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INDUCTION OF SELECTIVE INNER HAIR CELL DAMAGE BY CARBOPLATIN

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Abstract

Carboplatin (diammine [1,1 cyclobutane dicarboxylato (2)-0,0'] platinum) is an anti-cancer agent which can be toxic to the inner ear. We have explored the nature of this ototoxicity in the chinchilla. In this species, initial degenerative changes appear to be restricted to the inner hair cell (IHC) regions of the organ of Corti. This finding is intriguing and unusual since all other known ototoxic drugs, such as aminoglycosides, are predominantly associated with outer hair cell damage.

In the present study, the mechanism of ototoxicity was investigated by comparing two different routes of carboplatin administration. Carboplatin was administered either intravenously (i.v.) or intraperitoneally (i.p.). The mode of administration influenced electrophysiological and morphological changes. Hearing thresholds were elevated in the i.v. group significantly more than in the i.p. group at all tested frequencies. The degree of hair cell damage was evaluated by scanning electron microscopy at four frequency regions in each cochlea. IHC damage in the i.v. group was significantly more severe than in the i.p. group.

Carboplatin effects on a different species, the guinea pig, were also determined to clarify interspecies differences. In the guinea pig, outer hair cell damage occurred sporadically and inner hair cells remained intact. In contrast, chinchilla inner hair cells are susceptible to the ototoxic effects of carboplatin. The degree of hair cell damage appears to be dependent on the peak level of carboplatin rather than on the total dose. This animal model provides a new tool for the investigation of inner and outer hair cell function.

Key Words: Scanning electron microscopy, chinchilla, organ of Corti, inner hair cell, carboplatin, ototoxicity.

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Introduction

The development of the platinum group of anti-cancer drugs originated in 1965 with pioneering work in which the inhibitory effects of soluble platinum salts on cell division of *Escherichia coli* were discovered (Rosenberg *et al.*, 1965, 1969). Cisplatin was the first and the most potent drug from the platinum group and has been used for over a decade for the treatment of various cancers. However, cisplatin has serious dose-limiting side effects of nephrotoxicity and neurotoxicity. Ototoxicity, in the form of dose-dependent sensorineural hearing loss, is another major side effect of cisplatin treatment. In an attempt to reduce the dose-limiting toxicities, a second generation of platinum compounds was developed and entered clinical trials.

Carboplatin (diammine [1,1 cyclobutane dicarboxylato (2)-0,0'] platinum, CBDCA) is one of the cisplatin analogues. Carboplatin expresses its anti-neoplastic effect by forming inter/intra-strand DNA cross-links when converted into a hydrated species (van Hennik *et al.*, 1987). In clinical experience, carboplatin causes significant, dose-limiting, myelosuppression but is reported to be less nephrotoxic and ototoxic than cisplatin. The incidence of hearing loss in humans caused by carboplatin, generally restricted to the higher frequencies, ranges from O to 19% (Calvert *et al.*, 1982; Kennedy *et al.*, 1990; Macdonald *et al.*, 1994). This value is less than that of cisplatin which ranges from 25 to 100% (Lippman *et al.*, 1973; Piel *et al.*, 1974; Strauss *et al.*, 1983; Kopelman *et al.*, 1988; Myers *et al.*, 1991). The large variability in reported incidence of ototoxicity is influenced by: mode of administration, cumulative total dosage and maximal single dosage, age of patient, and presence or absence of a preexisting hearing defect. In experimental animal models, ototoxic effects of these two platinum compounds on the organ of Corti have been characterized by a range of outer hair cell (OHC) damage across various species including guinea pigs (Estrem *et al.*, 1981; Nakai *et al.*, 1982; Tange *et al.*, 1982; Schweitzer *et al.*, 1984, 1986; Marco-Algarra *et al.*, 1985; Saito *et al.*, 1989; McAlpine and Johnstone, 1990; Taudy *et al.*, 1992), monkeys (Stadnicki *et al.*, 1975), and rats (Anzai *et al*, 1987). The ototoxic potential has also been analyzed using organ culture models (Anniko and Sobin, 1990). All these data have consistently indicated that the predominant effect of

both compounds is OHC degeneration, carboplatin being less ototoxic than cisplatin.

We have recently found that in chinchillas carboplatin is highly ototoxic (Wake *et al.*, 1993; Takeno *et al.*, 1994). A single intravenous administration of 200 to 400 mg/m^2 carboplatin, which is equivalent to the clinically suggested therapeutic dose, is enough to induce significant hearing threshold elevation as determined by auditory brainstem evoked responses (ABR). Furthermore, the degenerative changes seen in the organ of Corti are highly selective to the inner hair cells (IHCs). An IHC lesion is very unusual since all known cochleotoxic drugs in any other animal model predominantly and initially affect OHCs. While IHC loss due to cisplatin ototoxicity has been observed both in humans (Wright and Schaefer, 1982; Strauss *et al.*, 1983) and in rhesus monkeys (Stadnicki *et al.*, 1975), it always occurred subsequent to extensive OHC damage. To the best of our knowledge, the only combination producing a lesion with missing IHCs and intact OHCs is carboplatin in chinchillas.

