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D-JNKI-1 Treatment Prevents the Progression of Hearing Loss in a Model of Cochlear Implantation Trauma

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Hypotheses: 1) Hearing loss caused by electrode insertion trauma has both acute and delayed components; and 2) the delayed component of trauma-initiated hearing loss can be prevented by a direct delivery of a peptide inhibitor of the c-Jun N-terminal kinase cell death signal cascade, that is, D-JNKI-1, immediately after the electrode insertion within the cochlea.
Background: Acute trauma to the macroscopic elements of the cochlea from electrode insertion is well known. The impact of trauma-induced oxidative stress within injured cochlear tissues and the efficacy of drugs (e.g., D-JNKI-1) to prevent apoptosis of damaged hair cells is not well defined.
Methods: Hearing function was tested by pure-tone evoked auditory brainstem responses (ABRs) and distortion products of otoacoustic emissions (DPOAEs). D-JNKI-1 in artificial perilymph (AP) or AP alone was delivered into the scala tympani immediately after electrode trauma and for 7 days. Controls were nontreated contralateral and D-JNKI-1-treated ears without electrode insertion trauma.
Results: There was no increase in the hearing thresholds of either the contralateral control ears or in the D-JNKI-1 without trauma animals. There was a progressive increase in ABR thresholds and decrease in DPOAE amplitudes after electrode insertion trauma in untreated and in AP-treated cochleae. Treatment with D-JNKI-1 prevented the progressive increase in ABR thresholds and decrease in DPOAE amplitudes that occur after electrode insertion trauma.
Conclusion: Hearing loss caused by cochlear implant electrode insertion trauma in guinea pigs has both acute and delayed components. The delayed component can be prevented by treating the cochlea with D-JNKI-1. **Key Words:** Cochlear Implantation—Oxidative Stress—Hearing Preservation—Electrode Trauma-initiated Apoptosis—c-Jun N-terminal Kinase—D-JNKI-1 Inhibitory Peptide.
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The insertion of a cochlear implant electrode array into the scala tympani of a guinea pig causes tissue trauma, which can directly kill any residual hair cells via necrosis and also generate oxidative stress within the damaged tissues of the cochlea resulting in the apoptosis of hair cells and a loss of hearing.

Histological evaluation of the cochlea after insertion of a cochlear implant electrode in human cadaver temporal bones has demonstrated immediate damage to different structures of the inner ear (1,2). In a previous cryohistological study of human temporal bones, we analyzed both the electrode position and structural damage to the cochlea and have formulated a scale of numerical values that range from 0 to 4 to rate the extent and severity of initial macroscopic trauma (1). Using this trauma scale, we have demonstrated that the severity of direct trauma to cochlear structures can be lessened by design and use of less traumatic electrodes and by modification of the surgical technique used for electrode insertion (1,3).

Recently, there has been an increase in the number of patients with some residual hearing that choose to have cochlear implantation surgery (4). There is also a new trend to use bimodal electroacoustic stimulation that combines the use of a hearing aid with a cochlear implant in patients with severe hearing loss that is mostly in the high-frequency range and who posses stimulable hearing

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in the low-frequency range (4,5). This type of bimodal approach accentuates the need to preserve the patient's functioning low-frequency-sensitive hair cells during and after implantation of an electrode array specifically designed for electroacoustic stimulation patients. There is recent evidence that a patient's preoperative hearing thresholds can be maintained after implantation with a short hybrid electrode array (6), or if there is some initial loss of residual hearing after implantation of a longer electrode array in that this initial decline in hearing is overcome in time postimplantation (7). There is a previous report of a delayed loss of patients' residual hearing after implantation of the electrode array (8); however, these patients were not implanted with a shortened electrode array designed to minimize the initial trauma (6). It is possible that the insertion of a nonhybrid short electrode array may initiate a loss of auditory hair cells and later of the auditory neurons by oxidative stress generated within damaged sensory tissues of the cochlea (9–11).

A recent animal study characterized the electrophysiological pattern of hearing loss postcochlear electrode insertion trauma in the laboratory rat (12). The results of this study documented an initial hearing loss of 25 to 35 dB sound pressure level (SPL) and then a progressive loss of hearing of an additional 15 dB SPL postimplantation (i.e., Days 0–7) for all of the frequencies tested (i.e., 4–32 kHz) and strongly suggest that electrode insertion trauma generates a damaging level of oxidative stress within injured cochlear tissues that occurs after the initial physical trauma.

