Neurochemistry of the Peripheral and Central Auditory System After Ototoxic Drug Exposure: Implications for Tinnitus

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Abstract: Platinum-containing drugs, such as cisplatin and carboplatin, are known to have ototoxic side effects causing hearing loss that may be accompanied by tinnitus. This study reviews recent studies on the ototoxicity of cisplatin and carboplatin and summarizes the effects of protective agents that may prevent hearing loss and tinnitus. The primary locus of ototoxicity is in the cochlea, but oxidative stress to the inferior colliculus has been reported recently with carboplatin. Enhanced spontaneous activity within the dorsal cochlear nucleus has been correlated with loss of outer hair cells in animal experiments using cisplatin. This may result from disinhibition of neurons within the dorsal cochlear nucleus caused by reduced input from spiral ganglion cells. Carboplatin may cause tinnitus by oxidative stress within the inferior colliculus or by loss of inhibition within the inferior colliculus resulting from cochlear damage. This could lead to compensatory gain and enhanced responses in neurons within the auditory cortex. Protective agents may prevent tinnitus by preventing damage to the cochlea, thereby obviating the development of disinhibition within central auditory pathways.

Key Words: carboplatin; cisplatin; cochlear nucleus; inferior colliculus; ototoxicity; tinnitus

Cisplatin and carboplatin are chemotherapeutic agents frequently used to treat a variety of malignant neoplasms in patients. Both these drugs have numerous side effects, including ototoxicity. The latter is manifested by tinnitus that may or may not be accompanied by hearing loss. These ototoxic side effects may be temporary or permanent. The purpose of this study is to review the ototoxicity of these two platinum compounds and to show the relationship between cochlear damage and tinnitus-like phenomena that can subsequently develop in the central auditory system. Furthermore, we propose that the use of chemoprotective agents to avert cochlear injury may ameliorate the onset or the severity of tinnitus and hearing loss caused by these drugs.

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CISPLATIN TOXICITY PROFILE

Cisplatin therapy has been associated with severe damage to the renal tubules with a decrease in glomerular filtration rate. This agent is the most emetogenic anticancer drug in common usage. It causes peripheral neurotoxicity in 30–100% of patients, which may be manifested as a loss of vibratory sense, paresthesias, or sensory ataxia. This can be a major dose-limiting side effect after cisplatin therapy [1].

Ototoxicity has been reported in a high percentage of patients receiving cisplatin. Some audiometric studies have demonstrated that 75–100% of patients administered cisplatin have elevated hearing thresholds [2]. Studies of cisplatin ototoxicity in pediatric patients also have shown a high incidence of hearing loss. This has implications for the future quality of life in children who may survive their cancer but who suffer for many years from deafness and tinnitus.

One study of children undergoing cisplatin chemotherapy revealed that 90.5% had significant sensorineural hearing loss at 8 kHz. Greater hearing losses were associated with being of young age at first dose of cisplatin, undergoing a greater number of chemotherapy cycles, and receiving a high cumulative dose of cisplatin [3]. Another study examined the ototoxicity of cisplatin or carboplatin in children. Pediatric cancer patients who received the so-called blast therapy with these agents had a high probability of developing hearing loss. In 86% of patients treated with cisplatin, bilateral perceptive deafness was detected with conventional audiometry. A smaller percentage (33%) were found to have hearing loss after carboplatin therapy. Children having stage 4 neuroblastoma treated with cisplatin suffered from hearing loss in 27% of cases [4,5].

A statistical analysis of audiograms from children treated with cisplatin was carried out. Logistical regression revealed that children younger than 5 years were at a greater risk of sustaining cisplatin ototoxicity than were children older than 15. Cumulative doses of cisplatin were also strongly correlated with hearing loss in these patients. The higher cumulative dose of 1,200 mg/m^2 occasioned a 90% risk of hearing loss [6]. A recent study showed that hearing loss after cisplatin treatment of pediatric malignant tumors continued to worsen 2 years after completion of therapy. Some 44% of patients were found to have hearing loss on audiometry. Children treated with cisplatin in cumulative doses approaching 400 mg/m^2 require long-term auditory surveillance to detect hearing losses [7].