In the present study, we describe detailed morphological features of this intriguing animal model using scanning electron microscopy (SEM). The results are compared with physiological data obtained by ABR recordings. In the chinchilla, two different routes of administration of carboplatin (intravenous: i.v.; and intraperitoneal: i.p.) were employed to determine potential differences in ototoxic effects. In an additional study, guinea pigs were treated with the same dosage of carboplatin to clarify the unique interspecies difference between chinchillas and other animals.

Materials and Methods

Adult chinchillas and albino (Hartley) guinea pigs, free from ear disease, were used in this study. Three treatment groups were set up as follows: **A)** 6 chinchillas treated with carboplatin injected into the internal jugular vein (i.v.); **B)** 6 chinchillas treated with i.p. injection of carboplatin; and **C)** 6 albino guinea pigs treated with i.p. injection of carboplatin. In all groups, carboplatin in 5% dextrose was administered as a single injection at the dose of 400 mg/m^2 (total volume 3 ml). This is equivalent to the maximum therapeutic dose clinically scheduled for single-agent regimens as a single i.v. injection. Additionally, two control studies were carried out: **D)** chinchillas identically treated with 3 ml i.v. injection of 5% dextrose without carboplatin; and **E)** guinea pigs treated with 3 ml i.p. injection of 5% dextrose without carboplatin.

Carboplatin for the study was supplied by Bristol Myers Canada (Paraplatin-AQ). The surface area of the animals was estimated using the formula of Yates and Kugler (1986). ABR recordings of all animals were carried out prior to and two weeks after treatment.

ABR recordings were obtained from animals lightly anesthetized using ketamine (15 mg/kg), xylazine (2.5 mg/kg) and atropine (0.004 mg/kg). Skin needle electrodes were used in a standard vertex to post-aural configuration.
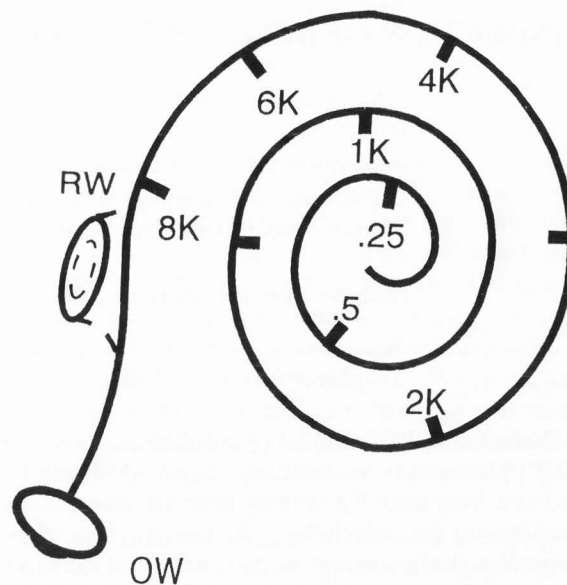

Figure 1. Frequency position map of the chinchilla cochlea. Characteristic frequencies are assigned along the cochlea length according to the data of Eldredge *et al.* (1981). Note that 8, 4, 2 kHz regions are located in the basal turn, and 1, 0.5 kHz regions are included in the second turn. RW: round window. OW: oval window.

Sound stimuli were tone pips (2 ms rise/fall, 2 ms plateau) at octave intervals between 0.5 and 8 kHz. These signals were calibrated in an artificial (chinchilla) ear using a standard reference. Sound was delivered free-field in a sound attenuated room. Potentials were band pass filtered (150 Hz - 3 kHz) and amplified conventionally. After analogue to digital conversion and artifact rejection, signals were averaged over 300 sweeps in a 25 ms window (Cambridge Electronic Design 1401 intelligent interface with 80286 microprocessor host). ABR waveform amplitudes were measured over a range of stimulus intensities allowing threshold determinations. Bilateral pre- and post-treatment ABR audiograms were determined for each animal.

All animals were prepared for morphological evaluation of the cochleas immediately following the final recording sessions. Under deep anesthesia with sodium pentobarbital (50 mg/kg), cardiac perfusion was carried out with fixative (1.25% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4), and the temporal bony capsules were removed. Cochleas were exposed and perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer through the oval and round windows. The specimens were then prepared for SEM. After initial fixation, the specimens were rinsed in 0.1 M phosphate buffer and conductive stained with 1% tannic acid for 30 minutes followed by post-fixation with 0.5% osmium tetroxide for 15 minutes. After dehydration through a graded ethanol series (50, 70, 80, 90, 95 and 100% twice), specimens were immersed in