In this study, the same objective measurements used to define hearing function and characterize the progressive loss of hearing in the rat (12) were adapted to the guinea pig and used to assess electrode trauma-initiated hearing loss and the otoprotective effect of direct delivery of the synthetic inhibitory peptide of c-Jun N-terminal kinases (JNKs), that is, D-JNKI-1. Apoptosis of auditory neurons as a consequence of oxidative stress damage has been shown to involve the downstream target of the JNK signal cascade, that is, c-Jun (13). A previous study using D-JNKI-1, which binds to all three JNK molecules and inactivates their downstream signaling capacity, has demonstrated that this inhibitory peptide can prevent loss of both hearing and hair cells in animals challenged with exposure to either a damaging level of acoustic trauma or to an ototoxic level of an aminoglycoside antibiotic (14).

MATERIALS AND METHODS

Experimental Animals and Surgical Procedures

The use of laboratory animals in this study was performed in accordance with the guidelines of the University of Miami Miller School of Medicine Animal Care and Use Committee and in compliance with the USDA and the NIH Guidelines for the Care and Use of Laboratory Animals.

Thirty-seven ears from 25 guinea pigs were evaluated for hearing acuity before and after exposure of one cochlea to an electrode insertion trauma paradigm. Only one ear from each guinea pig was used to study the effect of electrode insertion trauma and either a left or right ear was randomly chosen to be the experimental ear. Contralateral unoperated cochleae acted as internal controls. All surgery and testing were accomplished under general anesthesia using an intramuscular injection of a mixture of ketamine (90 mg/kg i.m.) and xylazine (10 mg/kg i.m.).

Artificial Perilymph Formulation and D-JNKI-1 in Artificial Perilymph

Artificial perilymph (AP) was prepared in pyrogen-free, sterile, double-distilled water at the following salt and buffer concentrations: NaCl, 145 mM; KCl, 2.7 mM; $MgSO_4$, 2 mM; $CaCl_2$, 1.2 mM; HEPES, 5 mM. This final AP solution was prepared on the day of use from stock solutions and filtered through a 0.45-μm sterile filter (Corning, Corning, NY). D-JNKI-1 peptide (Xigen, Lausanne, Switzerland) was stored at −80°C in a sterile stock solution of 1 mg/mL and diluted in sterile AP to a concentration of 10 μM before immediately loading into the Alzet 2001 pump (DURECT Corp., Cupertino, CA, U.S.A.). All Alzet mini-osmotic pumps loaded with either the treatment drug in AP or AP only were primed overnight at 37°C in a bath of sterile Ringer saline.

Trauma Group

One ear was randomly chosen in six animals that composed the trauma group. A retroauricular incision exposed the bulla. The bulla was opened with a scalpel, the round window membrane and promontory were identified, and a cochleostomy was performed in the basal turn of the cochlea using a straight surgical pick. A steel electrode with a 0.14-mm diameter ball on the tip was slowly inserted via the cochleostomy for a length of 3 mm into the scala tympani and then gently withdrawn. Care was observed during both insertion and withdrawal to avoid contact with the basal lamina of the scala media and the electrode. A temporalis muscle graft sealed the cochleostomy site.

Trauma with D-JNKI-1 Perfusion and Contralateral Control Groups

One ear was randomly chosen in a group of 12 guinea pigs, and we performed the same middle and inner ear operations and electrode insertion and withdrawal procedure; however, instead of packing the cochleostomy site with a muscle graft, we inserted a catheter and delivered a D-JNKI-1 peptide solution (i.e., at a 10-μM concentration in 200 μL of AP) via a 2001 Alzet mini-osmotic pump at a rate of 1 μL/h for a period of 1 week.

The catheter was secured within the bulla with dental cement and a muscle graft to close the cochleostomy site. The catheter was left out of the bulla and the pump secured in a pocket created under the skin. The bulla defect was closed and the skin wound closed with sutures. The hearing test results from the unoperated ears of this group of animals were used as the contralateral control group data for all statistical comparisons in this study, n = 12.

Trauma with AP Perfusion Group

One ear was randomly chosen from three animals to be the AP perfusion control group with a cochleostomy, and insertion of a catheter with a carrier solution of trauma + AP only was delivered at the same rate and during the same period of time with regard to the animals in D-JNKI-1 in the AP treatment group.

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Auditory Function Tests

Auditory brainstem response (ABR) and distortion products of otoacoustic emission (DPOAE) in response to pure-tone stimuli were performed presurgery, Day 0 (immediately post-surgery), and on postsurgery, Days 3 and 7.

