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TITLE: Preventing Ototoxic Synergy of Prior Noise Trauma during Aminoglycoside Therapy

PRINCIPAL INVESTIGATOR: Hongzhe Li, PhD

RECIPIENT: Loma Linda Veterans Association for Research and Education
Attn: Dr. Colin D. Rasmussen
25884 Business Center Drive, Suite A
Redlands, CA 92374-4516

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Hongzhe Li, PhD

E-Mail: Hongzhe.Li@va.gov

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VA Loma Linda Healthcare
System, Research (151)
11201 Benton Street
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14. ABSTRACT

We successfully identified two molecular targets among a handful candidates that may contribute to an escalated inner ear ototoxicity. One is the Duffy antigen receptor for chemokines (Darc), and the other is the transient receptor potential vanilloid 1 (TrpV1). Both receptors actively participate in the process of cochlear inflammation, a condition resulting from exposure to moderate and intense noise stimulation. During this reporting period, we continued research activities using electrophysiology, immunohistochemistry, and cochlear perfusion techniques to conduct proposed experiments. The effect of inflammation on drug- or noise-induced ototoxicity was further investigated, using the application of LPS or aminoglycosides. Acquired data were analyzed thoroughly and reported at conferences and prepared in manuscripts for peer-reviewed publication.

15. SUBJECT TERMS
Noise trauma, combat injury, otoprotection, aminoglycoside antibiotic, gentamicin, loop diuretic, furosemide, bacterial infection, LPS, ototoxicity, auditory function, hearing loss

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[SF298] Project Abstract

Background: Exposure to loud sounds causes temporary or permanent threshold shifts in auditory perception, with reversible or irreversible cellular damage in the cochlea. Noise trauma, or loud sound exposure, is particularly associated with military environments, especially when sustaining blast injuries. These injuries are frequently treated with aminoglycoside antibiotics that have broad-spectrum bactericidal activity for treating or preventing life-threatening infections. However, aminoglycosides are also toxic to the cochlea, leading to hearing loss and further degradation from pre-injury status. The combination of both prior noise trauma and aminoglycoside treatment can degrade auditory function greater than simple summation of the two insults. We have found that prior sound exposure enhances cochlear uptake of aminoglycosides, providing a mechanistic basis for the observed ototoxic synergy due to noise trauma and subsequent aminoglycoside treatment.

Objective/Hypothesis: The ultimate goal of this research is to prevent aminoglycoside-induced cochleotoxicity (as well as vestibulotoxicity and nephrotoxicity) that can severely debilitate the recovery of military personnel, including combatants and associated casualties to pre-injury effectiveness. In this proposal, we hypothesize that ***prior noise trauma induces synergistic ototoxicity with systemically-administered aminoglycosides by potentiating cochlear uptake of the drug.*** We also hypothesize that specific aminoglycoside-permeant cation channels directly facilitate noise trauma-enhanced uptake of aminoglycosides in the cochlea.

Specific Aims:

Aim 1: Determine the acoustic parameters that induce noise-enhanced aminoglycoside uptake in auditory sensory hair cells.

Aim 2: Determine if prior noise trauma modifies intra-cochlear trafficking of aminoglycosides.

Aim 3: Determine if aminoglycoside-permeant channels on the hair cell apical membrane contribute to aminoglycoside uptake by cochlear hair cells.

Aim 4: Determine if TRP channels on the basolateral membrane of cochlear hair cells also contribute to aminoglycoside uptake.

Study Design: In Aims 1, 3a and 4a, C57BL/6 mice, genetically-modified mice and Dunkin-Hartley guinea pigs will receive noise exposure followed by systemic aminoglycoside administration to determine the minimum and optimal acoustic paradigms that enhance hair cell uptake of aminoglycosides. Cochlear tissues will be examined by whole-mount preparation and confocal microscopy. In Aim 2 and 4b, cochlear perfusion will be performed with aminoglycosides administered either systemically or locally by scala tympani perfusion. In Aim 3b, noise-exposed organ of Corti will be prepared for scanning electron microscopy to correlate tip-link survival and drug uptake compared to control animals. In Aim 3c, cochlear explants will be prepared for MET blockade to determine if hair cells can take up aminoglycosides via TRPV4 and $P2X_2$ channels.