a) Group A   b) Group B   c) Group C
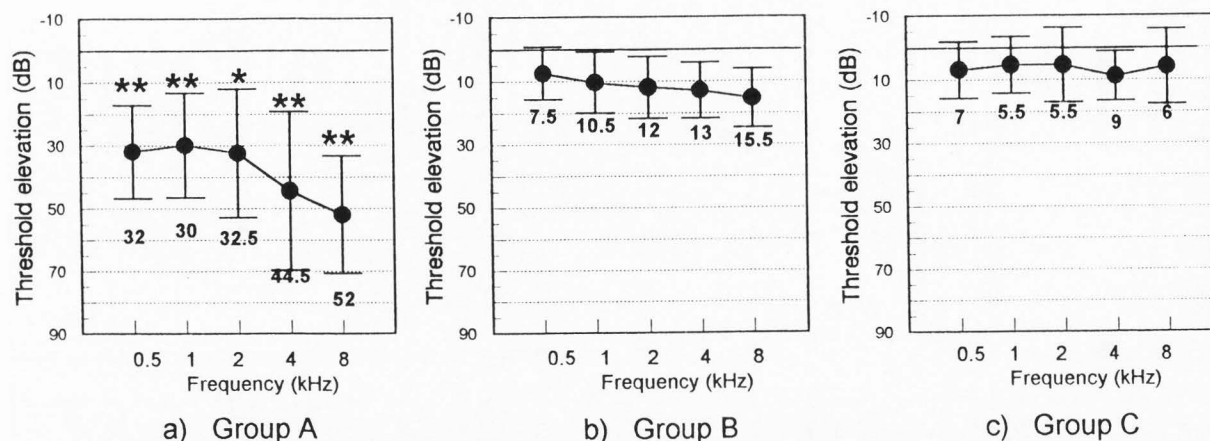
Figure 2. ABR threshold elevation values (mean ± standard deviation, S.D.) after carboplatin treatment for experimental groups A, B, and C (10 cochleas in each group). **a)** Group A (i.v. in the chinchilla) shows the most remarkable changes at all frequencies. **b)** Group B (i.p. in the chinchilla) shows less hearing loss compared to Group A. **c)** Group C (i.p. in the guinea pig) developed only minimal changes in threshold elevation. Statistical differences observed in the chinchilla between group A (i.v.) and group B (i.p.) are indicated by: * (p < 0.05), ** (p < 0.01); (two-sample t-test).

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amyl acetate for 30 minutes then critical point dried from CO_2. Specimens were sputter coated with 30 Å thick gold.

The extent of IHC and OHC damage observed by SEM was assessed in specific regions of the cochlea and compared to the corresponding ABR data. In the chinchilla, cochlear frequency-place maps derived by Eldredge *et al.* (1981) were used. Hair cell damage was assessed in the 0.5, 1, 2, 4 and 8 kHz regions (Fig. 1). In the guinea pig, the mid-portion of each turn of the cochlea was observed as described elsewhere (Saito *et al.*, 1989). At each assessed location, an area of the organ of Corti containing 100 IHCs and 100 each first, second and third row OHCs was examined to determine the degree of damage in each of the four rows. Hair cell condition was graded using the following criteria: Grade 0 = normal to slight damage (less than 20% disruption of the stereocilia); Grade 1 = moderate damage (disruption of stereocilia but with less than 50% of stereocilia missing); Grade 2 = severe damage (less than 50% surviving stereocilia); and Grade 3 = presence of fusion/extrusion bodies, or total loss of stereocilia or hair cell (this grading scheme was modified from that of Fukushima *et al.*, 1990). Final hair cell damage scores are calculated according to the formula below and expressed as a percentage (total loss = 100%).

$$\frac{(\text{total grade number})}{(\text{number of evaluated hair cells}) \times 3} \times 100$$

All procedures using animals were carried out with strict adherence to standards implemented by local Animal Care Committees and following national guidelines issued by the Canadian Council on Animal Care, as well as local (Ontario) legislation.

Results

The single administration of 400 mg/m^2 carboplatin caused the death of one subject in each group due to acute toxicity. The necropsy data suggested the existence of severe pulmonary infection triggered by myelosuppression. Therefore, 10 cochleas in total (5 animals) from each group were assessed for ABR threshold elevation and hair cell condition. The general condition of the surviving carboplatin treated animals was good throughout the experiments. They appeared healthy with normal behavior.

ABR threshold shifts

In Figure 2, the mean values (± 1 S.D.) of post-treatment ABR threshold elevations (i.e., the difference between the pre- and post-treatment threshold level in each subject) are plotted for the three experimental groups. The majority of cochleas from each group showed differences in pre- and post-treatment thresholds. Group C (i.p. injection in the guinea pig; right-hand plot) developed only minimal changes in hearing sensitivity with the administration of 400 mg/m^2 carboplatin. In this group, less than 10 dB threshold elevation was found across the tested frequencies. In group B (i.p. injection in the chinchilla; center plot), the mean threshold elevation ranged from 7.5 dB to 15.5 dB, the greater shifts occurred at higher frequencies.

