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Ototoxic Effects of Carboplatin in Organotypic Cultures in Chinchillas and Rats

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Introduction

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 μM for 48 h. Consistent with *in vivo* data, carboplatin selectively damaged IHC at low concentrations (50 μM-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 μM. 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.

Carboplatin: Second-Generation Platinum

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 μM 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, 37o, 5% CO2) overnight. On the second day, the serum-free medium was exchanged with new medium 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. 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 MPBS. After rinsing in 0.1 MPBS 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 MPBS. Specimens were rinsed three times in PBS, and then stained with Alexa Fluor 488 conjugated phalloidin (1:200) for 30 min.

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