Effects of Selective Inner Hair Cell Loss on DPOAE and CAP in Carboplatin-Treated Chinchillas

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In the chinchilla, systemic administration of the anti-neoplastic drug, carboplatin, damages inner hair cells (IHCs), leaving the outer hair cells (OHCs) morphologically intact (Wake et al., 1993; 1994). An animal model with total and selective IHC loss could be a particularly useful preparation if the OHCs are not functionally compromised by the drug treatment. The present experiments were undertaken to address this issue. Distortion product otoacoustic emissions (DPOAEs; 2f1-f2, f1-f2) and compound action potentials (CAPs) of the auditory nerve were recorded at a number of stimulus frequencies from carboplatin-treated and control chinchillas. Cochleas were subsequently retrieved and processed for light microscopic evaluation of hair cell status. Results of the histologic evaluation confirmed previous reports of selective loss of IHCs in carboplatin-treated animals. DPOAEs at 2f1-f2 were indistinguishable from control, even when IHC loss was essentially complete, whereas DPOAEs at f1-f2 were often diminished in magnitude. CAP “thresholds” were elevated when the IHC loss exceeded roughly 50% and were eliminated with total IHC loss. Results suggest that OHC function remains essentially normal in the carboplatin-treated chinchilla with extensive IHC loss, and are consistent with the view that IHCs are not required to generate a normal 2f1-f2 DPOAE, although they may play a role in the generation of f1-f2.

Keywords: Cochlea, sensorineural hearing loss, outer hair cells, otoacoustic emissions

Carboplatin [cis-diammine (1,1-cyclobutane-dicarboxylato) platinum] is an organometallic complex widely used as an anti-neoplastic drug (Rosenberg et al., 1969). The drug is thought to form inter- and intra-strand DNA crosslinks, and its therapeutic effects are thought to derive from interference with DNA replication (Pascoe and Roberts, 1974). Like its parent compound, cisplatin, carboplatin destroys sensory cells in the guinea pig cochlea, with outer hair cells (OHCs) significantly more vulnerable than inner hair cells (IHCs) (Schweitzer et al., 1986; Saito et al., 1989). However, it has been reported recently that, in chinchillas, treatment with carboplatin could result in selective loss of IHCs throughout the cochlea (Wake et al.,...
the first report of such a lesion pattern in the inner ear following cochlear insult. Treated animals also showed significant threshold elevations for auditory brainstem responses.

In the past, animal models with selective OHC destruction, for example by ototoxic antibiotics such as kanamycin (Kiang et al., 1970), have helped elucidate the functional differences between inner and outer hair cells and the respective contributions of each sensory cell type to auditory nerve response (Dallos and Harris, 1978; Liberman and Dodds, 1984). An animal model with selective IHC loss could be similarly useful in a variety of contexts, such as in the recording of activity of the afferent innervation of the OHCs in a preparation in which the (much more numerous) IHC afferents have been rendered non-responsive and/or have been removed. However, the usefulness of such a model depends, in large part, on the functional state of the remaining OHCs. It has been demonstrated that the round-window cochlear microphonic (CM) is only slightly diminished in ears with selective carboplatin-induced IHC loss (Takeno et al., 1994). Although the round-window CM is probably generated largely by the OHCs, it is dominated by contributions of cochlear regions near the cochlear base, even for low-frequency stimulation (Patuzzi et al., 1989), and is not a good measure of the physiologically vulnerable contribution of the OHCs (Murugasu and Russell, 1995).

The aim of the present study was to assess the functional state of the OHCs throughout the cochlea, following selective destruction of the IHCs with carboplatin. To accomplish this, we recorded distortion product otoacoustic emissions (DPOAEs) at 2f₁-f₂ and at f₂-f₁ from the ear canals of control and carboplatin-treated chinchillas. The DPOAEs are thought to arise from mechanical nonlinearities generated in the cochlea by the OHCs in cochlear regions tuned to the primary frequencies (Mountain, 1980; Siegel and Kim, 1982; Schmiedt, 1984). Although possible contribution of the IHCs to the generation of these nonlinearities has not been unambiguously ruled out, we reasoned that the maintenance of normal or near-normal DPOAEs in the face of nearly complete IHC destruction would 1) bolster the case that DPOAEs require only OHCs for their generation and 2) strongly suggest that the OHCs in carboplatin-treated chinchillas remain functionally normal.

