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# Protection against cisplatin ototoxicity by adenosine agonists

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**Abstract**

Cisplatin is a commonly used antineoplastic agent that causes ototoxicity through the formation of reactive oxygen species (ROS). Previous studies have shown that cisplatin causes an upregulation of $A_1$ adenosine receptor ($A_1$AR) in the cochlea, and that application of the adenosine agonist, *R*-phenylisopropyladenosine (*R*-PIA), to the round window (RW) results in significant increases in cochlear glutathione peroxidase and superoxide dismutase. These data suggest that adenosine receptors (ARs) are an important part of the cytoprotective system of the cochlea in response to oxidative stress. The purpose of the current study was to investigate the effect of various adenosine agonists on cisplatin ototoxicity using RW application. Auditory brainstem response (ABR) thresholds were recorded in anesthetized chinchillas at 1, 2, 4, 8 and 16 kHz. The auditory bullae were surgically opened, and 1 mM *R*-PIA, 10 μM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX)/*R*-PIA (1 mM) cocktail, 100 μM 2-chloro-*N*-cyclopentyladenosine (CCPA), 2-[4-(2-*p*-carboxy-ethyl)phenylamino]-5′-*N*-ethylcarboxamidoadenosine (CGS) or vehicle were applied to the RW. After 90 min, the remaining solution was removed and cisplatin was applied to the RW. The bullae were closed and the animals recovered for 72 h, after which, follow-up ABRs were performed. Cochleae were harvested for scanning electron microscopy (SEM) and for lipid peroxides. Pre-administration of the $A_1$AR agonists *R*-PIA or CCPA significantly reduced cisplatin-induced threshold changes at all but the highest test frequency. In addition, $A_1$AR agonists protected against cisplatin-induced hair cell damage and significantly reduced cisplatin-induced lipid peroxidation. Co-administration of the $A_1$AR antagonist, DPCPX, completely reversed the protective effects of *R*-PIA. In contrast, pretreatment with CGS-21680, an $A_{2A}$ adenosine receptor ($A_{2A}$AR) agonist, significantly increased cisplatin-induced threshold changes. Our findings are consistent with the notion that the $A_1$AR contributes significantly to cytoprotection in the cochlea, and thereby protects against hearing loss.


*Keywords:* Cisplatin; Ototoxicity; Adenosine receptor; *R*-PIA; Oxidative stress; Cytoprotection

## 1. Introduction

Cisplatin is a commonly used antineoplastic agent that produces a number of dose-limiting side effects, including ototoxicity that is manifested by permanent, sensorineural hearing loss, affecting the high frequencies first and progressing toward the lower frequencies [1–3]. Reactive oxygen species (ROS) have been implicated in the mechanism of cisplatin ototoxicity [4], and cisplatin administration has been shown to result in depletion of antioxidant enzymes and reduced gluthathione (GSH), as well as increased malondialdehyde levels in the cochlea [5,6]. Pretreatment with free radical scavengers or antioxidants has been shown to protect against cisplatin ototoxicity and to restore or maintain endogenous antioxidant and GSH levels [7–10].

The activity of the antioxidant defense system in the cochlea appears to be mediated, in part, by ARs. Pretreatment with adenosine agonists can protect against cisplatin ototoxicity and other forms of oxidative stress in the cochlea, such as noise trauma and ischemia [11–14]. Adenosine receptors are expressed in numerous tissues. Four subtypes of AR, namely $A_1$, $A_{2a}$, $A_{2b}$ and $A_3$, have

*Abbreviations:* $A_1$AR, $A_1$ adenosine receptor; $A_2$AR, $A_2$ adenosine receptor; *R*-PIA, *R*-phenylisopropyladenosine; CCPA, 2-chloro-*N*-cyclopentyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; CGS, 2-[4-(2-*p*-carboxy-ethyl)phenylamino]-5′-*N*-ethylcarboxamidoadenosine; PBS, phosphate-buffered saline; RW, round window; SEM, scanning electron microscopy; ABR, auditory brainstem evoked response; dB, decibel; kHz, kilohertz; MDA, malondialdehyde