Relevance: Eliminating ototoxic synergy is not possible when prior loud or traumatic noise exposure is followed by treatment with aminoglycosides for blast, burns or penetrative injuries. The proposed research will test specific mechanisms to determine how noise trauma enhances aminoglycoside entry into cochlear hair cells to induce synergistic ototoxicity. This knowledge will enable the development of countermeasures to preserve auditory function during sequential and synergistic ototoxic insults in military environments.

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1. INTRODUCTION

Exposure to loud sounds causes temporary or permanent threshold shifts in auditory perception, with reversible or irreversible cellular damage in the cochlea. Noise trauma, or loud sound exposure, is particularly associated with military environments, especially when sustaining blast injuries. These injuries are frequently treated with aminoglycoside antibiotics that have broad-spectrum bactericidal activity for treating or preventing life-threatening infections. However, aminoglycosides are also toxic to the cochlea, leading to hearing loss and further degradation from pre-injury status. The combination of both prior noise trauma and aminoglycoside treatment can degrade auditory function greater than simple summation of the two insults. We have found that prior sound exposure enhances cochlear uptake of aminoglycosides, providing a mechanistic basis for the observed ototoxic synergy due to noise trauma and subsequent aminoglycoside treatment.

In the mammalian inner ear – the cochlea, the auditory sensory cells, particularly outer hair cells (OHCs), are more susceptible to aminoglycoside-induced cytotoxicity than other cochlear cells, particularly at the base of the cochlea most sensitive to higher frequency sound. Once these OHCs are lost, these sensory cells cannot be endogenously regenerated, leading to life-long hearing loss and deafness. Thus, extensive efforts are underway to ameliorate and prevent aminoglycoside-induced hair cell death. Under normal physiological condition, aminoglycosides can rapidly cross the blood-labyrinth barrier (BLB) into the cochlear tissues and fluids and enter OHCs through a number of conduits. The best-characterized conduit is permeation through the mechanoelectrical transduction (MET) channel. The MET channel is mechanically-gated by the extracellular, heterodimeric tip links between two stereocilia. Other mechanisms by which aminoglycosides can enter hair cells include endocytosis, and/or other aminoglycoside cation channels (*e.g.* TRP channels) expressed by hair cells besides the MET channel, such as TRPV4 on the apical membranes, or TRPA1 on the basolateral membranes, of OHCs.

The ultimate goal of this research is to prevent aminoglycoside-induced cochleotoxicity (as well as vestibulotoxicity and nephrotoxicity) that can severely debilitate the recovery of military personnel, including combatants and associated casualties to pre-injury effectiveness. In this project, we hypothesize that prior noise trauma induces synergistic ototoxicity with systemically-administered aminoglycosides by potentiating cochlear uptake of the drug. We also hypothesize that specific aminoglycoside-permeant cation channels directly facilitate noise trauma-enhanced uptake of aminoglycosides in the cochlea.

2. KEYWORDS

Noise trauma, combat injury, otoprotection, aminoglycoside antibiotic, bacterial infection, ototoxicity, auditory function, hearing loss

3. OVERALL PROJECT SUMMARY

What were the major goals of the project?

Aim 1: Determine the acoustic parameters that induce noise-enhanced aminoglycoside uptake in auditory sensory hair cells.

This is completed at OHSU.

Aim 2: Determine if prior noise trauma modifies intra-cochlear trafficking of aminoglycosides.

Aim 2a: Use cochlear perfusion techniques to determine the contribution of endolymph or perilymph trafficking of aminoglycosides to hair cells with prior noise exposure. GTTR will be administrated either systemically or by scala tympani infusion to the animal.

This is completed at OHSU.

Aim 3: Determine if aminoglycoside-permeant channels on the hair cell apical membrane contribute to aminoglycoside uptake by cochlear hair cells.

Aim 3a: Determine if prior noise trauma enhances drug uptake in hair cells.

This is completed at Loma Linda.

Aim 3b: Examine tip-link integrity in noise-exposed rodents by scanning electron microscopy.

This is completed at Loma Linda.

Aim 3c: Confirm that hair cell $P2X_2$ and TRPV4 channels are aminoglycoside-permeant.

