastrand DNA cross links. Although the incidence of carboplatin side effects is reduced, however, the antitumor potential of carboplatin remains the same as its original design thanks to technological innovation[2]. Despite that carboplatin has a lower risk of producing adverse reactions than cisplatin, there has been a growing concern over its adverse neurotoxic and ototoxic potentials in recent years[10,14,15]. In different experimental animal species, carboplatin has been documented as predominantly affecting outer hair cells in rats and guinea pigs[34, 37, 89]. In contrast to the findings in these species, carboplatin-induced selective inner hair cell damage in chinchillas becomes a unique, species-specific toxicity[56, 59, 61-63, 65-67, 74, 75, 82]. An intriguing question that needs to be addressed is why the various experimental animal species have different responses to carboplatin ototoxicity. There has not been a definite answer. Generally, different drug-induced responses in different species are considered to be related to either species diversity or the cell difference. To comprehend if the difference in susceptibility to carboplatin ototoxicity in different species is determined by the cell itself rather than the systemic drug metabolism in living animal species, we compared the in vitro carboplatin ototoxic effects between chinchillas and rats. According to the results from inner ear organotypic cultures in the current study, the species-specific ototoxicity of carboplatin is believed to be characterized at the cell level rather than the systemic metabolism.

The biological activation of carboplatin requires hydration following cell entry. In order to bind to DNA and to exert its toxic effects, carboplatin must first enter the cell. Evidence suggests that platinum agents are mainly imported by copper transporter importer, Ctr1, and exported from cells or cellular organelles by copper transporter exporters, ATP7A, and ATP7B[74, 82, 84, 85, 90-93, 102]. Cells have the potential to adjust the activities of copper transporters properly according to the intracellular and extracellular copper/platinum concentrations[82, 84, 85, 95-103]. According to reports in the literature, enhanced extracellular concentration of copper by local application on round window can result in an intrinsic cytoprotective effect of hair cells against carboplatin by modulating the activities of copper transporters[53, 82, 84, 85, 102]. These important findings suggest that intracellular and extracellular copper/platinum is detectable by the cells. Experiments indicate that the high concentration of copper/platinum can lead to a reduction of uptake by withdrawing Ctr1 from the membrane into the cytoplasm where Ctr1 is quickly degraded, and also increased efflux of copper/platinum from cytoplasm by ATP7A and ATP7B to reduce the accumulation of copper/platinum in the cell[53, 82, 84, 85, 102]. The cellular intrinsic modulation of copper transporters is efficient for the cytoprotection from platinum injury in vitro, but may not appear in vivo natively. The cause is that in vitro concentrations of platinum can be high enough to alert the cell. However, the in vivo concentration of platinum in the inner ear is low due to the block by the barrier between the bloodstream and organic tissues[52, 102, 104-107]. Therefore, the infiltration of platinum in the inner ear by systemic absorption may not attain enough concentration to attract cell's attention. An interesting phenomenon discovered is that cochlear and vestibular hair cells from postnatal day 3 rats and adult rats showed a similar nonlinear response to carboplatin ototoxicity, while the damage to auditory nerve fibers and spiral ganglion neurons in postnatal day 3 rats was in a linear dose dependent manner. This is different from neurotoxic effects of cisplatin in rat cochlear organotypic cultures[82, 84, 85]. In comparison with rat cochlear organotypic cultures, cochlear hair cells of chinchilla were destroyed by carboplatin only at low concentrations, which is similar to the response from in vitro studies in various platinum explants[82-85, 102, 108]. The only difference is that inner hair cells in chinchilla

are more susceptible to carboplatin, while outer hair cells in rat are the first victims. The species-specific mechanism of this phenomenon is unclear, but will need to be addressed in our future studies. The rat vestibular hair cells have equal dose-response to carboplatin in that these cells are destroyed by low concentration of carboplatin treatment, but remain intact at high concentrations. However, chinchilla vestibular hair cells exhibited a dose dependent response to carboplatin. The cause of the differences between rats and chinchillas in responses to carboplatin ototoxicity in vestibular hair cells is not known. A protective response may be aroused in rat vestibular hair cells by high concentrations of platinum[82, 85, 102, 105]. In contrast, the vestibular hair cells in chinchilla do not show this intrinsic resistance to carboplatin for some unknown reasons[76, 77].

To prevent the ototoxic effects of platinum, many strategies can be considered for inner ear protection. Since platinum can arouse cellular injury by activation of free radicals[76, 109-113], many antioxidants have been used for protection against cisplatin toxicity[112,114-118]. Although antioxidation can effectively protect the cochlea from platinum damage, it can also compromise the anti-tumor activity of platinum when treated systemically[119,120]. Therefore,local antioxidant application may be required for cochlear protection against platinum ototoxicity [118]. Platinum toxicity specifically causes cell apoptosis. Therefore, anti-apoptotic agents may also provide protection against platinum toxicity[82,85,121-139]. In addition, neurotrophic factors have also been reported to reverse platinum induced cochlear injury[140-142]. Although above mentioned strategies have seen variable successes, it has to be pointed out that the key is the process of cell degeneration. When the cell has started the degeneration process, treatment effects will vary depending on the degree of lesions. Therefore, the protective actions by antioxidation or anti-apoptosis agents are limited. The results in our recent publications and the current study, by modulating copper transporters, may develop a new strategy against platinum ototoxicity, which keeps cisplatin out of the cell by reducing platinum influx and enhancing platinum efflux[50, 53, 74, 82-85, 102, 105, 108]. When the platinum is isolated from the cell while in the process of attack, its following toxic effects may be unfulfilled.

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