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been cloned and characterized [15]. The functions of these receptors vary depending upon the type of tissue in which they are expressed. At least three subtypes of ARs, the $A_1$AR, $A_2$AR and $A_3$AR, have been identified in the cochlea [16,17]. The $A_1$AR, and possibly the $A_3$AR, appear to be involved in cytoprotection [16]. The application of cisplatin to the RW of the chinchilla results in an upregulation of the $A_1$AR in the cochlea at 24 and 72 h after administration [18]. Similarly, the application of the adenosine agonist, *R*-phenylisopropyladenosine (*R*-PIA), to the chinchilla RW results in significant increases in cochlear glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) after 90 min [16]. These data suggest that ARs may promote the antioxidant defense system and the scavenging of free radicals in response to oxidative stress. The purpose of the present study was to identify the AR subtype associated with the cytoprotection against cisplatin ototoxicity produced by adenosine analogs and to determine whether cytoprotection is linked to a decrease in cisplatin-induced lipid peroxidation.

## 2. Methods

### 2.1. Animals

Forty-seven healthy adult male chinchillas (weight 500–700 g), free of external or middle ear pathology were used in this study. All animals had free access to commercial food and water and were maintained in an environment with controlled temperature and 12 h light–dark cycles. This study was conducted in accordance with the Animal Welfare Act of 1986.

### 2.2. Anesthesia and drug administration

Animals were anesthetized with injection of ketamine HCl (45 mg/kg, i.m.) followed by sodium pentobarbital (30 mg/kg, i.p.). Depth of anesthesia was maintained during the experiment using half doses of ketamine each hour or as required. Body temperature was maintained at 36 °C with an animal warming blanket. Respiration was not assisted.

After control auditory testing was performed, the auditory bullae were surgically opened to expose the RW, and 10 μl of either PBS (pH 7.4), *R*-PIA (1 mM) in PBS (pH 7.4), CCPA (100 μM) in PBS (pH 7.4), CGS (1 mM) in PBS (pH 7.4), DPCPX/*R*-PIA (10 μM/1 mM, respectively) in 10% DMSO (pH 7.4) or 10% DMSO in PBS (pH 7.4) was applied to the RW. After 90 min, any remaining solution was removed and 2 μl of either cisplatin (0.66 mg/ml in PBS, pH 6.0) or PBS was applied to the RW. The bullae were re-sealed with impression material (Westone Laboratories, Inc.) and the skin was sutured closed. The animals were housed for 72 h before follow-up testing was performed.

### 2.3. Auditory testing

Auditory brainstem evoked response (ABR) was performed prior to drug administration and 3 days later. Therefore, each ear served as its own control. Chinchillas were anesthetized and the head was immobilized in a small animal stereotoxic apparatus with hollow chinchilla ear bars (David Kopf Instruments). An insert earphone (Etymotic ER-2) was placed into the hollow ear bar. Subcutaneous electrodes were placed over the vertex (active) and over the ipsilateral bulla (reference). Ground electrodes were placed over the neck muscles. ABRs were recorded in an electrically shielded, double-walled, radio frequency shielded sound booth in response to 10 ms tone burst at 1, 2, 4, 8, and 16 kHz. Intensities were expressed in decibels sound pressure level peak equivalent (dB SPL pe). Auditory stimuli were presented at a rate of 5 $s^{-1}$ in 10 dB steps between 0 and 100 dB SPL pe. Responses were amplified 1000 times by a pre-amplifier and an additional 100 times by the averaging system, for a total amplification of 100,000 times. Twenty millisecond responses were recorded on a PC-based, signal averaging system (Tucker-Davis Technologies). The responses were synchronized with the onset of the stimulus, with a 1 ms delay to compensate for the length of the earphone sound delivery tube. Each ABR waveform consisted of 512 averaged responses. The intensity series recorded included a sub-threshold response and increased to 30 dB supra-threshold for each stimulus. Two averaged responses were recorded for each stimulus intensity. Each intensity series was observed to determine the threshold response based on the growth pattern of the waveform amplitude and shortening of wave latency with increasing stimulus intensity. Threshold was defined as the lowest intensity that displays a replicable waveform, with two distinct waves and a minimum amplitude of 0.5 μV. The pretreatment ABR thresholds were compared to post-treatment ABR thresholds and the threshold changes between groups were compared using a Student's two-tailed *t* test.

### 2.4. Scanning electron microscopy (SEM)

After the final ABR recordings, the anesthetized chinchillas were sacrificed and cochleae were removed and processed for SEM using the method previously described by Janning et al. [19]. The perilymphatic space was perfused with 2.5% glutaraldehyde in 0.1 M cacodylate (Cac) buffer. The following day, cochleae were rinsed with Cac buffer and then perfused with 1.5% osmium tetroxide. The cochleae were then rinsed with Cac buffer.