A group of 115 patients with advanced germ-cell tumors was studied for long-term ototoxicity. Of 76 patients in complete remission for at least 1 year after cessation of chemotherapy, 29 (38%) suffered from persistent tinnitus [8]. Young to middle-aged testicular cancer patients treated with cisplatin demonstrated symptomatic ototoxicity that was permanent in 20%. Of these patients, 18% had sensorineural hearing loss, 59% complained of tinnitus alone, and 23% suffered both hearing loss and tinnitus. Persistent ototoxicity was dose-related and was found in more than 50% of patients treated with cumulative doses of cisplatin exceeding 400 mg/m^2.

Previous noise exposure tripled the risk for cisplatin ototoxicity [9]. A study of patients with metastatic germ-cell malignancies reported audiometric threshold changes in 60% of patients 2 years after treatment, with symptomatic hearing loss in 21% and persistent tinnitus in 26% [10]. In a group of 400 patients receiving weekly high-dose cisplatin chemotherapy, 168 (42%) were found to have ototoxicity, including hearing loss or tinnitus or both. Logistical regression analysis demonstrated that anemia was statistically associated with the development of ototoxicity [11]. Histopathological studies of temporal bones removed from deceased patients who had been treated with cisplatin chemotherapy have revealed loss of inner (IHCs) and outer hair cells (OHCs) from the basal turn, loss of spiral ganglion cells, and atrophy of the stria vascularis [12,13].

An intriguing double-blind, placebo-controlled clinical trial was carried out in cancer patients treated with cisplatin-based chemotherapy. Patients were randomly assigned to receive either supplementation with an antioxidant cocktail, consisting of vitamin C, vitamin E, and selenium dissolved in a beverage, or an identical placebo liquid. No difference was demonstrated in the incidence of ototoxicity in the two groups. However, the patients who achieved the highest plasma concentrations of the three antioxidant micronutrients had significantly less severe loss of high-tone hearing. In addition, significant correlations were found between the reduced-oxidized vitamin C ratio and malondialdehyde (MDA)—markers of oxidative stress in the plasma—and cisplatin-induced ototoxicity and nephrotoxicity. A future study using a higher dose of antioxidants and combinations of additional antioxidants may prove that these provide better protection against cisplatin ototoxicity [14].

Cisplatin ototoxicity has been associated with the production of reactive oxygen species that can deplete cochlear antioxidant defense mechanisms and cause lipid peroxidation in cochlear tissues [15–17]. This prooxidant state may trigger apoptosis of OHCs [18] and strial marginal cells [19] in the cochlea, resulting in hearing loss. Animal experiments have used a variety of compounds to reduce the ototoxicity of cisplatin. A number of thiol compounds have been tested for efficacy in protecting against cisplatin ototoxicity. These compounds include sodium thiosulfate, D- or L-methionine, diethyldithiocarbamate, 4-methylthiobenzoic acid, lipoic acid, and L-*N*-acetylcysteine. Sodium thiosulfate perfused into the cochlea of guinea pigs completely prevented cisplatin-induced hearing loss and cellular damage to the stria vascularis and hair cells in those animals treated with cisplatin [20]. This protective agent was applied directly to the inner ear because thiosulfate can neutralize the antitumor effect of cisplatin when given systemically. D-Methionine provided excellent protection against cisplatin ototoxicity when administered systemically [21,22] or topically [23], as did l-methionine when applied to the round-window membrane. Systemic pretreatment with alpha-lipoic acid [24,25] or 4-methylthiobenzoic acid [26,27] also provided excellent protection against OHC loss and auditory brainstem response (ABR) threshold shift in rats. The selenium compound ebselen acts as a mimic for glutathione peroxidase and as a scavenger