The most significant threshold elevations occurred in group A (i.v. injection of carboplatin in the chinchilla, left-hand plot). In this group, seven of ten cochleas sustained severe hearing loss (more than 40 dB) including unrecordable potentials especially at high frequencies. A large inter-individual variability was also noted in this group as indicated by the standard deviation bars. Similar to group B, hearing losses are greater at higher frequencies. Threshold elevations at all frequencies of group A were significantly greater (p < 0.01 at 0.5, 1, 4 and 8 kHz; 0.01 < p < 0.05 at 2 kHz; two-sample t-test) than group B. Differences in threshold shifts (averaged of all frequencies) between the right and the left cochleas were also
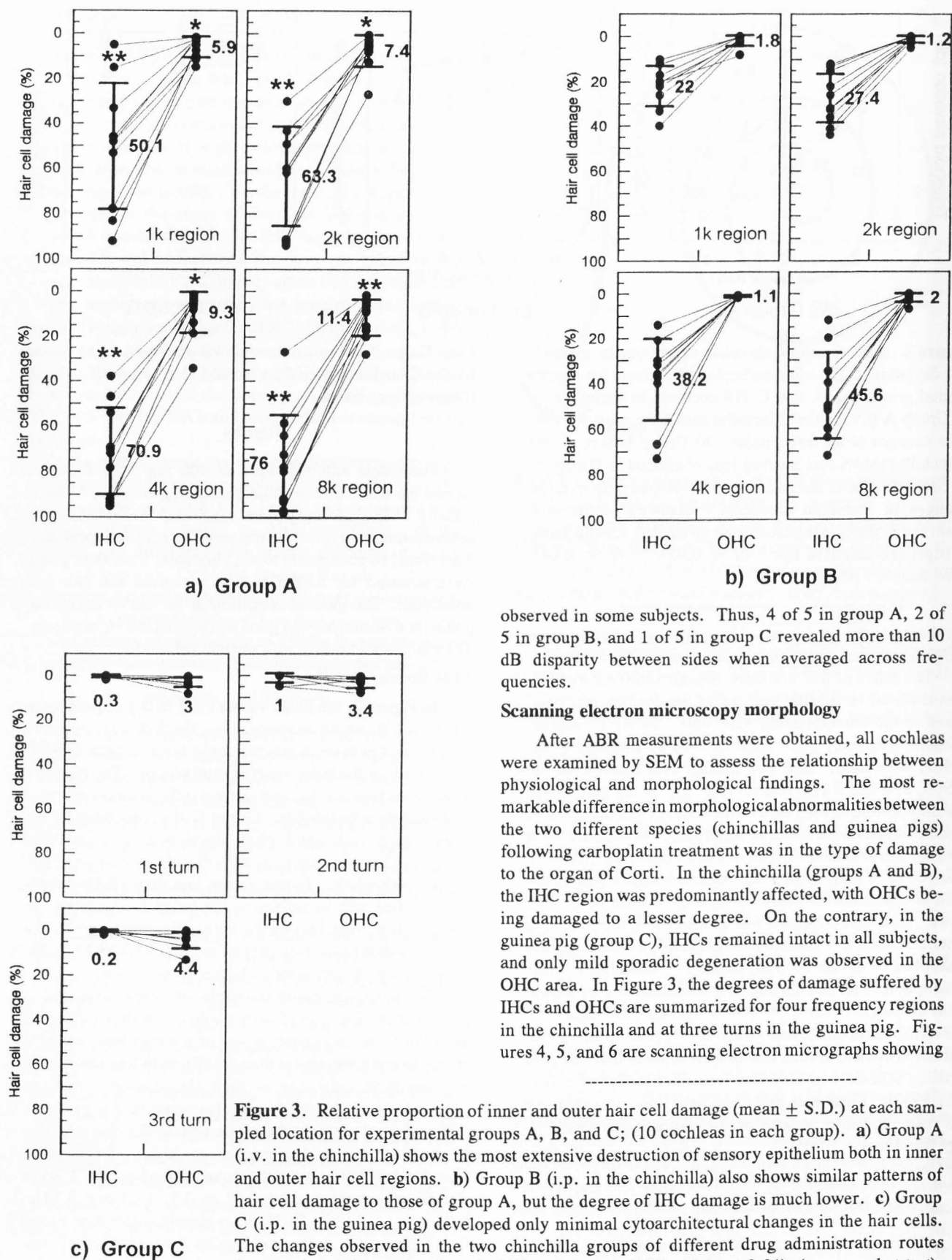
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a) Group A

b) Group B

c) Group C

observed in some subjects. Thus, 4 of 5 in group A, 2 of 5 in group B, and 1 of 5 in group C revealed more than 10 dB disparity between sides when averaged across frequencies.

Scanning electron microscopy morphology

After ABR measurements were obtained, all cochleas were examined by SEM to assess the relationship between physiological and morphological findings. The most remarkable difference in morphological abnormalities between the two different species (chinchillas and guinea pigs) following carboplatin treatment was in the type of damage to the organ of Corti. In the chinchilla (groups A and B), the IHC region was predominantly affected, with OHCs being damaged to a lesser degree. On the contrary, in the guinea pig (group C), IHCs remained intact in all subjects, and only mild sporadic degeneration was observed in the OHC area. In Figure 3, the degrees of damage suffered by IHCs and OHCs are summarized for four frequency regions in the chinchilla and at three turns in the guinea pig. Figures 4, 5, and 6 are scanning electron micrographs showing