Under the dissecting microscope, the bony capsule and lateral wall of cochlea were removed to expose the organ of Corti. Cochleae were then dehydrated in increasing concentrations of ethanol from 70 to 100%, and were critical point dried. Each specimen was mounted on a

SEM stub and sputter coated with 10 nm gold/palladium alloy. Cochleae were viewed and photographed with a Hitachi S-500 scanning electron microscope.

### 2.5. Lipid peroxidation assay

After the final ABR recordings, the anesthetized chinchillas were sacrificed and cochleae were removed, quick frozen in liquid nitrogen and stored at −90 °C until used. Cochlear MDA levels were determined using the method described by Ohkawa et al. (1979). Tissue extracts (200 μl) were added to 50 μl of 8.1% sodium dodecyl sulfate (SDS), vortexed, and incubated for 10 min at room temperature. Three hundred and seventy-five microliters of 20% acetic acid (pH 3.5) and 375 μl of thiobarbituric acid (0.6%) were added and placed in a boiling water bath for 60 min. The samples were allowed to cool at room temperature. A mixture of butanol:pyridine (15:1) (1 ml each) were added, vortexed and centrifuged at 1000 rpm for 5 min. Five hundred microliters of the colored (upper) layer was measured at 532 mg using 1,1,3,3-tetraethoxypropane as a standard. The levels of MDA were expressed as nanograms per milligram (ng/mg) protein.

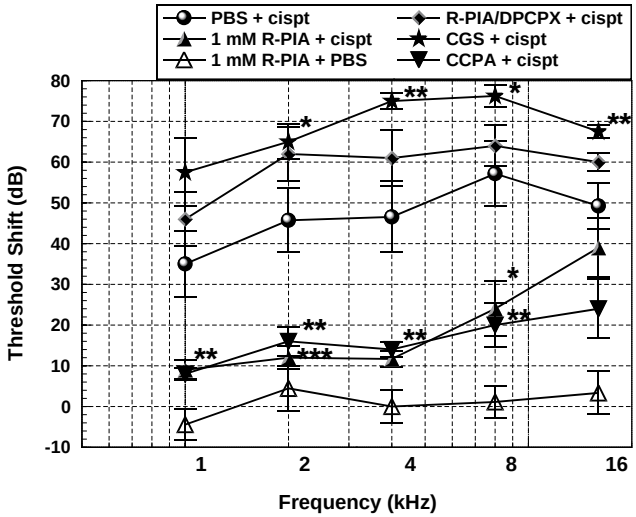


Fig. 1. ABR threshold changes (mean ± S.E.M.) from six treatment groups. Threshold changes were significantly lower in the *R*-PIA-treated and CCPA-treated groups, compared to the vehicle + cisplatin group, at all frequencies except 16 kHz. Co-administration of the $A_1$AR antagonist, DPCPX, completely reversed the protective effects of *R*-PIA. Similarly, administration of the $A_{2A}$AR agonist, CGS, significantly increased cisplatin-induced threshold changes at all frequencies except 2 kHz. No threshold changes were seen after the administration of *R*-PIA alone. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