This is partially completed at Loma Linda.

Aim 4: Determine if TRP channels on the basolateral membrane of cochlear hair cells also contribute to aminoglycoside uptake.

Aim 4a: Determine if noise enhances drug uptake in TrpA1 or TrpV1 mice.

This is partially completed at Loma Linda.

Aim 4b: Verify that scala tympani perfusion of TRPA1 or TRPV1 antagonists in noise-exposed guinea pigs inhibit noise-enhanced uptake of GTTR.

This is ongoing at Loma Linda.

What was accomplished under these goals?

1) Major activities

During this reporting period, we continued research activities using electrophysiology, immunohistochemistry, and cochlear perfusion techniques to conduct proposed experiments in this project. New members joined the lab and some senior lab members left. Trainings on essential techniques and protocols were provided upon the transitions. The effect of inflammation on drug- or noise-induced ototoxicity was further investigated, using the application of LPS or aminoglycosides. Acquired data were analyzed thoroughly and reported at conferences and prepared in manuscripts for peer-reviewed publication.

2) Specific objectives

a) We continued to host the mouse cohorts which are essential materials for the project. Major colonies include *TrpV1* mice (#3770), *Darc* mice and wildtype *C57BL/6* mice (#0664) in the animal facility (VMU) at Loma Linda VA Healthcare System. For mice that are available from accredited vendors (*e.g.* Jackson Laboratory, Bar Harbor, ME), breeding pairs were periodically purchased to keep the cohorts genetically consistent with the original source.

b) We investigated the effect of intratympanic LPS on cochlear uptake of ototoxic aminoglycosides, in both wildtype mice and in *TrpV1* mice. We studied the correlation between modulated drug uptake and inflammatory events, including the morphology and infiltration of cochlear macrophages and the variation of immune active cytokines' variation.

c) We continued to monitor a small *TrpV1* mouse cohort (as well as a *Darc* mouse cohort) as they are getting older, to reveal the effect of the mutant allele on the systemic aging process.

d) We investigated the dose-dependent effect of gentamicin and furosemide in combination on producing cochlear damage, by 1) characterizing the residual hearing function, 2) distinguishing the morphology of outer and inner hair cells, and ribbon synapses at various cochlear locations, and 3) determination of the synaptic variation in number, and in morphological distribution.

4. KEY RESEARCH ACCOMPLISHMENTS

a) Combining loop diuretics to aminoglycoside treatment is an effective approach for accelerating ototoxic damage, especially, in mice, which results in immediate cochlear trauma (Hirose and Sato, 2011; Taylor *et al.*, 2008). In this simplified version of a mouse ototoxicity model, the addition of loop diuretics, such as furosemide, disrupts the blood labyrinth barrier thereby greatly elevating the concentration of aminoglycosides, such as gentamicin, in the cochlea (Ding *et al.*, 2016; Taylor *et al.*, 2008). Furosemide, by itself, is not considered directly damaging to the inner-ear components including HCs, ribbon synapses or SGNs (Rybak, 1993). In the present study, the dose-dependent effect of gentamicin and furosemide (G/F) in combination was investigated to: 1) characterize the residual hearing function, 2) distinguish the morphology of OHCs and IHCs, and ribbon synapses at various cochlear locations, and 3) determine whether there is a change in the number, or the distribution, of synaptic ribbons.