for peroxynitrite free radicals. It was found to be highly efficacious in preventing hair cell damage and ABR threshold shifts in rats receiving cisplatin [17]. Such antioxidants as sodium salicylate [27], glutathione ethyl ester [28], and aminoguanidine [29] each provided protection against cisplatin ototoxicity in rats. Guinea pigs treated with vitamin E (alpha-tocopherol) prior to receiving cisplatin were found to have significant protection against ABR threshold shifts caused by cisplatin. Significant preservation of OHCs in the cochlea was found in animals pretreated with vitamin E [30]. These results were confirmed with local application of a water-soluble form of vitamin E, trolox [31]. Chinchillas pretreated with vitamin E prior to high doses of cisplatin were also protected against OHC loss and ABR threshold shifts [32,33]. A combination of alpha-tocopherol and tiopronin was reported to be more effective than either agent alone in preventing cisplatin ototoxicity in guinea pigs [34].

Melatonin (*N*-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland in humans and other mammals. It has been shown to act as a direct free radical scavenger and an indirect antioxidant [34]. The administration of melatonin in combination with other antioxidants ameliorated the ototoxicity of cisplatin in rats [35]. Adenosine receptor agonists prevented cisplatin-induced hair cell damage in vitro [36] and ABR threshold elevations and hair cell loss after application to the round window in chinchillas [37]. The effects of adenosine agonists appear to be mediated by the A1 adenosine receptor. The protective effects of adenosine agonists were blocked by pretreatment with the A1 adenosine receptor antagonist DPCPX. Adenosine A2 receptor agonist application exacerbated, rather than reduced, cisplatin ototoxicity [37]. Inhibition of cell death pathways by caspase inhibitors [38], the p53 inhibitor pifithrin-alpha [39], and inhibitors of c-*jun* activation [40] prevented auditory neurons from cell death. Whether these latter agents could be used clinically remains questionable. In summary, if cochlear damage from cisplatin could be reduced or prevented, hearing could be preserved, and tinnitus likely would be prevented or ameliorated.

CARBOPLATIN TOXICITY PROFILE

Carboplatin was introduced into clinical chemotherapy because it was found to cause less nephrotoxicity than does cisplatin. Initial reports also suggested that carboplatin was less ototoxic than was cisplatin. The primary dose-limiting toxicity of this drug has been bone marrow toxicity. This toxic effect has been overcome by the use of autologous stem cell rescue, a finding that has allowed medical oncologists to use larger doses of carboplatin in an effort to increase antitumor efficacy. However, the increased efficacy has come at the expense of greater ototoxicity than that which was initially appreciated. Nine of 11 children with neuroblastoma (82%) who were treated with high-dose carboplatin followed by autologous bone marrow transplantation had, in their speech frequencies, hearing losses that were so severe that hearing aids were recommended. All these children had previous treatment with cisplatin, and several had also received aminoglycoside antibiotics in the past [41]. Another study of pediatric neuroblastoma patients (stage 4) treated with high-dose carboplatin chemotherapy with autologous stem cell reinfusion showed hearing losses in 34.5% (65 of 188 patients) [5]. A very recent study of children with neuroblastoma treated with carboplatin and hematopoietic stem cell transplantation demonstrated hearing loss in 20 of 45 patients (44%) by audiometry or otoacoustic emission testing [42].

Carboplatin has been used successfully to treat malignant brain tumors in combination with osmotic blood-brain barrier disruption by use of mannitol. A large percentage of patients (79%) developed hearing loss with this protocol. When another group of patients was treated with carboplatin followed by sodium thiosulfate after the blood-brain barrier was allowed to close, very little hearing loss was observed [43].