## 3. Results

### 3.1. ABR thresholds

The administration of cisplatin resulted in profound ABR threshold changes. The ABR threshold changes in the PBS + cisplatin treated ears were 35 ± 8.0 dB, 45.7± 7.8 dB, 46.5 ± 8.7 dB, 57.1 ± 8.0 dB and 49.2 ± 5.6 dB for 1, 2, 4, 8 and 16 kHz, respectively. However, in animals pretreated with *R*-PIA or CCPA, the cisplatin-induced changes in ABR thresholds were significantly less at 1, 2, 4 and 8 kHz (Fig. 1). The ABR threshold changes in the *R*-PIA + cisplatin treated ears were 9 ± 2.3 dB, 12 ± 2.9 dB, 11.7 ± 2.0 dB, 24 ± 6.7 dB and 39 ± 7.2 dB for 1, 2, 4, 8 and 16 kHz, respectively, and the ABR threshold changes in the CCPA + cisplatin treated ears were 8 ± 1.4 dB, 16 ± 3.6 dB, 14 ± 1.8 dB, 20 ± 5.5 dB and 24 ± 7.3 dB for 1, 2, 4, 8 and 16 kHz, respectively. The ABR threshold change at 16 kHz was not significantly different between these treatment groups. The administration of *R*-PIA alone had no effect on ABR thresholds (Fig. 1). RW administration of the selective $A_1$AR antagonist, DPCPX, in combination with *R*-PIA, prior to cisplatin, resulted in increased ABR thresholds at all frequencies, compared to cisplatin alone. However, the increases were not statistically significant. The ABR threshold changes in this group were 46 ± 6.7 dB, 62 ± 6.6 dB, 61 ± 6.9 dB, 64 ± 5.0 dB and 60 ± 2.1 dB for 1, 2, 4, 8 and 16 kHz, respectively. Administration of the $A_{2A}$AR agonist, CGS, to the RW prior to cisplatin, significantly increased ABR thresholds at all frequencies except 2 kHz when compared to cisplatin alone. The ABR threshold changes in this group were 57.5 ± 8.4 dB, 65 ± 4.2 dB, 75 ± 1.9 dB, 76.3 ± 2.6 dB and 67.5 ± 1.6 dB for 1, 2, 4, 8 and 16 kHz, respectively. Administration of CCPA, DPCPX, *R*-PIA/DPCPX cocktail or 10% DMSO to the RW did not significantly affect ABR thresholds (data not shown).

### 3.2. Organ of Corti surface morphology

Fig. 2 shows representative scanning electron micrographs of the organ of Corti from three treatment groups. Extensive loss of inner and outer hair cells could be seen in the hook and basal turns of ears treated with cisplatin. Moderate hair cell loss was seen in the middle turns of these ears. In contrast, the organ of Corti was well preserved in all turns of the ears pretreated with *R*-PIA prior to addition of cisplatin. The *R*-PIA treated animal with the highest threshold changes had some cytoplasmic extrusions from the inner hair cells and disruption of the stereocilia bundles in the hook region (Fig. 2D). This is consistent with greater threshold changes seen at the highest test frequency for this group. Ears treated with DPCPX and *R*-PIA prior to cisplatin show more extensive damage in the middle turns, as compared to the cisplatin-treated group alone. However, the difference between these treatment groups was negligible in the apical turn (not shown).

### 3.3. Lipid peroxidation

Cochleas from cisplatin-treated animals showed a 73 ± 16% increase in MDA over baseline levels of