Seven days after the one-time G/F treatment, synaptic ribbons that were identified by the anti-CtBP2 labeling exhibited various degrees of morphological change. On the *xy* plane at the cochlear location equivalent to 12 kHz (Müller *et al.*, 2005), the ribbon distribution appeared normal for some low-dose G/F treatments, demonstrated by a vacant zone basally located to the IHC nuclei, without a reduction in the number of ribbons, *i.e.*, ribbon density, as illustrated by the micrographs of Figs. 1A1-A4. Among them, Figs. 1A2-A4 are the confocal images reconstructed in the *yz* plane from three adjacent IHCs. Similar to our previous findings in healthy control cochleae, the size of ribbons near the nuclei was relatively small, while those located at the IHC basal pole were larger (Edderkaoui *et al.*, 2018). In addition, it is noteworthy that with the mounting technique used, the IHCs from the apex and the middle coils of the cochlea generally orientate in parallel to the cover slide, which allowed a convenient quantifying of the distance of individual ribbons to the nucleus along the y axis, *i.e.*, the main axis of the IHC. Thus, a shape of normal distribution along the main axis was typically anticipated, as indicated in Fig. 1A5. Yet, under most G/F treatment conditions, a certain distribution disorder was observed, for example, in Figs. 1B1-B5, manifested by some ribbons being shifted toward the IHC nucleus without a reduction in the number of ribbons. In addition, the "nucleus-ward or upward" shift appeared predominantly toward the pillar side of the IHC as shown on the left side of Figs. 1B2 and 1B4. The axial gradient based on the ribbon size was also disturbed. In the extreme ototoxic scenario, the ribbon numbers did decline after some high-dose G/F treatments, with extensive upward shift of the ribbons as illustrated in Fig. 1C2, and enlarged ribbons in many cases as demonstrated in Fig. 1C1.