METHODS

Nineteen chinchillas of either sex weighing between 425 and 725 grams were used as experimental animals. All procedures were approved by the IACUC of the Massachusetts Eye and Ear Infirmary. Twelve of the animals were given an intravenous injection of carboplatin in the external jugular vein under sterile surgical conditions while anesthetized with sodium pentobarbitol (50mg/kg i.p.); the remaining seven animals received no injections and were used as controls. The carboplatin dosage varied from 400–500 mg/m² (Wake et al., 1993; 1994) and was delivered in 2 to 3 ml of aqueous solution via slow infusion over 5 to 10 minutes (see Table I). Surface area (m²) was estimated by the formula: m² = (1.045 * (weight in grams)⁰.⁶⁵)/1000.

After the injection, animals were sutured and returned to the animal-care facility for a survival period ranging from 14 to 132 days. Two of the twelve carboplatin-treated animals died within 2 days after surgery. Each surviving treated animal and each control animal was surgically prepared for acute physiological experiments to measure distortion product otoacoustic emissions (DPOAEs) and compound action potentials (CAPs). Surgical levels of anesthesia were produced by a combination of

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sodium pentobarbital (44 mg/kg i.p) and ketamine (44 mg/kg i.m.). Booster injections of sodium pentobarbital (1/2 of original dose) were delivered every 4–5 hours and ketamine (1/2 original dose) every hour. Once anesthetized, the animal was placed on a respirator (breathing room air). The skin and muscles overlying the dorsolateral parts of the skull were reflected, and the cartilaginous ear canals were severed to allow insertion of acoustic couplers. The posterior aspects of both bullae were exposed and a small hole made (with a scalpel blade) to vent the bulla space and to allow placement of a thin silver wire on the bone just ventral to the round window (to record the CAP).

Measurements of the CAP were made using a calibrated acoustic system consisting of a 1" Bruel and Kjaer condenser microphone used as a sound source and a 1/4" Bruel and Kjaer microphone coupled to a probe tube (see Kiang, 1965) to measure sound pressures at the entrance to the bony ear canal. Stimuli were 5-msec tone pips, with a 0.5 msec rise/fall time (cos² shaping) delivered at a rate of 10/sec. CAPs were recorded from the silver wire near the round window referred to the mouthbar, amplified (10,000X), filtered (0.3-3 kHz passband) and averaged (tone-pip stimuli were alternated in phase to eliminate cochlear microphonics). Under computer control, the sound pressure was varied (first in 2-dB steps and then in 1-dB steps) to determine the level required to produce a 10µV peak-to-peak CAP. Tone pip frequency was systematically varied, in 15 logarithmically spaced steps, descending in frequency from roughly 25 to 2 kHz.

Measurements of the DPOAEs were made using an Etymotic Research ER10c system which includes two sound sources for generation of primary tones (f₁ and f₂) and a low noise microphone connected to a preamplifier providing 40dB of gain for measurement of ear-canal sound pressure. The sensitivity vs. frequency of the low-noise microphone (dBV/Pa) was measured before each experiment by coupling the ER10c system to a calibrated Bruel and Kjaer 1/4" microphone. The DPOAE at 2f₁-f₂ was measured in all chinchillas for a number of primary frequencies. The frequency of the higher primary, f₂, varied from 1.92 to 10.10 kHz, and the f₂/f₁ ratio was always 1.2; f₂ frequencies were chosen to match a subset of the frequencies at which CAP was measured. Primaries were produced by two computer-controllable oscillators and were equi-level. Sound pressure of the primaries was varied in 5 dB steps from −0 dB SPL to, at most, 80 dB SPL. At each level, the amplified microphone output was filtered (high-pass above 1.0 kHz) and fed to a spectrum analyzer (Hewlett Packard 35660A) where 10 spectra were obtained (with a frequency span of 800 Hz centered at the DPOAE frequency) and averaged together. No other low-pass filtering was introduced other than that inherent in the frequency response of the ER10c microphone. From this spectral average, the amplitudes of the primaries, the distortion component at 2f₁-f₂ and the noise floor (the average of three spectral values just above, and three just below, the 2f₁-f₂) were obtained. Depending on frequency, the noise floor ranged from −20 to −10 dB SPL. In later animals, the DPOAE at f₂-f₁ and its associated noise floor were also measured (with the high-pass filter cut-off moved to 200 Hz). System distortion at 2f₁-f₂ or f₂-f₁ (measured in a passive cavity of appropriate volume) was not detectable above the noise floor for any of the primary-tone pairs at levels below 75 dB SPL.