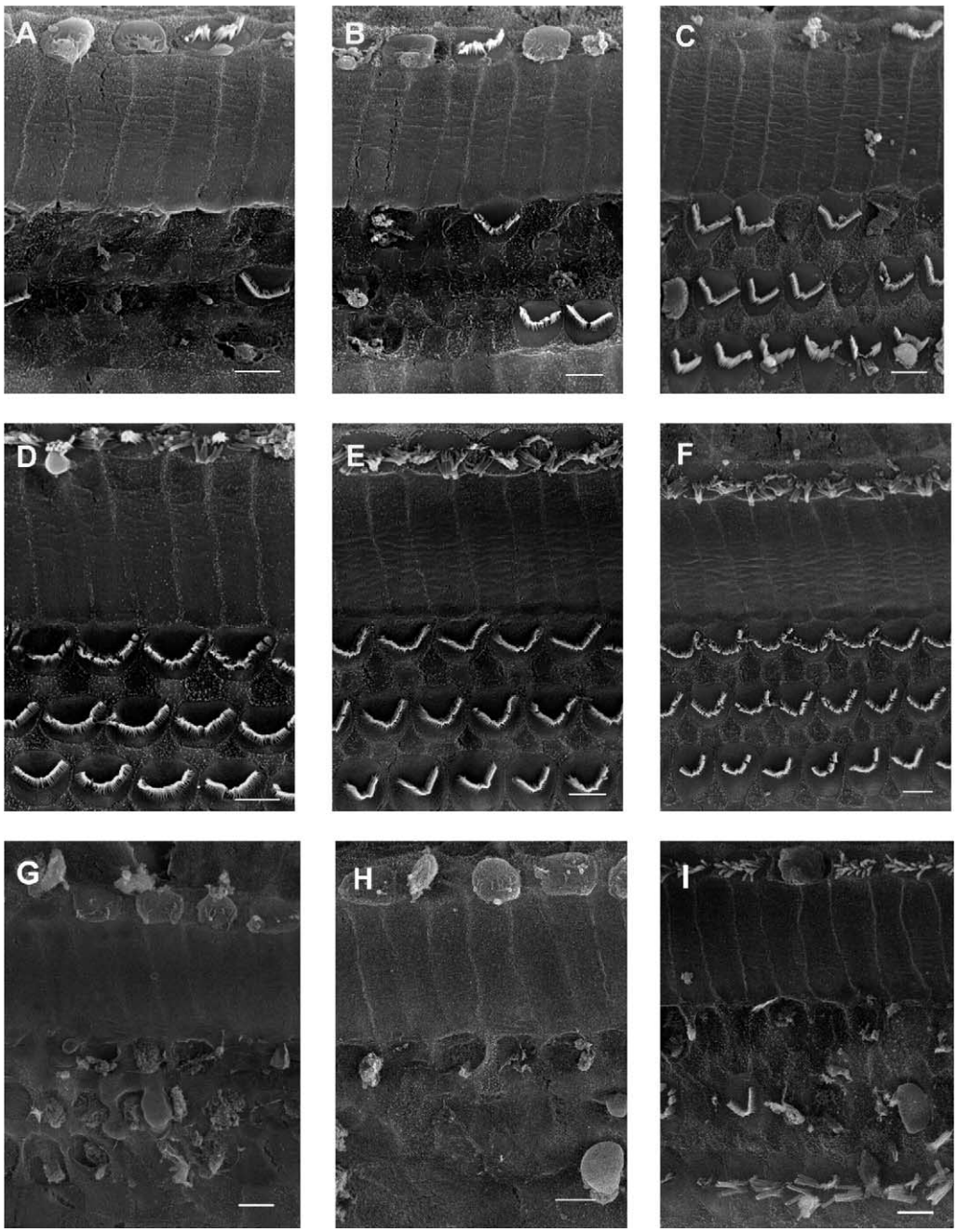


Fig. 2. Comparison of organ of Corti morphology between an ear treated with saline + cisplatin, one treated with *R*-PIA + cisplatin and one treated with DPCPX/*R*-PIA + cisplatin. Application of saline + cisplatin resulted in almost complete IHC and OHC loss in the hook (A) and basal turn (B) and moderate loss in the middle turn (C). In contrast, the organ of Corti morphology was very well preserved in the hook, basal and middle turns (D, E and F, respectively) of ears treated with *R*-PIA + cisplatin. Some cytoplasmic extrusions from the IHCs and disruption of stereocilia were evident in the hook region of the *R*-PIA + cisplatin-treated ear that displayed the largest threshold changes (D). The administration of DPCPX/*R*-PIA cocktail prior to cisplatin resulted in extensive hair cell loss in the hook, basal and middle turns (G, H and I, respectively). The morphological differences between the apical turns from the three groups were negligible (not shown). Bars = 5 μm.

$250 \pm 10$ ng/mg protein. The addition of *R*-PIA (1 mM) reduced the levels of MDA by 37%. Furthermore, the addition of CCPA (100 μM) to the round window prior to cisplatin administration, reduced the level of cisplatin-induced malondialdehyde by $25 \pm 8\%$. Inhibition of cisplatin-mediated lipid peroxidation by CCPA was statistically significant ($P < 0.05$).

## 4. Discussion

Upregulation of the $A_1$AR has been observed in response to conditions of oxidative stress [20] and noise trauma [21] and protection against these conditions by the application of adenosine agonists has been demonstrated. The administration of *R*-PIA to the RW prior to high level

continuous or impulse noise exposure was shown to improve evoked potential recovery in the chinchilla [12,13]. In addition, pretreatment with CCPA protected against auditory threshold shifts resulting from transient ischemia [14]. Previous studies from our laboratory have demonstrated that induction of $A_1$AR expression also occurs in response to cisplatin in the kidney and in the cochlea, and that this is accompanied by increases in the activity of key enzymes in the antioxidant and GSH pathways [18]. Ford et al. demonstrated that the application of *R*-PIA to the RW in chinchillas resulted in significant increases in cochlear SOD and GSH-Px after 90 min [16]. In a second study, Ford et al. demonstrated a fivefold increase in $A_1$AR expression in the cochlea after the application of cisplatin to the RWM [18]. In addition, adenosine has recently been shown to block the production of ROS by phagocytes by inhibiting the movement of NADPH oxidase to active sites within these cells [22]. The reduction of ischemia-induced ROS formation by $A_1$AR activation has also been observed in ventricular myocytes [23]. In the current study, MDA levels were greatly reduced in the cochleas of animals administered CCPA or *R*-PIA + cisplatin, compared to those treated with PBS + cisplatin, indicating that adenosine analogs can reduce the degree of oxidative stress in the cochlea. Therefore, adenosine appears to both reduce ROS production and increase the activity of key enzymes in the GSH and antioxidant pathways.