ABR Recording

To obtain ipsilateral ABR information, recording electrodes were attached to the ipsilateral superior postauricular area (−) and to the vertex (+). A ground electrode was inserted deep into the muscles of the left leg. Electrophysiological responses were amplified using an Opti-Amp bioamplifier from Intelligent Hearing Systems (IHS, Miami, FL) connected to the Smart EP system. Tone bursts of 0.5, 1, 4, and 16 kHz were delivered with the rate of stimulation set at 29 Hz. The level of stimulation was decreased in 10-dB steps until there was no identifiable ABR response. This ABR method is described in a previous report (15).

DPOAE Recordings

Measurements were conducted for pure tones from 0.5 to 16 kHz. An Etymotic 10B+ Probe was inserted into the external ear canal and used with two different types of transducers depending on the range of the stimulation frequency. Three stimulation levels ranging from 65 to 25 dB SPL in 20-dB steps were used. This DPOAE method is described in a previous report (15).

FITC-Phalloidin/Propidium Iodide Labeling of Corti's Organ Surface Preparations

Cochleae from control and electrode insertion trauma guinea pigs were fixed at 12, 24, and 36 hours posttrauma by intracardiac perfusion with freshly prepared 4% paraformaldehyde and then immersed overnight in fixative at 4°C. The Corti's organ surface preparations were microdissected from the lower middle turns of the fixed, undecalcified guinea pig cochleae. Tectorial membranes were carefully removed before FITC-phalloidin/propidium iodide (PI) staining. The double-stained lower middle turn Corti's organ specimens were observed under a confocal microscope under a 495-nm wavelength light for the FITC-phalloidin staining of the hair cells and under a 530-nm wavelength light for PI staining of the nuclei. Z-series images were collected for both FITC and PI staining of the same area and then these two Z-series-stacked images were merged to create a single image that showed both HC staining (FITC-phalloidin) and nuclear staining (PI). This allowed the identification of outer hair cell (OHC) and inner hair cell (IHC) nuclei and the observation of changes in nuclear staining in these sensory hair cells that is consistent with apoptosis, that is, nuclear condensation and the formation of highly condensed apoptotic bodies in contrast to necrosis where the nuclei are swollen.

Statistics

Statistical analysis was performed using standard parametric techniques. Significance testing was performed between groups (e.g., ANOVA single factor test and appropriate post hoc tests). For within-group testing, Student's t test was used. A p value of <0.05 was considered statistically significant. The following are the number of animals in each experimental and control group in this study: D-JNKI-1 in AP, n = 4; electrode insertion trauma, n = 6; electrode trauma + D-JNKI-1 in AP, n = 12; contralateral control, n = 12; electrode trauma + AP, n = 3.

RESULTS

Preoperative ABR thresholds for tone bursts stimuli of frequencies 500 Hz, 1 kHz, 4 kHz, and 16 kHz and DPOAE amplitude response for L1 = L2 = 65 dB SPL showed no significant differences between all groups.

ABR and DPOAE losses were flat across tested frequencies; ANOVA demonstrated no significant frequency-based differences. Thus, for clarity, group mean results are discussed for ABR thresholds at 0.5, 4, and 16 kHz and for DPOAE amplitude at 8 kHz. Immediate changes refer to differences in results immediately recorded before (pre) and after (Day 0) electrode insertion. Progressive changes refer to further differences between results of Days 0 and 7.

The trauma with perfusion of D-JNKI-1 group had a less immediate loss of ABR threshold (ABR mean difference of 11.9 ± 9.9 dB) than observed in the trauma group (ABR mean difference of 15.6 ± 7.0), but this difference in threshold values did not achieve significance ($p = 0.27$). The DPOAE amplitude had a smaller decrease in the trauma group with D-JNKI-1 peptide in AP perfusion (DPOAE mean difference −5.7 ± 7.4) than the trauma group without treatment (DPOAE mean difference −8.0 ± 4.6), but again the difference between these values did not achieve significance ($p = 0.51$) (Table 1). However, the progressive loss of auditory function posttrauma (i.e., from Days 0–7) did represent a significant change; the trauma with perfusion of D-JNKI-1 in AP group had less immediate loss of ABR threshold (ABR mean difference of −4.9 ± 10.1 dB) than the trauma group (ABR mean difference 13.6 ± 11.9 dB), and the difference between these two groups was very significant ($p < 0.0001$). The DPOAE amplitude also had less of a decrease in the trauma group with D-JNKI-1 in AP perfusion (DPOAE mean difference 1.5 ± 5.6) than the trauma group without treatment (DPOAE mean difference −10.7 ± 8.4), again significant with $p < 0.001$

TABLE 1. *Mean immediate changes of ABR threshold and DPOAE amplitude*

	Trauma	Trauma + D-JNKI-1	p
Mean of ABR threshold changes	15.6 ± 7.0	11.9 ± 6.9	0.27
Mean of DPOAE amplitudes	−8.0 ± 4.6	−5.7 ± 7.4	0.51

Note that ABR thresholds were counted for 500 Hz, 4 kHz, and 16 kHz tone bursts.