After DPOAEs and CAPs were recorded, the cochleas were removed from all carboplatin-treated animals and from four of seven control animals and fixed by intralabyrinthine perfusion of 2.5% glutaraldehyde in phosphate buffer. After post-fixation overnight at 4°C, the cochleas were osmicated, dehydrated in graded ethanols and embedded in epoxy resins. After polymerization of the epoxy, the cochleas were drilled and dissected into roughly 15 pieces, each containing ~1 mm of the organ of Corti and osseous spiral lamina. These pieces were thinned with a Dremel drill and glued onto microscope slides to be viewed in the light microscope as surface preparations. Cytocochleograms were prepared by an observer blind to the physiological data in each animal.

1 Three control animals were added at the end of the study to increase the database for DPOAEs at f₂-f₁. These additional animals were not subjected to histological analysis.
RESULTS

Histological Results

As reported previously by Wake et al. (1993), the carboplatin-treated chinchillas showed selective loss of inner hair cells. Of the treated animals in the present study, 9 out of 10 had significant IHC loss: the carboplatin dosages and survival times of all treated animals are given in Table 1. As illustrated by the sample cytocochleograms in Figure 1, IHC could be virtually total (MCL535L) and almost completely restricted to the IHCs. When IHC lesions were more moderate, the loss was always more extensive in the base than in the apex (e.g., CPC 10L), and when minimal, the loss was restricted to a small cochlear region roughly 20–30% of the distance from the base (e.g., CPC 7L). According to a cochlear frequency map for the chinchilla (Eldredge et al., 1981), this region is normally tuned to frequencies near 5–10 kHz.

When lesions were moderate, the IHC loss was patchy: clusters of 5 to 10 adjacent missing IHCs were interspersed among larger patches of intact cells. The patchy nature of the loss is clear in the cytocochleograms, which are plotted with a binwidth equal to 1% of cochlear length, i.e., about 20 IHCs long. Even when the loss was nearly complete, occasional islands of 1–5 intact IHCs were seen which could appear normal in our light-microscopic analysis. On the other hand, some of the remaining IHCs clearly showed grossly abnormal stereocilia: stereocilia fusion of the type seen after ototoxic drugs or acoustic overstimulation (Liberman and Dodds, 1984) was not uncommon.

The IHC lesion patterns in the two ears of individual animals were always symmetrical: the data in Figure 2A display the average IHC loss in corresponding regions of the left vs. the right cochlea for all the treated animals in the present study. There was no strong tendency for the loss to be more severe on one side or the other.

OHC loss was minimal in all ears. As can be seen from Figure 2B, first-row OHC loss in treated ears rarely exceeded 10% (when averaged over ~2 mm cochlear lengths). The same was true for the other two OHC rows (not illustrated), and there was no difference in the vulnerability among the three OHC rows (see Fig. 1). In the treated ears, OHC loss tended to be greater in those cochlear regions affected by the carboplatin (i.e., with more than 10% IHC loss). However, the correlation between IHC loss and OHC loss was minimal (Fig. 2B). Furthermore, the degree of OHC loss in carboplatin ears did not differ from that seen in control ears (Fig. 3). OHC loss, when present, typically occurred as small foci, with 2–3 adjacent cells missing from each of the three rows. Such foci were often associated with pillar cell loss as well. The stereocilia of the OHCs in treated ears appeared normal at the light-microscopic level. However, a systematic analysis was not undertaken.

Physiological Results

Compound Action Potentials

CAPs were measured in both ears from 9 of the 10 carboplatin-treated animals and in 14 ears from 7 control animals. The “iso-CAP” measure consists of a computer-controlled determination of the sound pressure required to produce an N1 potential of 10 μV peak-to-peak as a function of stimulus frequency. As shown in Figure 4A, these “iso-CAP” curves in control animals range from about 10 to 40 dB SPL for most stimulus frequencies below 10 kHz and rise precipitously for stimulus frequencies above 15 kHz. Such values are consistent with existing studies of cochlear electrophysiology in the chinchilla (Salvi et al., 1983).