Patients with ovarian cancer treated with cisplatin and carboplatin were evaluated for toxicity. Both thrombocytopenia and ototoxicity were associated with high area-under-the-curve (AUC) blood concentrations of carboplatin. No patient in the low AUC group developed ototoxicity, but 12% of patients in the high AUC group demonstrated ototoxicity, and 45% had thrombocytopenia [44]. All nine patients pretreated with up to four cycles of cisplatin chemotherapy followed by three cycles of carboplatin and peripheral blood-derived stem cell support for cisplatin-resistant tumors suffered hearing impairment. Three required hearing aids, and six complained of tinnitus. Ototoxicity in these patients was strongly related to the cumulative carboplatin AUC [45].

Animal experiments have shown that D-methionine prevents hair cell damage by carboplatin [46]. Future experiments using protective agents likely will reduce the incidence of hearing loss and tinnitus in carboplatin-treated patients.

ROLE OF THE DORSAL COCHLEAR NUCLEUS IN TINNITUS

Cisplatin ototoxicity could induce tinnitus by causing central auditory neuronal hyperactivity in various centers, including the dorsal cochlear nucleus (DCN). Kaltenbach

et al. [47] treated hamsters with cisplatin using various dosage regimens ranging from 1.5 to 3.0 mg/kg/day given as a single intraperitoneal injection on alternate days over a 10-day period. Most animals were allowed to recover for 1–2 months, but some were maintained for longer periods after treatment. After the recovery interval, animals were studied by performing recordings of multiunit action potentials along the tonotopic axis of the DCN in a blinded fashion. Afterward, animals were sacrificed for scanning electron microscopic cytocochleograms. Hyperactivity of spontaneously active units in the DCN was observed in cisplatin-treated hamsters as compared with untreated controls. This spontaneous activity was greatest in the high-frequency region of the DCN, consistent with the fact that cisplatin preferentially damages the OHCs of the basal turn of the cochlea. Those animals with OHC loss and little or no accompanying IHC damage were found to have a strongly positive correlation with the mean maximal rate of spontaneous activity in the DCN ($r = 0.89$) (i.e., the greater the loss of OHCs, the higher the spontaneous rate of neuron firing in the appropriate region of the DCN). Among those hamsters with damage to the IHCs, the correlation was weaker ($r = 0.51$). Thus, the damage to the IHC tended to offset the condition of hyperactivity triggered by the loss of OHCs. The authors did not believe that the hyperactivity noted in the DCN was caused by a direct neurotoxic injury to the DCN. They discussed several potential mechanisms by which loss of OHCs caused by cisplatin could lead to DCN hyperactivity.

One possible mechanism is that altered cochlear mechanics caused by the loss of OHCs could increase the excitability of type 1 primary afferent neurons in the spiral ganglion that could be relayed centrally. They concluded that this was unlikely because OHC loss from aminoglycoside ototoxicity does not significantly affect spontaneous discharge rates of type 1 primary afferent auditory nerve fibers [48].

A second possible way by which cisplatin ototoxicity could induce hyperactivity in the DCN is through a change in the balance of the input to the DCN from the two types of primary afferent channels. The loss of OHCs caused by cisplatin would lead to the loss of tonic drive from the type 2 spiral ganglion cells to the granule cells, leading in turn to the reduction in activity of the cartwheel and stellate cells. This could lead to disinhibition of fusiform cells, resulting in an increased firing rate. This mechanism would be consistent with the findings of Brozoski et al. [49] that spontaneous activity of fusiform cells increases after acoustic trauma in chinchillas, which selectively damaged their OHCs.

A third potential explanation is that the loss of normal input from afferent auditory nerve fibers might result in compensatory changes in the influence of intrinsic or descending inputs to the DCN neurons. Compensatory increases in the sensitivity of granule cells to cholinergic input from the medial olivocochlear bundle could lead to increased activation of cartwheel or stellate cells. Conversely, if the sensitivity to cholinergic input is reduced, granule cell activation of cartwheel or stellate cells would decrease and result in disinhibition of fusiform cells, allowing them to become hyperactive.