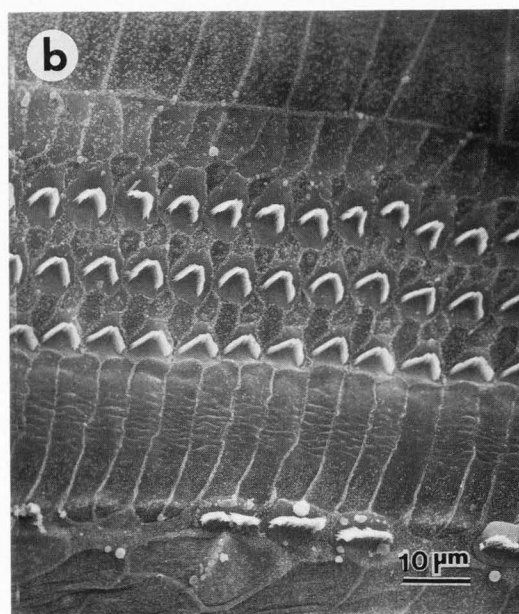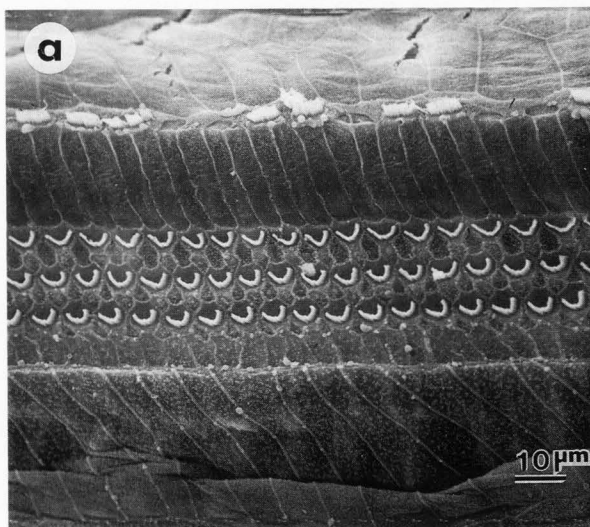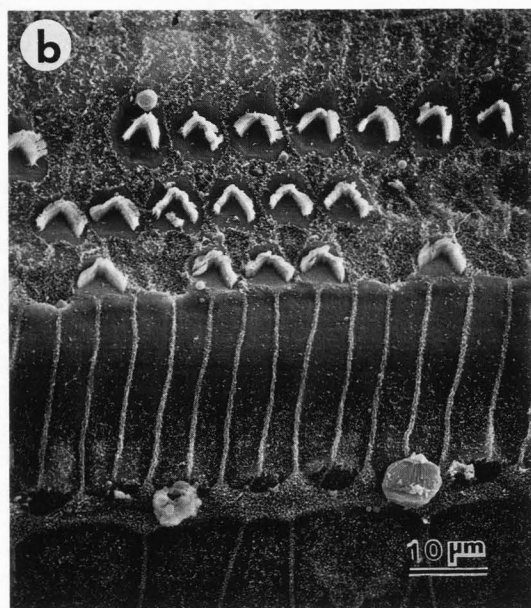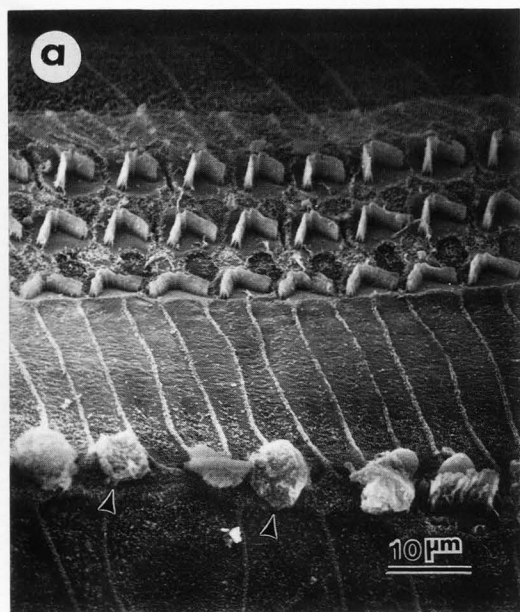---

Figure 3. Relative proportion of inner and outer hair cell damage (mean ± S.D.) at each sampled location for experimental groups A, B, and C; (10 cochleas in each group). **a)** Group A (i.v. in the chinchilla) shows the most extensive destruction of sensory epithelium both in inner and outer hair cell regions. **b)** Group B (i.p. in the chinchilla) also shows similar patterns of hair cell damage to those of group A, but the degree of IHC damage is much lower. **c)** Group C (i.p. in the guinea pig) developed only minimal cytoarchitectural changes in the hair cells. The changes observed in the two chinchilla groups of different drug administration routes (groups A and B) are significantly different: * (p < 0.05); ** (p < 0.01); (two-sample t-test).

Figure 4. Scanning electron photomicrographs of the organ of Corti showing representative samples from group A. **(a)** In this 8 kHz region, i.v. injection of carboplatin caused almost total IHC destruction. Characteristic formation of fusion/extrusion bodies is demonstrated on the surface of reticular lamina. The OHCs remain intact in this area (IHC loss; 97%, OHC loss; 10%). **(b)** Two of ten cochleas in group A showed considerable OHC degeneration along with complete IHC loss. This relatively rare case is demonstrated in the 4 kHz region (IHC loss: 94%; OHC loss: 30%).

Figure 5. Scanning electron photomicrographs showing representative samples from group B. **(a)** The gross view of the 4 kHz region in this subject demonstrates a loss of > 50% IHCs with a normal OHC population. In general, i.p. injection of carboplatin induced milder damage to the hair cells than i.v. injection. Furthermore, formation of fusion/extrusion bodies, which occasionally occurred subsequent to IHC degeneration in i.v. cases, is not commonly detected in i.p. cases (IHC loss: 67%; OHC loss: 0.6%). **(b)** In this 2 kHz region of group B, moderately preserved IHCs are demonstrated (IHC loss: 33%; OHC loss: 0.8%).