CAPs in the carboplatin-treated chinchillas are illustrated in Figure 5A, where they are superimposed
FIGURE 1  Cytocochleograms for four ears from four carboplatin-treated animals, chosen to illustrate differing degrees of selective IHC loss. Each panel displays the percentage of hair cells (IHCs and three rows of OHCs) remaining in each cochlea as a function of position along the cochlear spiral, with apical-basal position translated into frequency via a cochlear map (Eldredge et al., 1981). The bin width for this analysis was 1% of cochlear length (i.e., roughly 0.2 mm or 20 IHC locations per bin). The key in the upper left panel applies to all four cytocochleograms. In some regions of some cases, the hair cells were difficult to see due to preparation artifact: in such cases, no points are plotted.
FIGURE 2  Bilateral symmetry of IHC loss in the two ears (panel A) and the lack of correlation between IHC and OHC loss in individual ears (panel B). For both panels, all cochlear regions from all carboplatin-treated ears are included; each point represents the percentage of hair cells remaining in a region spanning 10% of cochlear length (~2 mm). Panel A: each point compares the percentage of IHCs remaining in corresponding cochlear regions of the right and left ears of a carboplatin-treated animal. Panel B: each point compares the percentage of IHCs and first-row OHCs in the same cochlear region of the same ear of a carboplatin-treated animal.

on thick lines indicating the range of values obtained in our control group. The data from treated animals has been divided arbitrarily into two groups: those with normal or near normal “thresholds” (open symbols) and those with significantly elevated thresholds (filled symbols). The CAP-based functional measures were usually similar in the two ears: in only 1 of 9 cases (CPC 8) were thresholds near normal in one ear and significantly elevated in the other. The threshold shifts were clearly greater at high test frequencies than at low frequencies. Indeed, several cases showed normal thresholds at frequencies below roughly 4 kHz and significant threshold shifts at higher frequencies.

The data in Figure 5A may underestimate the “true” threshold shift in many cases, due to incomplete cancellation of the cochlear microphonic potential at these elevated sound pressure levels. When using tone-pip stimuli, the extraction of a neurally based

FIGURE 3  Comparison of OHC populations in control (hatched histogram) and carboplatin-treated (solid histogram) ears. The frequency distributions of first-row-OHC values for each group of ears comprises measurements of the percentage of first-row OHCs remaining in each 10%-length of all cochleas in the group. Thus, each cochlea contributes ten measurements to its relevant sample. The values from carboplatin-treated animals are the same as those plotted in Figure 2B.
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FIGURE 4  Iso-response measures of cochlear function from control animals: the "criterion" response amplitude is given at the lower left of each panel. Panel A shows "iso-CAP" curves from 14 control ears. Each curve, displayed as a function of tone-pip frequency, the sound pressure in the ear canal required for a tone pip to elicit an N1 in the compound action potential (CAP) of 10µV p-p from the round window electrode. Panels B and C show "iso-2f1-f2" and "iso-f2-f1" curves for control ears. Each curve, displayed as a function of the frequency of the higher primary tone (f2), the sound pressure in the ear canal required for two equilevel primary tones to produce a distortion product otoacoustic emission (DPOAE) at 2f1-f2 (panel B) or at f2-f1 (panel C) equal to 0 dB SPL. Data from the two ears of each animal are shown with the same symbol type (solid line for the right ear, dashed lines for the left ear), and each symbol type represents the same animal in all three panels. Data at f2-f1 and 2f1-f2 are only available for subsets of the control ears. Iso-CAP curves are directly measured under computer control (See Methods); iso-DPOAE curves are computed by interpolation from DPOAE-amplitude-vs.-f2-level functions.