Thus, a number of different processes could account for the increased spontaneous multiunit activity in the DCN after cisplatin ototoxicity, which could result in a tinnitus-like perception [47]. Elevated fusiform cell activity in the DCN of chinchillas after acoustic trauma that damages OHCs appears to be related to psychophysical performance consistent with tinnitus [49]. In this model, the onset of tinnitus may result from a loss of glycinergic inhibition within the DCN, resulting from a loss of DCN afferent input [50].

Persistent tinnitus after cisplatin ototoxicity could be related to neuronal hyperactivity in the DCN (Fig. 1) [51]. Therefore, the DCN may be a pivotal structure within the auditory central nervous system related to

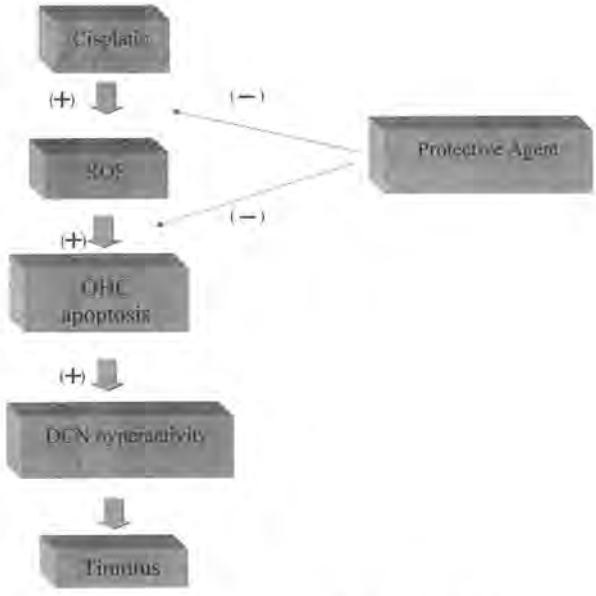

Figure 1. Proposed mechanisms for cisplatin-induced tinnitus. Cisplatin administration can result in the production of reactive oxygen species (*ROS*) in cochlear tissues. This can lead to apoptosis of the outer hair cells (*OHC*). The loss of OHCs can permit the onset of hyperactivity in neurons in the dorsal cochlear nucleus (*DCN*). Such hyperactivity can represent or produce tinnitus-like phenomena. Pretreatment with protective agents may prevent the production of ROS, thereby preventing OHC apoptosis. Other agents, such as caspase inhibitors, can prevent apoptosis of OHC despite ROS generation by cisplatin.

the etiology and the maintenance of permanent tinnitus after cisplatin ototoxicity [51].

ROLE OF THE INFERIOR COLLICULUS IN TINNITUS

Recent studies in our laboratory have investigated the effect of carboplatin on the neurochemistry of the inferior colliculus (IC) of rats. Animals were injected with carboplatin (256 mg/kg) or saline by intraperitoneal injection. ABRs were measured before and 4 days after treatment. The animals then were sacrificed, and the IC and the cerebellum were removed and analyzed for reduced and oxidized glutathione (GSH and GSSG, respectively), antioxidant enzymes, nitric oxide, lipid peroxidation products, MDA, and xanthine oxidase. Significant ABR threshold elevations for clicks and for tone burst stimuli at 2, 4, 8, 16, and 32 kHz were found, with the greatest elevations occurring at the highest frequencies. Carboplatin-treated animals were found to have significant increases in nitric oxide, MDA, xanthine oxidase, and manganese superoxide dismutase activities in the IC but not in the cerebellum. These findings suggested a specific increase in free radical production in the IC. Carboplatin-treated rats had significant reductions in the ratio of GSH/GSSG and antioxidant enzyme activities, including copper-zinc superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase and enzyme protein expression in the IC but not in the cerebellum (Fig. 2). These findings suggest that carboplatin produces oxidative stress specifically in the IC, which is correlated with hearing loss [52] and perhaps to tinnitus.