TABLE 2. *Mean progressive changes of ABR threshold and DPOAE amplitude*

	Trauma	Trauma + D-JNKI-1	p
Mean of ABR threshold changes	13.6 ± 11.9	−4.9 ± 10.1	<0.0001
Mean of DPOAE amplitudes	−10.7 ± 8.4	1.5 ± 5.6	<0.001

Note that ABR thresholds were counted for 500 Hz, 4 kHz, and 16 kHz tone bursts.

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TABLE 3. *Mean total changes of ABR threshold and DPOAE amplitude*

	Trauma	Trauma + D-JNKI-1	*p*
Mean of ABR threshold changes	29.2 ± 13.0	7.1 ± 7.1	<0.0001
Mean of DPOAE amplitudes	−18.7 ± 7.5	−4.3 ± 3.6	<0.0001

Note that ABR thresholds were counted for 500 Hz, 4 kHz, and 16 kHz tone bursts.

(Table 2). The total protective effect (combined immediate and progressive) of D-JNKI-1 treatment in trauma animals is represented in Table 3. There is a significant increase of hearing threshold in trauma + D-JNKI-1 in AP group compared with the trauma group that did not receive treatment at Day 7 ($p <$ 0.0001), and there is a significant decrease of amplitude of DPOAE in the trauma group compared with the trauma + D-JNKI-1 group at Day 7 ($p < 0.0001$).

Contralateral Unoperated Ear and D-JNKI-1 Peptide without Trauma Control Animal Groups

There was no significant increase in the ABR thresholds of either the contralateral unoperated cochleae (see Fig. 1A) of electrode insertion trauma animals or in the cochleae of the animals perfused with D-JNKI-1 peptide in AP without any electrode insertion trauma (see Fig. 1C) for the duration of the experiment, that is, 7 days.

There was also no significant change in the amplitudes of the DPOAEs obtained from the cochleae of either of these two groups of animals (see Fig. 1, B and D).

The Pattern of Hearing Loss in Electrode Insertion Trauma with and without D-JNKI-1 Peptide and Electrode Insertion Trauma with AP Groups

There was a significant immediate increase of ABR thresholds after electrode insertion trauma across all frequencies tested with a similar decrease in the amplitudes of DPOAE responses and then a progressive, gradual loss of auditory function that occurred over the next 7 days (Fig. 2, A and B).

The auditory function results of the trauma with D-JNKI-1 in AP perfusion group are presented in Figure 2, C and D. The same type of immediate loss in auditory function occurred in this group of animals; however, the progressive loss of function that occurred in the untreated trauma group (Fig. 2, A and B) did not occur in this group of trauma + D-JNKI-1 animals (see Fig. 2, C and D).

The hearing thresholds in traumatized cochleae perfused with AP alone were not protected by the perfusion of this carrier solution (Fig. 2E), and these animals had a pattern of hearing loss (immediate and then a progressive loss of auditory function) similar to untreated traumatized cochleae (Fig. 2A). The amplitude of the DPOAE responses in electrode insertion-traumatized cochleae perfused with AP was significantly decreased (see Fig. 2F) and had a pattern of DPOAEs responses that were similar also to those of untreated traumatized cochleae (Fig. 2B).

Thus, an immediate loss of auditory function is present in all electrode insertion animals, this immediate loss is lessened in the trauma + D-JNKI-1 group but still occurs. Additionally, there is a progressive loss of function after all electrode insertion traumas occurring between Days 0 and 7 in all animals of the trauma group. This progressive loss of function is significantly reduced ($p < 0.0001$) only