FIGURE 5  Iso-response measures of cochlear function from all carboplatin-treated animals, superimposed on the maximum and minimum values seen in control animals (thick grey lines in each panel). Data from the two ears of each animal are shown with the same symbol type (solid line for the right ear, dashed lines for the left ear), and each symbol type represents the same animal in all three panels. The symbol key and carboplatin treatments for each case are shown in Table I. Those cases with the highest CAP "thresholds" are shown with filled symbols; in panel A, points plotted at 100 dB SPL signify that the CAP had not reached criterion amplitude (10µV) at the highest SPLs achievable by the acoustic system. In panel C, points plotted within the hatched box had not reached criterion amplitude (0 dB SPL) at the highest SPLs tested. Other aspects of data display are as described for Figure 4.
"N₁" potential from the composite round-window response waveform relies on the cancellation of these microphonic potentials by adding equal numbers of responses evoked by tone pips of opposite polarities. Given the large size of the microphonic, non-linearities produce a significant hair-cell-based response due to incomplete cancellation. Although the software that generates the iso-CAP curves “windows” the response waveform around latencies appropriate for N₁, the resultant waveforms at these high SPLs were distinctly aberrant and could well include components generated by non-neural elements.

**DPOAEs**

To facilitate comparison with the CAP data, the DPOAE data are displayed as “iso-DPOAE” curves (although they were obtained as a series of iso-frequency level sweeps). The curves extracted from control animals are displayed in Figure 4: panel B for the DPOAE at 2f₁-f₂ and panel C for the DPOAE at f₂-f₁. These curves display the sound pressure of f₁ and f₂, the equilevel primary tones, required to produce a DPOAE of 0 dB SPL. Most of the iso-2f₁-f₂ curves fall between levels of 25 and 40 dB SPL; thus in the most sensitive cases, this most prominent distortion product is only 25 dB below the level of the primaries. Such results are similar to those previously described for chinchilla (Zurek et al., 1982). The noise floor for our measurements was typically between −20 and −10 dB SPL, thus a 0 dB distortion product has a signal-to-noise ratio of at least 10 dB.

In these control animals, there are a number of similarities in the cochlear sensitivity patterns reflected in the iso-CAP curves (Fig. 4A) and the iso-2f₁-f₂ curves (Fig. 4B). For example, the two ears from the case with the highest CAP “thresholds” at frequencies below 3 kHz (circles with continuous or dotted lines) also shows the highest DPOAE “thresholds” for f₁ frequencies below 3 kHz. Similarly, the case with the highest DPOAE thresholds at 4 kHz (triangles with dotted lines) also has the highest CAP thresholds at 4 kHz. One apparent discrepancy, i.e., that the high-threshold value at 6kHz in the iso-CAP data (filled circle) is not reflected in the DP curves, may be due to coarser frequency sampling; the DPOAEs in this case were only measured for f₁ near 4 and 8 kHz.

The DPOAE at f₂-f₁ is smaller in these control animals than that at 2f₁-f₂. Thus, the iso-DPOAE curves for f₂-f₁ (Fig. 4C) are 10 to 20 dB higher (in the control ears) than those for 2f₁-f₂. Measurements at f₂-f₁ were not obtained in any of the higher threshold control cases discussed above; thus, the correlation, or lack of it, between iso-CAP measures and iso-DPOAE measures is harder to ascertain for this DPOAE.

The DPOAE data from carboplatin-treated animals are shown in Figures 5B and C, superimposed on thick lines indicating the range of values seen in the control ears. According to this analysis, the DPOAEs at 2f₁-f₂ (panel B) are unaffected by the carboplatin treatment. Virtually all the 2f₁-f₂ DPOAE curves fall within the range seen for the smaller number of control ears; a few points fall slightly above the range, however, a few points also fall slightly below the range. None of the points falling above the normal curves are from the most severely affected cases (as assessed by the iso-CAP curves shown in panel A, and as depicted in filled symbols). Thus, even in cases where a CAP could not be measured at 100 dB SPL (i.e., filled circles and filled triangles in Fig. 5), the 2f₁-f₂ DPOAEs were indistinguishable from control values. The data in Figure 5 include only low-level primaries and low-level DPOAEs; however, the same discrepancy between CAP shifts and DPOAE shifts holds for higher level primaries. For example, as illustrated in Figure 6A, the primary SPLs required in the treated animals to generate a 30-dB 2f₁-f₂ DPOAE all fall within 4 dB of the normal range, even from the most severely affected cases. The same was true for iso-DPOAE curves at 10, 20 and 40 dB SPL (not illustrated).