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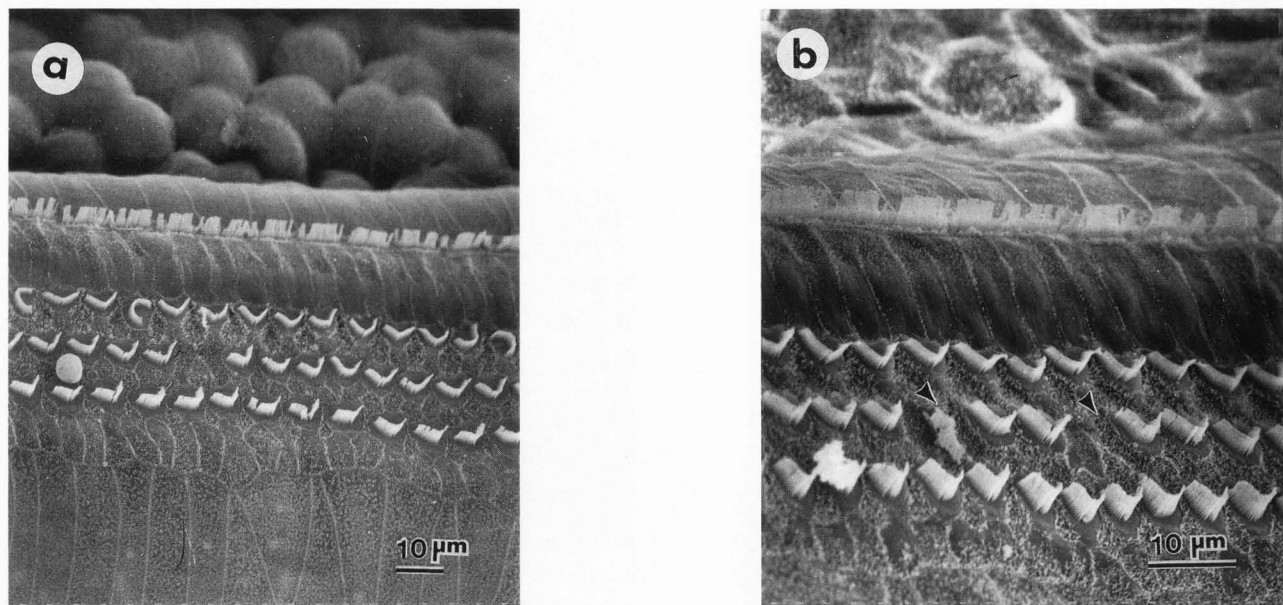
Figure 6. Scanning electron micrographs showing representative samples from group C. (a) The most intriguing finding here is that carboplatin induces no IHC damage in the guinea pig in complete contrast to the chinchilla. This micrograph from the 3rd turn of a guinea pig's cochlea demonstrates an intact row of IHCs with occasionally missing OHCs (IHC loss: 1%; OHC loss: 2%). (b) Mildly scattered loss of OHCs is observed in the 1st turn region of this specimen (IHC loss: 0%; OHC loss: 4%).

representative samples of the damaged sensory epithelium from treatment groups A, B, and C respectively.

Inner hair cell damage was greatest in group A animals at all frequency locations. Mean values of group A damage scores ranged from 50% at 1 kHz to 76% at the 8 kHz region. A general finding of carboplatin ototoxicity in the chinchilla (both i.v. and i.p. treated subjects) was that more IHC damage was noted in the higher frequency regions than in the apical lower frequency areas. Group A animals have a high degree of variability of inter-cochlea susceptibility to IHC damage (Fig. 3a), as is also reflected in the variability in ABR threshold shifts (Fig. 2a). In group B, the pattern of hair cell damage caused by carboplatin was similar to that of group A in all four frequency areas. However, the degree of IHC damage was less (from 22% damage at 1 kHz to 46% at the 8 kHz region) than that of group A, and the variability between cochleas was reduced particularly in the lower frequencies. OHC damage in this group was minimal with generally less than 2% OHC damage observed across all frequencies. The degree of IHC and OHC degeneration assessed at four frequency regions was statistically different from those of group A ($p < 0.01$ at 1, 2, 4, 8 kHz for IHCs and at 8 kHz for OHCs; $0.01 < p < 0.05$ at 1, 2, 4 kHz for OHCs; two sample t-test). The guinea pigs of group C did not show significant cytoarchitectural changes in the sensory epithelium except for some occasional scattered loss of OHCs. In general, there was less than 5% OHC damage and almost no IHC damage in this group.

Figure 7 summarizes the distributions of the surviving three rows of OHCs in each group. In general, carboplatin toxicity results in only minimal damage to OHCs of the three rows in groups B and C. In group A, two of ten cochleas indicate significant OHC loss (20-30% OHC damage), which is dominant toward the basal region and more pronounced in the first row. IHCs of these subjects are more extensively damaged (70-90% damage) than others in the group. Results of the statistical analysis (ANOVA; single factor) revealed no significant difference in damage to OHCs among the three rows either at any frequency region or at any turn in the three groups.

The control studies in both species gave the following results: normal animals treated with 5% dextrose, either i.v. (chinchillas) or i.p. (guinea pigs), showed no audiometric threshold elevation and retained morphologically normal cochleas.