The beneficial effects of AR upregulation in the cochlea could have resulted from activation of $A_1$AR and $A_3$AR. The $A_1$AR and $A_3$AR subtypes have been identified in the cochlea and are at their highest concentrations in the organ of Corti and stria vascularis, and their role in the cochlea appears to be related to cytoprotection, rather than function [16]. In Ford's study, the addition of *R*-PIA or adenosine deaminase to the RWM had no effect on ABR or whole eighth nerve, compound action potential (CAP) thresholds or on the endocochlear potential (EP). Similarly, Tabuchi et al. saw no effect by CCPA on CAP thresholds [14]. In the current study, the application of *R*-PIA, CCPA or DPCPX to the RW had no effect on ABR thresholds. However, pretreatment with *R*-PIA or CCPA significantly attenuated cisplatin ototoxicity at all but one of the tested frequencies. The introduction of the $A_1$AR-specific antagonist, DPCPX, completely reversed the protective effects of *R*-PIA. In addition, pretreatment with the $A_{2A}$AR agonist, CGS, significantly increased cisplatin-induced threshold changes. These data strongly support the role of $A_1$AR as part of a cytoprotective mechanism in the cochlea and also indicate that activation of the $A_{2A}$AR may produce the opposite effect.

The lack of protection against cisplatin-induced threshold changes at 16 kHz by *R*-PIA or CCPA may be the result of the drug application method itself. In our experiments, the excess *R*-PIA or CCPA solutions were removed from the RW after 90 min, and cisplatin solution was subsequently applied to the RW. The cisplatin solution was not removed from the RW, but remained for the full 72 h duration of the experiment. Therefore, ototoxic intracochlear cisplatin concentrations could remain elevated longer than an effective concentration of *R*-PIA or CCPA, and eventually overcome the protective effects afforded by these adenosine agonists. At that time, one would expect the cisplatin-induced damage to follow its typical pattern, by affecting the higher frequency regions first.

Concentration gradients of cisplatin within the cochlea could also partially explain our results. After RW application, the concentration of cisplatin in the perilymph might remain higher in the hook region of the cochlea, where the 16 kHz region of the chinchilla organ of Corti would be located, compared to other turns. Recent computer simulations of perilymph flow dynamics would suggest that this is the case [24]. However, we have observed a similar pattern with other protective agents, whether cisplatin is administered topically or systemically. For example, in a recent study from our laboratory, systemic aminoguanidine, an inducible nitric oxide synthase (iNOS) inhibitor and free radical scavenger, was used to protect against the ototoxicity of systemically applied cisplatin [25]. These results followed a similar pattern of protection to that seen in the current study, namely, significant reduction in threshold changes in all but the highest frequencies. These data suggest that our results from RW application are not the result of cisplatin concentration gradients in the perilymph, but are possibly due to differences in metabolic rates and antioxidant enzyme activities in different regions of the cochlea. For example, it has recently been demonstrated that the concentration of glutathione (GSH), an important endogenous free-radical scavenger and antioxidant, is much lower in the hook and basal turn of the cochlea compared to the middle and upper turns [26].

The ototoxic effect of cisplatin when applied to the RW can depend on its concentration. Ford et al. reported that no hair cell loss was observed until 72 h after the administration of 0.54 mg/ml cisplatin to the RW, at which time both outer hair cell (OHC) and inner hair cell (IHC) loss was seen [18]. However, Tsukasaki observed hair cell loss as early as 14 h after the administration of 1 mg/ml cisplatin to the RW. In addition, they observed that IHC loss occurred prior to OHC loss [27]. In the current study, we used a cisplatin concentration of 0.66 mg/ml and observed the organ of Corti morphology at 72 h after administration. At this time, we could see extensive loss of both IHCs and OHCs in the PBS + cisplatin ears. In ears that were protected by pre-administration of *R*-PIA, both IHCs and OHCs were preserved in all turns. However, some cytoplasmic extrusions could be observed adjacent to IHC cuticular plates, which corresponded with larger threshold changes at 16 kHz. Data from our laboratory indicate that the threshold ototoxic concentration of cisplatin solution applied to the RW is approximately 0.22 mg/ml, for the chinchilla. This concentration would be approximately