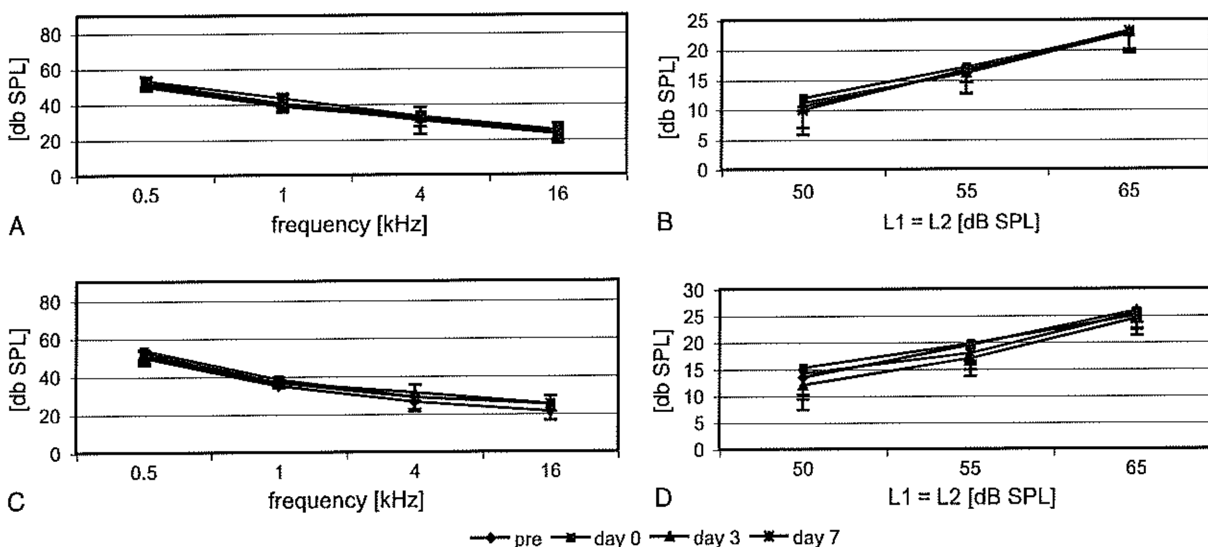

FIG. 1. The contralateral unoperated cochleae of trauma animals and the perfusion of an untraumatized cochleae with D-JNKI-1 peptide do not cause any loss of auditory function. Graphic presentation of ABR threshold (*A and C*) and DPOAE amplitude (*B and D*) changes for presurgery, immediately posttrauma (Day 0), and Days 3 and 7 posttrauma. ABR threshold and DPOAE amplitude values are plotted as means with the error bars representing the ±SD of these mean values. *A and B*, Electrode trauma, contralateral unoperated control cochleae (n = 12 animals). *C and D*, Untraumatized cochleae perfused with D-JNKI-1 peptide for 7 days (n = 4 animals).

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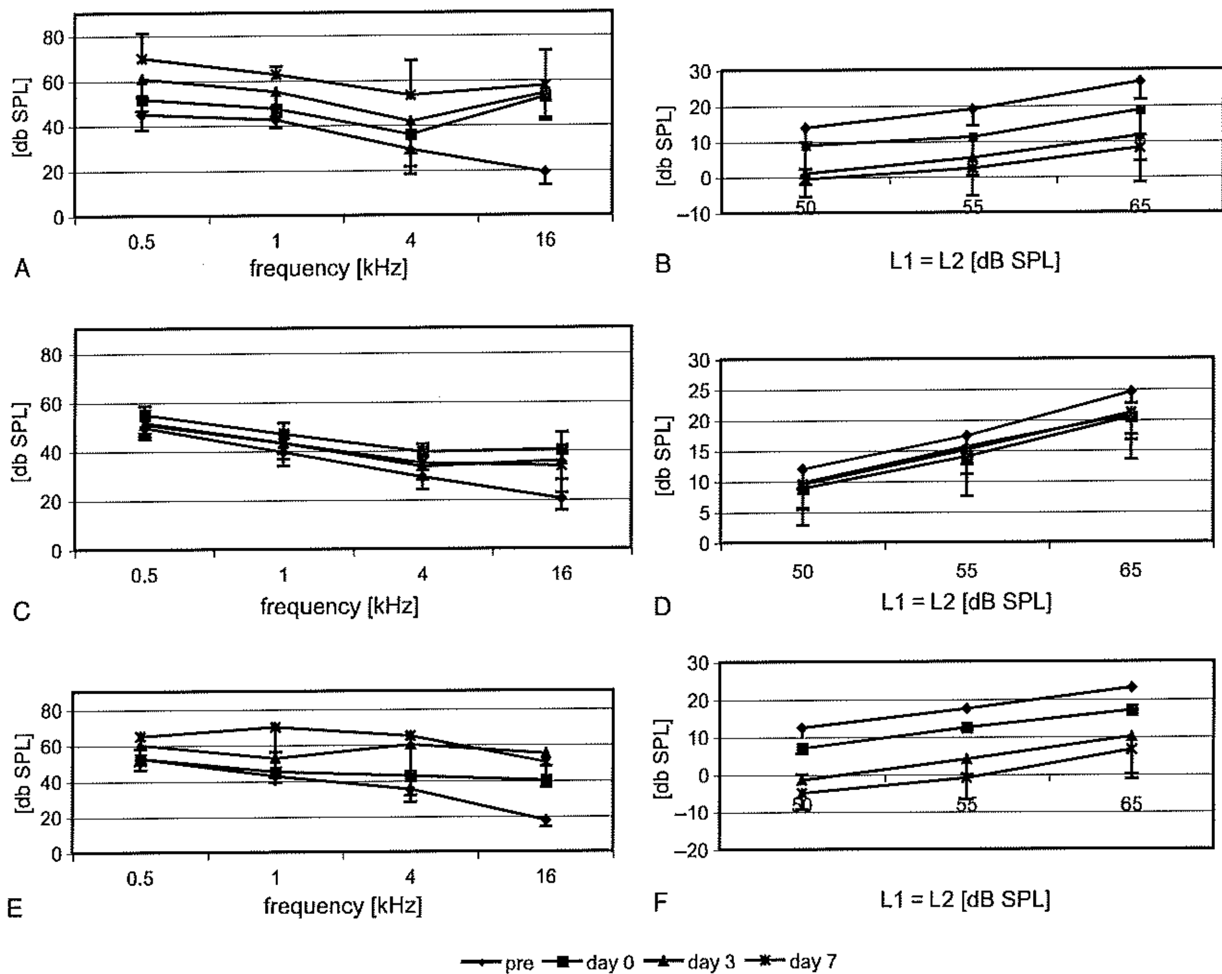