Although there are fewer data available for the DPOAE at f₂-f₁, the data obtained from 5 treated ears suggest that this distortion component has been diminished in magnitude by the carboplatin treatment. As shown in Figure 5C, several of the iso-DPOAE curves at 0 dB SPL fall outside the normal range, some by as much as 20 dB. Furthermore, the most elevated points (within the hatched area) represent minimum estimates of the shifts, since the criterion DPOAE level had not been reached at the highest
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FIGURE 6  Iso-DPOAE measures from carboplatin-treated ears for higher response criteria than illustrated in Figures 4 and 5. In panel A, the curves show the level of primary tones in the ear canal required to produce a 2f1-f2 DPOAE of 30 dB SPL. In panel B, the curves show the primary levels required to produce an f2-f1 DPOAE of 10 dB SPL. Other aspects of data display are as described for Figure 5. The correspondence between symbol types and ears is the same as for Figure 5 (see Table I).

SPL primaries tested. Note that one of the treated ears (shown by the open squares) showed essentially normal CAP data; corresponding f2-f1 DPOAE data fell entirely within or below the normal range. Also note, however, that one case with very high CAP thresholds (closed triangles, solid lines) also demonstrated f2-f1 values that fell largely within the normal range. The data for the iso-DPOAE curves at 10 dB SPL (Fig. 6B) are harder to interpret because, even in control ears, the primary levels required to produce a 10 dB f2-f1 were often close to the highest levels presented (70–80 dB SPL).

Correlation of Histopathology and Pathophysiology

The relation between IHC loss patterns and the cochlear iso-response measures for two carboplatin-treated cases is shown in Figure 7. One of these cases, CPC10L, showed normal CAP and DPOAE measures even though there is significant IHC loss throughout the cochlea. The other case, MCL535L, had the most severe IHC loss of any of the treated ears, with virtually total loss throughout most of the basal half of the cochlea. Although a CAP was reliably elicitable only for tone pips below ~5 kHz, and then only at substantially elevated SPLs, the 2f1-f2 DPOAE amplitude was indistinguishable from control at all frequencies tested.

The relation between IHC loss and cochlear iso-response measures is shown more systematically in Figure 8, where the shift in iso-response measures is plotted against the degree of IHC loss for all data from the carboplatin-treated ears. The relationship is clearly different for the iso-CAP measure (panel A) and the iso-DPOAE measure (panel B). Although severe loss of IHCs (panel A) is clearly associated with an attenuation of the CAP response, the particular CAP metric we have used is not a very sensitive indicator of the degree of IHC loss. For example, there can routinely be as much as 50% loss of IHCs in localized cochlear regions with little, if any, change in the mean value of the iso-CAP values for tonotopically appropriate stimulus frequencies. Even when the IHC loss reaches 90% and greater, a few cases show small CAP shifts, although the mean value is shifted by roughly 60 dB. For the DPOAE measures, on the other hand, there is no indication of any correlation with IHC loss: even those regions with greater than 90% destruction show mean iso-2f1-f2 values indistinguishable from normal.

DISCUSSION

Selective IHC Loss and the Utility of the Carboplatin-Treated Chinchilla

The present experimental series was undertaken to evaluate the utility of the carboplatin-treated chinchilla as a model of selective IHC loss. Our aim was to assess
FIGURE 7 Comparison of cochlear iso-response measures and cytocochleograms for two carboplatin-treated ears: CPC10L and MCL535L. The iso-CAP curves (top two panels) and iso-2f1-f2 are superimposed on grey lines indicating the range of appropriate control values, as described for Figure 5. The vertical arrows in the iso-CAP panels indicate frequencies for which the CAP failed to meet criterion amplitude at the highest SPLs generated. The conventions for cytocochleogram display are described in the caption to Figure 1.
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OHC function in ears in which the IHCs had been almost completely eliminated by the drug regimen. Both the CAP and the DPOAE data from the present experiments strongly suggest that OHC function in these cases is normal, at least to a first approximation.

The CAP data clearly show that significant carboplatin-induced IHC loss need not produce significant reductions of cochlear sensitivity, and normal cochlear thresholds strongly imply normal OHC function (e.g., Kiang et al., 1970; Dallos and Harris, 1978; Liberman and Dodds, 1984). As illustrated by the case in Figure 7 (left), and by the scatterplot in Figure 8A, even in cochlear regions where the average IHC destruction approaches 50%, the CAP “thresholds” can remain within normal limits. Similar results were obtained in ABR measures from mutant mice with selective IHC lesions (Schrott et al., 1989).