These findings contrast with those reported by Burkard et al. [53], who found that chinchillas treated with smaller doses of carboplatin (50 mg/kg intraperitoneally) had little or no changes in threshold or peak latencies in the IC potential. In contrast, the amplitude of the IC potential was reduced by an average of one-third. The failure to observe a greater increase in IC potential latency with increasing click rate suggested that the effects of carboplatin in that study were limited to the periphery and that carboplatin had little or no neurotoxic effects on the central auditory system. However, in the study by Husain et al. [52], we employed a fivefold greater dose of carboplatin in the rat. The findings of oxidative stress specific to the IC could indicate that in rats exposed to higher doses of carboplatin, direct neurotoxic effects to the IC did occur. The differences between the latter study and that of Burkard et al. [53] could be related to species differences and to dosing differences. Further studies will be needed to determine whether cell losses in the IC occurring in rats

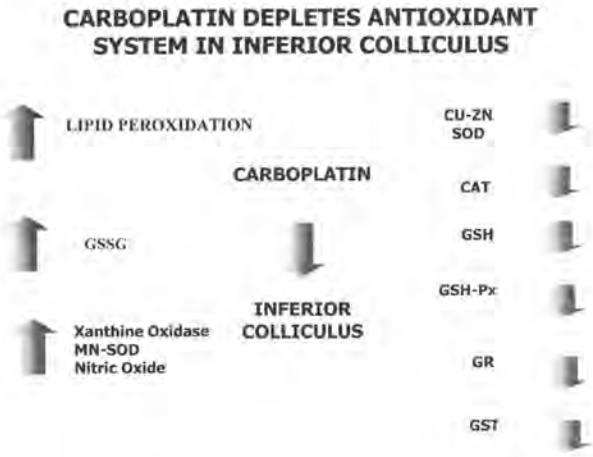

Figure 2. Effect of carboplatin on the antioxidant enzymes, the ratio of reduced glutathione (*GSH*) to its oxidized form (*GSSG*); nitric oxide; prooxidant enzyme activity (xanthine oxidase, manganese superoxide dismutase [*MN-SOD*]) and lipid peroxidation in the inferior colliculus after ototoxic doses of carboplatin in rats. The antioxidant system was compromised by an increase in oxidative stress caused by carboplatin. No significant change occurred in these molecules in the cerebellum of these animals as compared to controls. (*CU-ZN SOD* = copper-zinc superoxide dismutase; *CAT* = catalase; *GSH-Px* = glutathione peroxidase; *GR* = glutathione reductase; *GST* = glutathione-S-transferase.)

exposed to doses of carboplatin result in oxidative stress to this structure.

In chinchillas, widespread IHC lesions from carboplatin exposure reduced the amplitude of the eighth nerve compound action potential (CAP) over a wide range of frequencies. Despite this change in the CAP amplitude, the IC showed little amplitude reduction except at high intensities [54]. This lack of change in response of the IC indicated that the gain of the central auditory system had increased to compensate for the substantial reduction in the CAP. Possibly, the IHC loss resulting from carboplatin exposure resulted in a reduction of the GABA-mediated inhibition that controls the firing rate in IC cells that are excited by acoustic stimuli. The enhanced neural response in the IC after loss of IHCs may explain neuronal mechanisms that underlie tinnitus (Fig. 3) [55].

Other investigators have looked at the effect of ototoxic agents on the IC in relation to tinnitus. Rats treated chronically with salicylate develop behavioral evidence of tinnitus. A neurochemical study of the IC from these animals by Bauer et al. [56] using Western blotting revealed a significant elevation of the enzyme glutamic acid decarboxylase (GAD) in this structure. Muscimol saturation analysis showed an increase in the affinity of the $GABA_A$ receptors in the IC of rats displaying behavioral evidence of tinnitus after chronic