Discussion

Comparison of drug administration routes

In the present study, two different routes of administration of carboplatin were employed in the chinchilla, and the resulting physiological and morphological changes were analyzed statistically. The mode of administration of platinum compounds has been previously investigated by others with respect to both the therapeutic index in treating disease and systemic toxicity, especially nephrotoxicity, bone marrow suppression and ototoxicity.

a) Group A

b) Group B

c) Group C

In dogs which received a single cisplatin i.v. injection, the serum concentration showed a peak level after a few minutes (Litterst *et al.*, 1976; Pretorius *et al.*, 1981). The distribution was a distinct biphasic clearance pattern with a short initial half-time (less than 1 hour) and a prolonged biologic elimination phase (about 5 days). After i.p. administration of cisplatin, the initial concentration of platinum compounds within the peritoneal cavity exceeds the serum level, and a portion of the i.p. injected dose is gradually absorbed from the peritoneal cavity over the course of several hours. Serum platinum reaches peak levels around 4 hours after i.p. injection in dogs and then decreases in a way similar to the i.v. levels. These authors also reported that peak serum levels after i.v. injection were around ten times higher than after i.p. injection of an equal dose. The same phenomenon has been also reported in humans (Jones *et al.*, 1978).

Peak plasma concentrations and clearance rates differ between species. Van Hennik *et al.* (1987) reported that, after i.v. administration of 495 mg/m^2 carboplatin, the peak

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Figure 7. Degrees of damage in each of the three OHC rows (mean $\pm$ S.D.) at each sampled frequency location of the cochlea in groups A, B and C (10 cochleas in each group). **a)** In group A (i.v. in the chinchilla), two of ten cochleas show noticeable (20-30%) OHC damage, while the others maintain well-preserved OHCs. **b)** Group B (i.p. in the chinchilla) shows almost intact OHCs in all subjects. **c)** In group C (i.p. in the guinea pig), despite totally intact IHCs, about 5% OHC damage was observed across four frequency regions as depicted in Figure 6. In all three groups, no significant increase in damage to OHCs of any particular row is detected at the locations demonstrated here (ANOVA; single factor).

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platinum level measured in mice was nearly 7-fold higher than in humans, but the initial half-time was 6 times shorter in mice. In the present study, we did not measure actual levels of carboplatin with respect to serum and inner ear tissues. However, based on the above mentioned studies, we expect the peak serum level to be significantly higher in the i.v. injected group than in the i.p. injected group.

The route of administration (i.v. versus i.p.) does not affect systemic toxicity with reference to renal and bone marrow functions in dogs treated with a relatively high dose (3 mg/kg) of cisplatin for that species (Pretorius et al., 1981). However, regarding ototoxicity, Laurell and Engström (1989) reported different effects of cisplatin between a single high-dose i.v. injection and multiple low-dose i.p. injections in guinea pigs. With i.v. injection, the loss of endocochlear potentials (EP) was detected as well as ABR threshold elevation, while no permanent change in EP was seen with multiple low-dose treatment despite the occurrence of a more profound hearing loss. Our present study also showed significant difference in carboplatin oto-toxicity, both physiologically and morphologically, between the two administration routes. ABR threshold elevation in chinchillas induced by i.v. carboplatin was greater than that by i.p. injections at all measured frequencies.

Consistent with degree of auditory threshold elevation, we observed that pathological changes to the organ of Corti differ in degree of damage to the hair cells between the i.v. and i.p. treated groups. We might conclude that the significant differences in ABR threshold elevations primarily reflect degenerative changes occurring in the IHC regions. However, after administration of 400 mg/m^2 i.v. carboplatin in chinchillas, in addition to the total loss of IHCs, degenerative changes were occasionally seen in the stria vascularis. The observed pathology included gross capillary dilatation, widening of intercellular spaces and marginal cell flattening (Wake et al., 1993). Marginal cells of the stria vascularis have also been documented as a target of platinum compounds by others (Tange and Vuzevski, 1984; Kohn et al., 1988, 1991). In relation to strial damage, Konishi et al. (1983) reported a decrease in the endocochlear potential and suppression of the cochlear microphonic in cisplatin treated guinea pigs. The stria vascularis seems to be less susceptible than hair cells of the organ of Corti, and the strial degeneration might manifest itself under limited conditions when exposed at relatively high concentrations of platinum compounds (Nakai et al., 1982). On balance, however, the ototoxic effects of carboplatin in the chinchilla are mainly on the IHC, and the effects seem to be dependent on the peak levels of the drug rather than the total dosage. Future studies should include correlation of hair cell damage with drug levels and with other potential toxicities, after acute and chronic treatment.