According to current understanding of peripheral auditory function, pure IHC lesions would not be expected to produce significant elevations of CAP iso-response measures (at least for small response criteria) until the loss approached 100%, especially given scattered loss of the type seen in the present series of carboplatin-treated ears. For example, a 50% scattered loss of IHCs, associated with normal OHCs, should decrease the CAP amplitude by 50% at all stimulus levels, since each auditory nerve fiber contributes the same unitary potential to the round-window CAP (Kiang et al., 1976). This anticipated decrease in the slope of the CAP-vs-level function, say from 4 μV/dB to 2 μV/dB, would translate into a small increase (less than 5 dB) in the SPL required to produce a 10 μV response. Given the interanimal variance in CAP “thresholds” in control animals, such a small change would be undetectable (without a very large number of animals, or without monitoring CAP responses in individuals animals before and after the carboplatin treatment). Presumably, for higher CAP-response criteria, the effects of this IHC loss on CAP “threshold” shift should become proportionately larger and easier to detect.

The DPOAE data also suggest that carboplatin-induced IHC loss, even when essentially complete, does not significantly affect OHC function. As shown by the data in Figures 7 and 8, the 2f1-f2 DPOAE appears to be unaffected by complete IHC destruction in the cochlear regions of the primary tones. A number of experimental observations have suggested that this

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FIGURE 8 Correlation between IHC loss and CAP (panel A) or DPOAE “thresholds” (panel B) in all carboplatin-treated animals. Each iso-CAP or iso-2f1-f2 data point from each treated animal (Fig. 5) becomes a point in panel A or B, respectively. The X-axis value is the difference, in dB, between the measured iso-CAP or iso-2f1-f2 value and the mean control value at the same tone-pip or f2 frequency. The Y-axis is the percentage of IHCs remaining in that ear in a cochlear region (20% of the total length) centered at the locus appropriate to the tone-pip or f2 frequency.
DPOAE is a good measure of OHC function, among the most clearcut being the observation that electrical stimulation of the OHC efferent pathway affects its amplitude as it decreases cochlear sensitivity (Mountain, 1980; Siegel and Kim, 1982) and that loss of OHCs greatly reduces the DPOAE amplitudes (e.g., Brown et al., 1989; Schrott et al., 1991). The results of the present study clearly show that the 2f1-f2 DPOAE amplitudes at low and high levels remain within normal limits despite the carboplatin treatment and despite virtually complete loss of IHCs. Although the f2-f1 DPOAE showed signs of abnormality in some of the treated ears, the IHCs might be involved in the generation of this DPOAE (see below). Furthermore, even the f2-f1 DPOAE is reduced only slightly in the treated animals. As such, our results are consistent with those of Takeno et al. (1994) who showed that selective IHC loss in the carboplatin-treated chinchilla caused significant elevation of CAP “thresholds” (also defined as the SPL required to produce a 10 μV response) while producing much smaller effects on round-window cochlear microphonics. Thus, existing data strongly suggest that OHC function is, at most, only slightly compromised by a carboplatin treatment which can completely eliminate the IHCs.

The ability to produce a cochlea with selective subtotal or nearly total loss of IHCs, associated with otherwise normal cochlear sensitivity, can have significant utility in addressing a number of behavioral and physiological issues in audition. The present results suggest that carboplatin provides a means to reduce the number of IHCs and associated primary sensory fibers in the ear without affecting the response properties of the remaining IHCs and their associated innervation. This implication needs to be directly tested with single-fiber recordings. However, if true, such a pathology is functionally identical with a primary neural degeneration, homogeneously scattered along the tonotopic continuum, and varying in different treated animals from 0 to almost 100% loss of active neural “channels”. Existing studies suggest that neuronal loss following partial auditory-nerve section does not significantly affect behavioral thresholds in cat, even if the loss approaches 75% (Schuknecht and Woellner, 1953). With behaviorally trained, carboplatin-treated animals, one could address this issue and a number of other longstanding questions concerning the effect of pure loss of neural “channels” on threshold sensitivity, loudness perception, frequency discrimination, etc. It is also possible that this selective loss of IHCs and their innervation could prove useful in electrophysiological studies of the small population of OHC afferent fibers, which thus far have eluded physiological study (e.g., Robertson, 1984).