FIG. 2. Electrode trauma causes both an immediate and a progressive loss of auditory function, this progressive loss of auditory function can be prevented by treating the cochlea with D-JNKI-1 peptide in AP but not by treating with AP alone. Graphic presentation of ABR threshold (*A, C, E*) and DPOAE amplitude (*B, D, F*) changes for presurgery, immediately posttrauma (Day 0), and Days 3 and 7 posttrauma. ABR threshold and DPOAE amplitude values are plotted as means with the error bars representing the ±SD of these mean values. *A and B*, Electrode trauma cochleae (n = 6 animals). *C and D*, Electrode trauma + D-JNKI-1 peptide in AP (n = 12 animals). *E and F*, Electrode trauma + AP only (n = 3 animals).

in the trauma + D-JNKI-1 in AP perfusion-treated group when compared with the trauma-only group.

FITC-Phalloidin/PI Labeling

The preliminary results of FITC-phalloidin/PI labeling of the lower middle turn Corti's organ surface preparations (n = 1 animal/time point) from contralateral control and electrode insertion-traumatized cochlea at 12, 24, and 36 hours posttrauma show a progressive increase in PI-labeled hair cell nuclei with changes that are characteristic of apoptosis in the traumatized cochleae (see Fig. 3B) over time posttrauma, whereas these changes were not observed in the Corti's organ surface preparation of the contralateral control cochleae (see Fig. 3A). The preliminary data obtained from the counts of PI-labeled hair cell nuclei with changes characteristic of apoptosis and the counts from the 12-hour posttrauma control cochlea are presented in Figure 3C. These data provide preliminary evidence that the apoptosis of hair cells that occurs postelectrode insertion trauma begins at 24 hours posttrauma and continues to increase until at least 36 hours posttrauma. Because each time point is represented by one animal, no statistical analysis of this preliminary data was possible. The presence of apoptotic nuclei at the 24-hour posttrauma time point in the trauma specimen and not in the control specimen has been confirmed with a TUNEL-stained set of lower middle turn specimens (Van De Water and Mou, data not shown).

DISCUSSION

The pattern of hearing loss caused by electrode insertion trauma in the guinea pig documented in the current