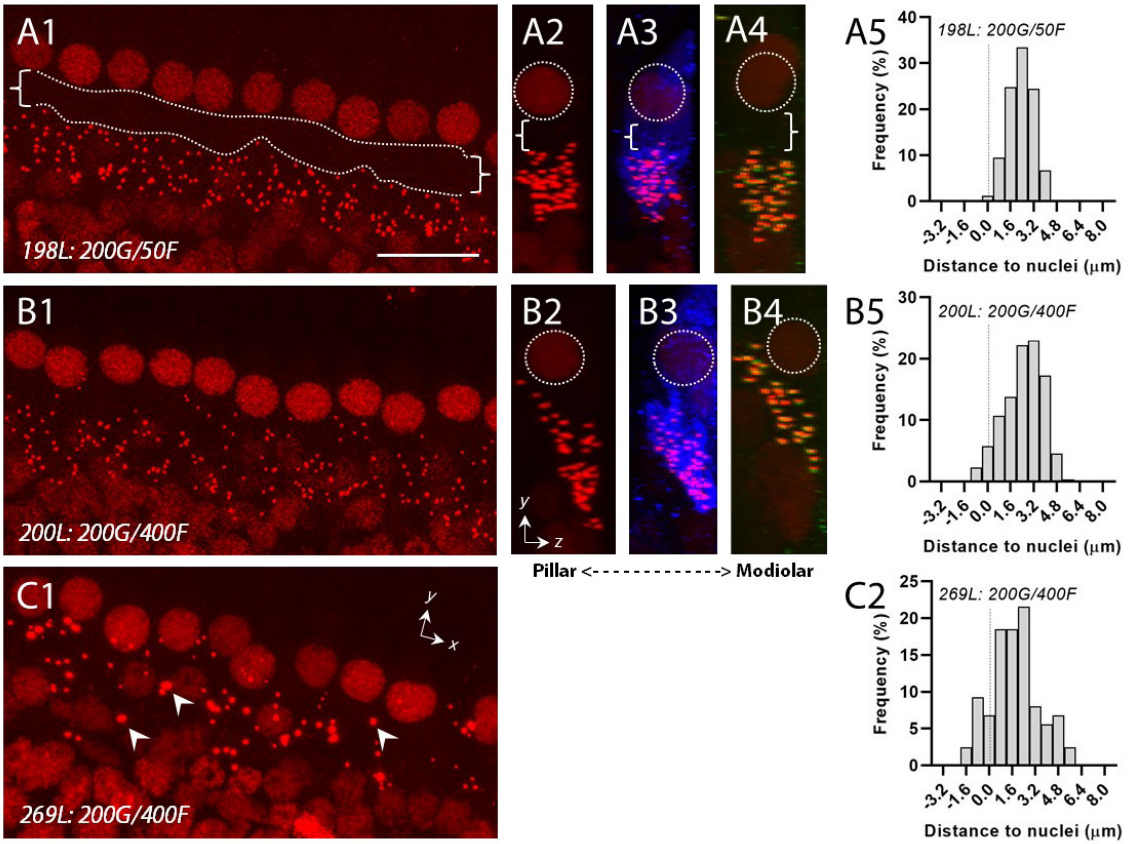

Figure 1. Alteration of synaptic ribbons after G/F treatment. A: In the apical and middle cochlear locations free of OHC damage, low-dose G/F treatment might not induce any visible change in CtBP2-labeled synaptic ribbons (red puncta), showing a characteristic vacant zone depicted by brackets and dotted lines basal to the IHC nucleus in the xy plane (A1). Error bar=20 μm. Reconstructed images in the yz plane from three adjacent IHCs exhibited the distribution of ribbons along the pillar-modiolar axis (A2), with identification of the cytoplasm of the IHC by anti-Myo7a immunolabeling (A3, blue), and paired postsynaptic AMPA receptors by anti-GluR2 immunolabeling (A4, green). A semi-normal distribution of the ribbon along the main cell axis was often observed (A5). B1-4: Seven days after G/F treatment, at cochlear locations free of OHC damage, a pillar-side upward shift of synaptic ribbons was often seen without a reduction in ribbon number. B5: ribbon distribution along the main cell axis was skewed to the nucleus. C1: With severe cochlear damage induced by G/F treatment, the ribbon density could be drastically reduced and many individual ribbons enlarged (arrowhead), and again, often exhibiting an upward shift (C2).

b) It is known that intensive noise exposure elevates cochlear immune activities, partly manifested by the increased number of tissue specific macrophages in the cochlea. Accordingly, we rationalized that the prior noise trauma-induced cochlear immune activity includes TRPV1 channel activation which subsequently enhances aminoglycoside ototoxicity. To test above hypothesis, we induced reliable cochlear inflammation by systemic endotoxemia. In these mice, we observed increased expression of TrpV1 in the cochlea and increased uptake of circulating aminoglycosides (GTTR as a tracer) by hair cells (Jiang, Li *et al.*, 2019).

Alternatively, we also induced reliable cochlear inflammation by topical lipopolysaccharide (LPS) treatment, and investigated cochlear macrophages by Iba1 labeling. In recent years, Iba1 antibody has been increasingly used in hearing research labs as a cell marker to identify tissue macrophages/microglia. After LPS inoculation, we observed increased number of Iba1+ cells in many cochlear locations, including the spiral ligament, the basilar membrane, the spiral limbus and the spiral lamina, but not in the stria vascularis (Chai *et al.*, 2020).

Thus, both noise and bacterial exposure induce cochlear inflammation, and subsequently enhance aminoglycoside ototoxicity. Here, we also question if aminoglycoside treatment itself activates cochlear immune cells, by using tandem intraperitoneal gentamicin and loop diuretic furosemide (G/F) to challenge the inner ear tissue. We did observe increased Iba1-positive activities along the basilar membrane, specially at the spiral laminar, 3-day and 7-day after the G/F treatment (Fig. 2). The Iba1+ activity appears more prominent on 3-day posttreatment compared to 7-day. Although higher gentamicin dose (400 mg) resulted in hair cell loss, but not necessarily more Iba1+ activity.