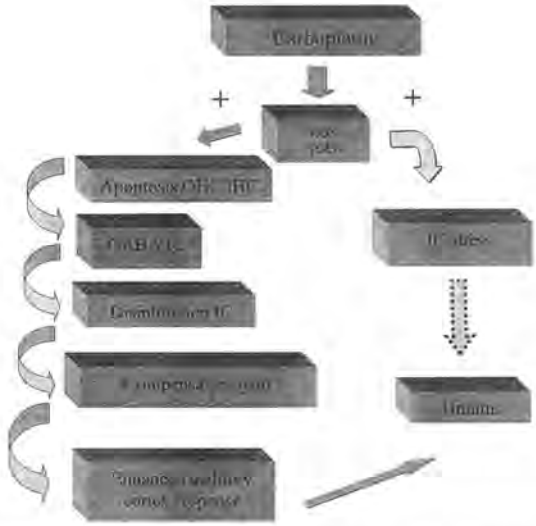

Figure 3. Proposed scheme for the induction of tinnitus by carboplatin. Ototoxic doses of carboplatin can induce oxidative stress within the cochlea and the inferior colliculus (*IC*). This may lead directly to tinnitus-like activity within the inferior colliculus. Also, the production of oxidative stress within the cochlea can lead to apoptosis of the outer (*OHC*) and inner hair cells (*IHC*), causing disinhibition of the inferior colliculus. This can result in compensatory gain and enhanced responses within the auditory cortex, leading to tinnitus. (*ROS* = reactive oxygen species; *RNS* = reactive nitrogen species.)

salicylate administration. These findings suggest that GAD expression and $GABA_A$ receptor–binding characteristics may be altered with chronic exposure to the ototoxic drug salicylate and that these changes could indicate plasticity of the auditory central nervous system experienced as the phenomenon of tinnitus [56]. However, tinnitus perception may develop because of increased activity in the cochlea, altered GABAergic activity within the IC, or both [56]. Noise trauma results in significant decreases in GAD_{65} levels within the IC of rats. Possibly, GABA neurons or their terminal fields may have been lost after acute noise exposure, resulting in a diminished inhibitory state. The increase in $GABA_A$ receptor binding observed in these rats may represent a compensatory mechanism for altered levels of inhibition or excitation within the IC [57].

ROLE OF THE AUDITORY CORTEX IN TINNITUS

The loss of IHCs in chinchillas after carboplatin ototoxicity leads to enhancement of the response amplitudes of the auditory cortex. This enhancement was found to be transient and returned to baseline after 5 weeks. The gain of the central auditory pathway after IHC loss may tend to compensate for the decreased input from the auditory periphery. The enhancement of auditory cortex potential after loss of IHC may indicate a functional reorganization of the central auditory pathways at, or below, the level of the auditory cortex [54]. These changes could represent plasticity within the auditory central nervous system after peripheral injury from ototoxic agents, which could lead to tinnitus (see Fig. 3). Eggermont [58] believed that tinnitus is likely to be caused by a discontinuity in the spontaneous or low-level stimulus-induced neural activity across auditory nerve fibers with different characteristic frequency. Such discontinuities may be caused by loss of OHCs in cochlear regions where IHCs are preserved, as noted by Kaltenbach et al. [47]. This may lead to a loss of lateral inhibition at more central levels in the auditory system. Neurons losing lateral inhibition may become hypersensitive and hyperactive. If these changes persist, the auditory cortical tonotopic map may be reorganized. The spontaneous firing of cortical auditory neurons may become synchronous, thus mimicking responses to external sound stimulation [58]. The prevention of hair cell loss caused by platinum chemotherapy would prevent hearing loss and likely prevent the development of tinnitus (see Fig. 1).

SUMMARY

Cisplatin and carboplatin ototoxicity for auditory hair cells may be mediated by the production of reactive oxygen species within the cochlea. Carboplatin ototoxicity has been found to alter the antioxidant balance within the IC. OHC loss after cisplatin ototoxicity may lead to disinhibition of central auditory structures and result in hyperactivity and hypersensitivity in the DCN, IC, and auditory cortex. These plastic changes in these structures could result in tinnitus. Prevention of ototoxic insults to the auditory system with protective agents might abrogate the production of tinnitus by protecting cochlear hair cells from damage and cell death.

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