Selective IHC damage in the chinchilla

Another remarkable aspect of the present study is the distinct interspecies difference between the chinchilla and the guinea pig. Both species are commonly used as experi-mental animal models of various ototoxic agents to supplement data from human clinical studies. In guinea pigs treated with carboplatin, hair cell damage occurred only sporadically in the OHC regions, and the IHC remained intact, confirming previous observations (Schweitzer et al., 1986; Saito et al., 1989; Taudy et al., 1992). Taudy et al. (1992) reported that carboplatin at cumulative doses of 120 mg/kg i.p. in the guinea pig induced ABR threshold shifts ranging from 8 to 15 dB across the frequencies, consistent with our data. Their hair cell counts in the carboplatin-treated animals also showed less than 15% loss of the normal population and this was limited to the OHC area. On the other hand, in our study, chinchillas, after i.p. carboplatin administration with the same dose and time schedule, showed degenerative changes restricted to the IHC regions. Mean IHC loss ranged from 22 to 46% with preference toward the basal turn, while more than 98% of OHCs survived at all locations. Degrees of IHC damage were characterized by separation and splaying of stereocilia, bleb formation on the plasma membrane, presence of fusion/extrusion bodies into endolymphatic space, and total loss of IHCs. It should be noted that the morphological changes observed here have some shared similarity with those induced by cisplatin in guinea pigs, an animal model which has been most thoroughly studied (Comis et al., 1986; Barron and Daigneault, 1987). Comis et al. (1986) also indicated a significant increase in calcium levels inside the distorted hair cells during early periods after single-dose cisplatin treatment and the onset of hair cell degeneration closely associated with the calcium increase. Since platinum compounds are known to inactivate various plasma membrane enzymes and, as a result, affect cellular homeostatic functions (Aggarwal and Niroomand-Rad, 1983; Aggarwal, 1993), it has been suggested that in both animal models changes such as the formation of blebs and fusion/extrusion bodies may be due to the primary effect of the drugs which directly act on hair cell metabolic mechanisms.

In addition to providing insights into the mechanisms of drug ototoxicity, we believe that the model presented here will allow further elucidation of cochlear mechanisms. Just as selective OHC destruction using aminoglycosides has revealed the contributions of hair cell groups in the transduction process (Dallos and Wang, 1974; Evans and Harrison, 1976; Zwislocki, 1984), the present model provides for a complementary study examining the function of the cochlea in the absence of IHCs.

Acknowledgements

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Discussion with Reviewers

Y. Nakai: Are there any special degenerative features in the inner hair cell cytoplasm, nerve endings or fibers associated with the ototoxic effects of carboplatin?

S.D. Comis: Are the authors planning to confirm by transmission electron microscopy (TEM) that there are no untoward changes in the morphology of the OHC synapse-nerve fiber unit after carboplatin administration? It would be interesting to know if there are any degenerative changes in the nerve fibers associated with the IHCs.

Authors: Our previous study (Wake *et al.*, 1993) demonstrated abnormal IHC stereocilia with evidence of fusion/extrusion bodies by light microscopy (LM) observations. OHC stereocilia and cell bodies appeared normal at the LM level. In addition, the spiral ganglion showed scattered Type I cell degenerative changes with pyknosis or nuclear disruption. Those features were observed two weeks after a single i.v. treatment of 400 mg/m^2 carboplatin. We are currently preparing specimens for TEM to investigate the ultrastructure of selectively damaged IHCs and their associated nerve endings, both at acute and chronic stages. The results will be reported in the near future.

Y. Nakai: Do the authors have any idea why IHCs in the chinchilla are more vulnerable than OHCs to carboplatin?

S.K. Aggarwal: Do the authors expect the mechanism for hair cell damage after carboplatin to be the same as after cisplatin treatment, i.e., through intracellular calcium changes?

G. Laurell: The present study describes species differences between the chinchilla and the guinea pig. Can the selective IHC damage in the chinchilla be explained by a specific drug distribution inside the cochlea or can it be explained by cellular mechanism of carboplatin ototoxicity at the IHC level?

V.G. Schweitzer: It would be interesting to compare the toxicities in the chinchilla model of carboplatin versus cisplatin including: morphological and electrophysiological function of OHCs and IHCs; serum drug levels after acute dosing; and other types of toxicities manifested (nephrotoxicity, neurotoxicity, bone marrow suppression, GI toxicity, as well as ototoxicity).

Authors: We do not have any direct evidence to postulate on the mechanism of selective IHC damage in the chinchilla. However, we would like to draw attention to the fact that cisplatin in the chinchilla does cause the usual OHC damage (Gratton *et al.*, 1990; our preliminary data). The effect of cisplatin on the chinchilla cochlea is similar to its effect on the guinea pig as reported by Comis *et al.* (1986); they noted significant increases in calcium levels in abnormal outer hair cells of the guinea pig cochlea. It is possible to speculate that the difference in molecular structure between carboplatin and cisplatin causes discriminative affinity for the two types of cochlea hair cells in the chinchilla. The combination of these two toxic effects in the same species are amazing and worth further investigation since they may enable us to elicit the key mechanism of platinum ototoxicity.

S.D. Comis: Do the authors have any data regarding the possible long term (many months) effects of carboplatin on OHCs?

Authors: We are maintaining a small colony of carboplatin treated chinchillas to investigate long term effects of the drug. Preliminary data has shown that the selective IHC damage can be maintained with complete OHC preservation at least six months. Results of this chronic study will be reported in the future.

S.D. Comis: Have any studies been performed on these chinchillas to look for changes in otoacoustic emissions?

V.G. Schweitzer: Do the authors consider the use of additional modalities to evaluate inner hair cell function, such as single unit hair cell recording and evaluation of the IHCs on a subcellular level?

Authors: We agree that studies of both single unit hair cell recording and otoacoustic emissions using this animal model would be of great value. The project is currently in progress and results will be reported in the future.