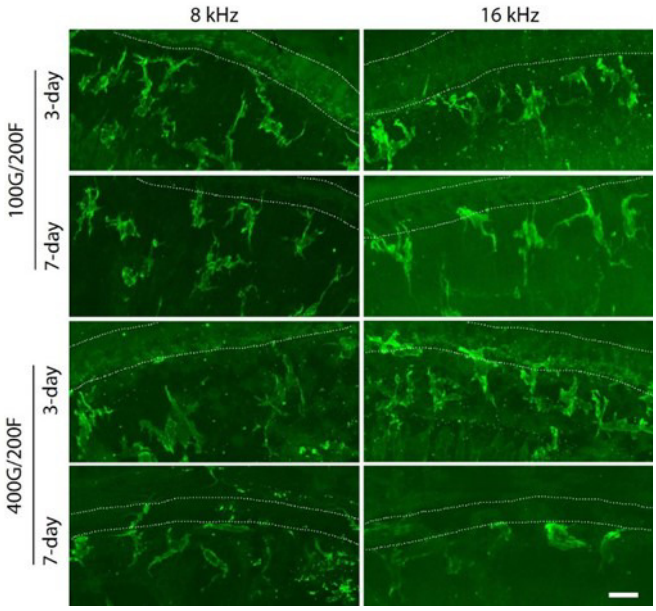

Figure 2. Induction of cochlear macrophages after combined treatment of gentamicin and furosemide. Three days or seven days after the combined treatment using 100 mg/kg gentamicin and 200 mg/kg furosemide (top panels), or 400 mg/kg and 200 mg/kg furosemide (bottom panels), macrophages or microglia cells were immunolabeled by anti-Iba antibodies with green fluorescence. Micrographs indicated elevated Iba+ activity at the cochlear locations equivalent to 8 and 16 kHz in the spiral laminar. Dotted lines depict the location of inner hair cells, and scale bar=20 μm.

c) We previously reported that Duffy antigen receptor for chemokines (DARC) is implicated in the peripheral auditory function, specifically in the context of inner ear damage and repair. Mice lacking DARC exhibited expediated recovery after noise exposure in terms of the duration and magnitude of ABR thresholds (Edderkaoui *et al.*, 2018). DARC is expressed on the membrane of erythrocytes in a selective human population, and also detectable in endothelial cells of postcapillary venules, as well as epithelial cells of kidney collecting ducts

and Purkinje cells in the cerebellum. Based upon our observation, DARC must exist in the inner ear, but their exact location and expression pattern is unknown. Here, using immunofluorescence technique, we observed DARC expression in the organ of Corti.

Using monoclonal DARC antibody (ABCam, 137044), fluorescence labeling and confocal imaging, we observed positive fluorescence signals (Fig. 3, red) in the cytoplasm of both outer and inner (asterisks) hair cells, as well as interdental cells (arrowheads). Most significantly, membrane bounding DARC was found at the basal pole of the outer hair cell (arrows). It has been postulated that DARC serves as a reservoir of its chemokine ligands including both selective C-C and C-X-C chemokines, we suspect that the presence of DARC in the organ of Corti slows down the dynamics of chemokines that typically come in waves after traumatic, inflammation-causing events, which results in prolonged but likely attenuated chemokine exposure in the inner ear.

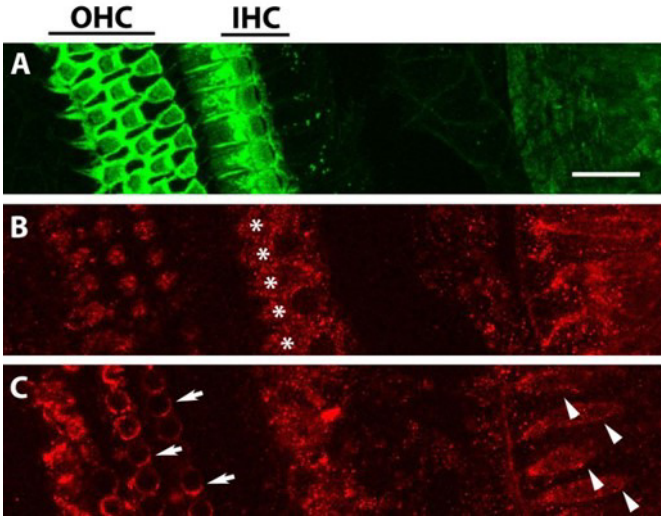

Figure 3. Darc expression in the cochlear hair cells. Phalloidin counter-labeled the cellular actin component, depicting the location of the cuticular plate of outer hair cells (OHC), inner hair cells (IHC) and portion of the pillar cell in between (A). Scale bar=20 μm. Cytoplasmic fluorescence signals can be seen at the neck region of OHCs (B) and membrane bounding signal at the basal pole of the OHC (C).

d) We performed cochlear perfusion experiments in mice. More specifically, the perfusate was sent through the round window membrane into the Scala tympani, and allowed to exit from an outlet hole opened on the tip of the cochlea (Li and Steyger 2011). Fluorescently conjugated gentamicin (GTTR) was dissolved in the perfusate here. This setting provides GTTR to the cellular structures in the basilar membrane. Due to the barrier created by the tight junctions lining the Scale media, GTTR was only exposed to the basolateral membrane of the outer hair cells, not the apical membrane where hair bundles and MET channels are located.