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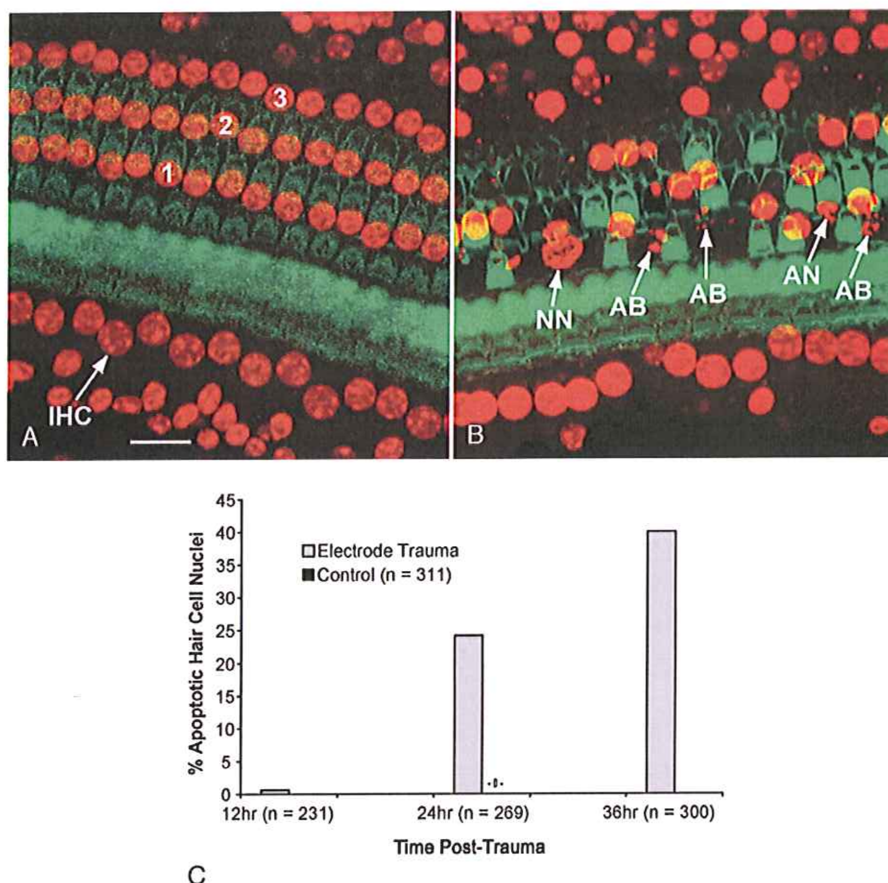

FIG. 3. Electrode insertion trauma initiates apoptotic changes in the nuclei of auditory hair cells in an area distal to the site of overt physical trauma. *A and B*, Confocal images (*Z*-series) from surface preparations of the lower middle turns of guinea pig cochleae stained with FITC-phalloidin/PI showing labeling of hair cell luminal surfaces (*green*) and the nuclei of the Corti's organ hair cells and support cells. *A*, A 36-hour posttrauma specimen from the contralateral untraumatized control cochlea showing four well-defined rows of hair cells with well-defined rows of intact healthy looking nuclei associated with the hair cells. *B*, A 36-hour posttrauma specimen from the traumatized cochlea showing disruption of all three rows of OHCs with associated apoptotic changes in almost all of the OHC nuclei. *C*, A graphic presentation of the counts of PI-stained hair cell nuclei with apoptotic changes in their staining pattern as seen in *panel B* presented as a percentage total HC nuclei counts for the electrode trauma lower middle turn specimens at 12, 24, and 36 hours posttrauma and a contralateral, unoperated cochlea at 24 hours posttrauma. AB, apoptotic bodies; AN, apoptotic nucleus; NN, necrotic nucleus.

study (Figs. 1 and 2) agrees with the pattern of hearing loss reported for a rat model of cochlear implantation trauma (12). The pattern of an initial loss and then a progressive loss of hearing is similar to that observed in both the guinea pig and the chinchilla cochleae after exposure to a damaging level of noise and with the progressive component of this noise trauma-induced hearing loss shown to involve the apoptosis of auditory hair cells (16,17). Noise exposure has also been shown to generate both oxidative stress and release of cytochrome-C from the mitochondria of noise-damaged hair cells within the auditory receptor (18) that leads to the accumulation of a toxic byproduct of membrane lipid peroxidation, that is, 4 hydroxyl 2,3-nonenal (HNE) (19). HNE has been demonstrated to be ototoxic to auditory sensory cells in vitro and to involve activation of the mitogen-activated protein kinase (MAPK)/JNK cell death signal cascade that leads to the death of the affected sensory cells (12,20). A novel inhibitor of the MAPK/JNK cell death signal cascade was developed to treat diabetes (21). When this novel blocking peptide that targets and competitively binds JNK molecules (i.e., JNK1, JNK2, JNK3) was directly applied into the scala tympani of guinea pigs exposed to a damaging level of noise, it prevented the conversion of almost all of the temporary noise-related hearing loss into a permanent hearing deficit (14). Noise-induced hearing loss and the pattern of hearing loss that results from electrode insertion trauma (12) have many similarities in that both are the product of a physical insult and both seem to have apoptotic loss of auditory hair cells as a mechanism for progressive loss of hearing after the initial trauma ((17); Fig. 3). Therefore, it is not surprising that the results reported in association with the current study (Figs. 1–3) are quite similar to the results obtained from the sound

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trauma experiments that blocked the MAPK/JNK signal cascade (14,22) in that the application of D-JNKI-1 immediately after electrode insertion trauma prevents almost the entire progressive component of the trauma-associated hearing loss (see Fig. 2). This is in agreement with the preliminary result of the FITC-phalloidin/PI labeling (Fig. 3) that demonstrates apoptotic changes in hair cell nuclei of the middle turn Corti's organ surface preparation at a site that is distal to the initial site of electrode insertion trauma much as the presence of apoptosis of hair cells has been demonstrated in the cochlea of sound trauma-damaged guinea pigs (16,23). The preliminary data presented in Figure 3 are in agreement with a very carefully done study that examined the frequency of both apoptosis and necrosis of OHCs after exposure of chinchillas to several different levels of continuous noise trauma (23). Studies are in progress to dissect this process at a molecular level, such as the sequential activation of caspases that have been demonstrated to occur in sound trauma-damaged cochleae (18,24).