equivalent to a maximum intra-cochlear concentration of 44 μg/ml if the entire application crossed the RW and remained in the cochlea. This is about 10 times the peak perilymph concentration of 4 μg/ml observed by Laurell after i.v. administration in the guinea pig [28], which suggests that only a fraction of the cisplatin that we applied to the RW was present in the perilymph at any given time. With this in mind, the perilymph concentration of cisplatin in the current study is likely to be in the range of 13 μg/ml. Therefore, the lack of protection afforded by *R*-PIA at 16 kHz may result from the high concentration of cisplatin applied to the RW.

Adenosine agonists are only selective in a specific concentration range. For example, *R*-PIA is selective for $A_1$AR in the 1 μM range, but begins to lose specificity near 10 μM. At higher concentrations, the effect of *R*-PIA on multiple ARs may have potentially adverse effects, such as altering cochlear blood flow. Indeed, cochlear perfusion with adenosine increased cochlear blood flow in the guinea pig [29]. This increase in cochlear blood flow was reduced by theophylline, a non-specific AR antagonist, while specific $A_1$AR and $A_{2A}$AR antagonists had no effect. There also appears to be cross-regulation between ARs. For example, $A_2$AR activation appears to inhibit $A_1$AR in the central nervous system [30,31]. With our RW application model, a U-shaped dose–response pattern was seen for *R*-PIA and for CCPA using a $\log_{10}$ dosage scale. Both 100 μM and 10 mM concentrations of *R*-PIA provided no protection against cisplatin ototoxicity (data not shown). However, 1 mM *R*-PIA significantly reduced cisplatin ototoxicity at all frequencies except for 16 kHz. A similar result was seen with CCPA, except that the response curve was shifted to the left. The 10 μM and 1 mM concentrations of CCPA did not protect against cisplatin (data not shown), while 100 μM CCPA was protective. The lower concentrations of these agonists likely did not activate enough receptors to invoke a protective effect, while the higher concentrations may have activated multiple AR subtypes or resulted in AR down-regulation. It is unlikely that the concentrations of these agonists are the same on the RW as they are at their site of action in the cochlea. Although low molecular weight compounds cross the RW readily [32,33], the rate of diffusion and the degree of dilution by perilymph and endolymph are not known. We estimate that 1–10% of the solution applied to the RW diffuses into the scala tympani over the 90-min period. Since the volume of perilymph in the chinchilla is approximately 10 μl [34], the solution would be diluted to 10% by perilymph. Therefore, the final concentration of *R*-PIA would likely be 1–10 μM and that of CCPA would be 0.1–1 μM. These concentrations would be in the same range as those shown to be effective in vitro.

The protective effects afforded by *R*-PIA and CCPA in the current study, appear to be mediated by the $A_1$AR. We estimate that the concentrations of *R*-PIA and CCPA at the target site are likely to be similar to the concentrations that produce selective action on the $A_1$AR in vitro. Additionally, the $A_{2A}$AR agonist, CGS, failed to protect against cisplatin ototoxicity. The administration of DPCPX, a specific inhibitor of $A_1$AR, completely reversed the protective effect of *R*-PIA. In fact, DPCPX administration resulted in a slight, but not significant, potentiation of cisplatin-induced threshold changes above those of the PBS + cisplatin values at all frequencies tested. This effect may be due to the inhibition of endogenous $A_1$AR, as well as induced $A_1$AR [18]. In fact, similar findings were observed when DPCPX was administered 90 min prior to various concentrations of cisplatin, in the absence of *R*-PIA (data not shown). The DPCPX-treated ears demonstrated slight, but not significant, increases in ABR thresholds compared to PBS + cisplatin-treated ears. The reduction in cisplatin-induced intracochlear MDA levels by CCPA, suggests that the protective effects of $A_1$AR agonists are due, in part, to a reduction in oxidative stress. Our results strongly support $A_1$AR as an important step in protection against ROS-induced cell damage in the cochlea. We conclude that $A_1$ARs play an important role in protecting the cochlea from oxidative stress, and that adenosine agonists, such as *R*-PIA and CCPA, may be clinically important compounds for protection against ototoxic agents.

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