Implications for Mechanisms of DPOAE Generation

The present results show clearly that the generation of a 2f1-f2 DPOAE is fundamentally unaffected by the total regional loss of IHCs (Figs. 7 and 8). As such, our results are consistent with the prevailing view that this odd-order DPOAE, at least for low level primary tones, is generated by the OHCs and is, therefore, a useful measure of their functional state. However, the present results cannot rule out the possibility that IHCs also produce distortion at 2f1-f2 and make a measurable (if small) contribution to the DPOAEs measured in the ear canal.

Previous reports of the DPOAE amplitudes in mutant mice with selective loss of the IHCs reported significant diminution in DPOAE (as well as in CAP) amplitudes (Horner et al., 1985; Schrott et al., 1991). Our results suggest that these response abnormalities, as the authors point out, may have been due to pathology in the remaining OHCs. Our results clearly argue against the suggestion (Schrott et al., 1991) that the loss of IHCs leads to mechanical changes in the organ of Corti which diminish the magnitude of the 2f1-f2 DPOAE.

In the present study, we show evidence for selective diminution in the amplitude of the f2-f1 DPOAE (Figs. 5 and 6). The idea that the DPOAEs at 2f1-f2 and f2-f1 are, at least to some extent, independent of each other has been suggested by a number of previous studies. For example, it has been shown by a number of authors that the f2-f1 DPOAE varies significantly in amplitude with prolonged continuous presentation of the primaries (over several minutes),
whereas the amplitude of the simultaneously measured 2f1-f2 DPOAE is quite stable (Brown, 1988; Whitehead et al., 1991; Kirk and Johnstone, 1993; Kujawa et al., 1995; Lowe and Robertson, 1995). Caution must be exercised in interpreting the suggestion, in our data, that the f2-f1 DPOAE is more vulnerable to carboplatin treatment than that at 2f1-f2. First, the evidence concerning f2-f1 is based on relatively few animals and needs to be repeated with a larger sample. Second, it has been reported (Mills et al., 1993) that the sensitivity of the 2f1-f2 DPOAE as a measure of cochlear dysfunction varies with differences in the level ratios for the primaries, specifically that DPOAEs from equilevel primaries are not as sensitive to cochlear dysfunction as when the primaries differ by 10 dB (f1 > f2). Thus, it is possible that 2f1-f2 DPOAEs measured with different level-ratios would have shown carboplatin-induced abnormalities similar to those seen in the iso-f2-f1 data (Figs. 5 and 6). Such concerns are especially relevant given the suggestion that carboplatin treatment can also affect the stria vascularis (Anzai et al., 1987), and therefore might decrease the endolymphatic potential, as is known to occur with furosemide-induced changes in cochlear thresholds as when the primaries differ by 10 dB (f1 > f2). It is possible that 2f1-f2 DPOAEs measured with different level-ratios would have shown carboplatin-induced abnormalities similar to those seen in the iso-f2-f1 data (Figs. 5 and 6). Such concerns are especially relevant given the suggestion that carboplatin treatment can also affect the stria vascularis (Anzai et al., 1987), and therefore might decrease the endolymphatic potential, as is known to occur with furosemide (e.g., Sewell, 1984).

Nevertheless, if a selective decrease in the f2-f1 DPOAE is indeed associated with carboplatin treatment, this has interesting implications for the mechanisms of f2-f1 generation. Although the changes in the f2-f1 DPOAE could arise from alterations in OHC or stria function, alternatively, they may be causally related to the observed IHC loss. It has been reported that, in the guinea pig, a large f2-f1 distortion component in the cochlear microphonic can be recorded at the round window, and that this component arises from the IHCs (Nuttall and Doan, 1993). Although Nuttall and Doan speculate that the IHC-generated potential at f2-f1 arises from the OHCs, it is possible that it arises independently from non-linearities in IHC transduction. It is further possible that this IHC-based electrical distortion normally serves as a driving force for the electromotile response of the nearby OHCs which, in turn, transduce this voltage into cochlear motions which are transmitted to the external ear canal as a major component of the measured f2-f1 DPOAE. If true, one would expect a selective decrease in the f2-f1 DPOAE associated with selective IHC loss. On the other hand, this IHC-based electrical distortion cannot be the only source of the f2-f1 DPOAE, as this distortion component is only reduced, not eliminated, by the IHC lesion.

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**References**