It is not yet clear that a normal-hearing animal can serve as a model of residual hearing for cochlear implantation. Electrode insertion trauma in a normal-hearing animal is not the same as implanting a patient with a profound hearing loss with only audible residual hearing present in the low-frequency region of the cochlea (6–8); however, it is our experience that an electrode insertion trauma-lesioned normal-hearing animal provides a more consistent and reproducible lesion and pattern of both initial and progressive hearing loss (12) than does an ototoxin-damaged cochlea subjected to electrode insertion trauma because of the large variability in the levels of hearing loss in animals given equivalent amounts of an aminoglycoside antibiotic (Van De Water and Eshraghi, unpublished data). Therefore, the use of a normal-hearing animal in our studies allows us to study the effect of electrode insertion trauma and otoprotective drugs on auditory hair cells damaged by our electrode insertion paradigm (12) that have not experience previous oxidative stress damage such as occurs with exposure to a toxic level of an aminoglycoside antibiotic (25).

The functions of the JNKs under physiological conditions are diverse and not completely understood (e.g., mitosis, cell survival, motility, DNA repair); however, under pathological conditions, there is also increasing evidence that JNK molecules are potent effectors of apoptosis of oxidative stress-damaged cells in both the brain and the mammalian inner ear after trauma and/or exposure to a toxic substance (13). The molecule used in this study that has proven to be so effective in preventing electrode trauma-induced hearing loss has its action by competitively binding the JNK molecules, thereby preventing the interaction of activated JNK molecules with their downstream targets, for example, c-Jun, N-FAT, ELK-1, and bcl-2 family of anti- and proapoptotic molecules (see Fig. 4). Because it uses physical binding and not a broad chemical action, the specificity of this molecule is high and therefore should limit the occurrence of any unwanted side effects. The mode of action of D-

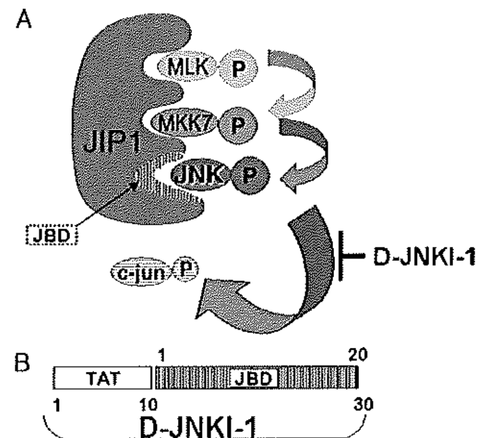

FIG. 4. Illustrations representing (*A*) the action of D-JNKI-1 peptide in binding-activated JNK molecules and (*B*) construction of the D-JNKI-1 peptide. P, phosphorylated site; JIP1, JNK interactive peptide; MLK, mixed lineage kinase; MKK7, mitogen-activated kinase kinase 7; JNK, c-Jun N-terminal kinase; c-Jun, c-Jun protein; TAT, a transport sequence from the HIV virus; JBD, c-Jun N-terminal kinase binding domain from the JIP1 molecule.

JNKI-1 peptide needs to be further defined, such as its ability to block the apoptosis of auditory hair cells that is initiated by electrode insertion trauma (Fig. 3) and the transcription of apoptosis regulatory genes such as *harakiri* (26). The possibility of delivering D-JNKI-1 peptide via the RMW route of delivery (27) also needs to be explored because if this route of direct delivery to the inner ear is possible, it would greatly expand the application of this high effective molecule to many other otological diseases that involve the apoptosis of inner ear sensory cells.

CONCLUSION

1. Hearing loss caused by cochlear implant electrode insertion trauma in guinea pigs has both an acute component (direct trauma) and a delayed component that develops during the period of at least a week after the initial trauma.
2. The delayed, progressive component of electrode insertion trauma-initiated hearing loss can be prevented by treating the cochlea immediately after electrode insertion with a peptide inhibitor of the JNK cell death pathway, that is, D-JNKI-1. This protection of hearing persists after the perfusion has been stopped for at least a period of 1 week.

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