GTTR uptake in the organ of Corti by Scala tympani perfusion was deviant from the traditional experimental settings, where GTTR is acutely administrated with an intraperitoneal injection (Fig. 4). First, OHCs did present clear GTTR fluorescence throughout the cell body, including the nucleus (asterisks). Second, the phalangeal process

of the Deiter's cell also presented intense fluorescence (arrows). Third, GTTR signal was drastically lower in inner hair cells, while overt GTTR uptake was seen in the terminals (bracket) of auditory nerve fibers. In sum, these observations provided a foundation to study the mechanism of gentamicin uptake by hair cells through basolaterally located conduits.

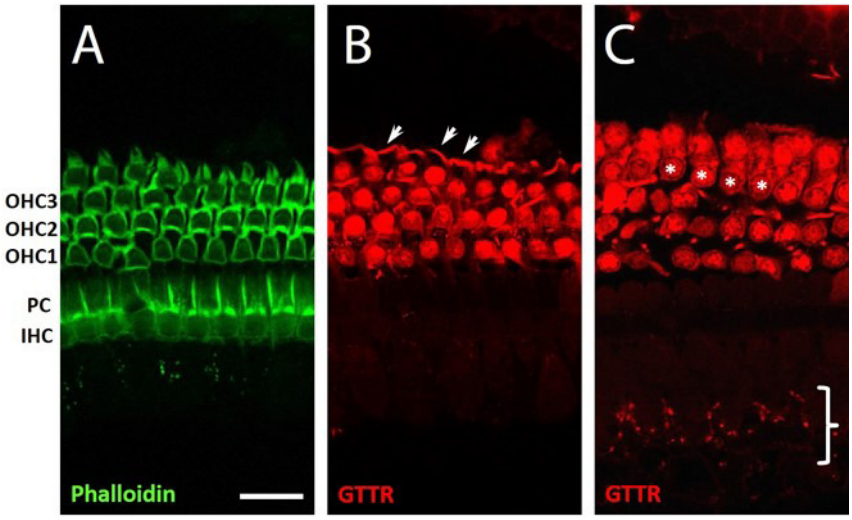

Figure 4. GTTR uptake in the organ of Corti by Scala tympani perfusion. A. Phalloidin labeling depicts the cellular structure of the organ of Corti. Scale bar=20 µm. B. GTTR fluorescence in the outer hair cell and the phalangeal process of the Deiter's cell (arrows). C. GTTR fluorescence in the nucleus of hair cells (asterisks) and the terminals of the auditory nerve fibers (bracket).

5. CONCLUSION

Cochlear inflammation has gradually becoming a major research topic in recent years in auditory neuroscience. It is first known that intensive noise exposure elevates cochlear immune activities, manifested by increased number of tissue specific macrophages and elevated cytokine levels in the cochlea. In addition, we recently found that aminoglycosides also trigger cochlear immune response without other extrinsic inflammatory triggers. This finding raised the clinical significance of this line of research, given that aminoglycoside antibiotics are often prescribed to patients with systemic or local infection to suppress a potential or ongoing bacterial presence. The exact role of these cochlear macrophages and their elevated activities is not totally clear. It is known that the function of tissue macrophages is multifaced. We studied the event sequence upon aminoglycoside-induced cochlear damage, including hair cell loss, synaptic damage and macrophage behavior. The recruitment or proliferation of cochlear macrophages appeared as a prelude to hair cell loss, and definitely occurred ahead of synaptic damage. These findings suggest that cochlear macrophages, at least the ones located in the spiral laminar, likely underpin a protective mechanism to the neuronal elements in the cochlea that is essential for a reliable signal transmission, and certainly warrant further investigation.

Over the project period of XW81XWH-14-1-0006, we have successfully identified two

molecular targets among a handful candidates that may contribute to an escalated inner ear ototoxicity. One is the Duffy antigen receptor for chemokines (Darc), and the other is the transient receptor potential vanilloid 1 (TrpV1). Understanding their presence in the untreated cochlea and modulation in the inflammatory cochlea are essential in the development of countermeasures to prevent cochlear damage by variety of insults, including but not limited to acoustic overexposure and drug-induced ototoxicity.

6. PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Peer reviewed publication

Liana Sargsyan, Alisa P. Hetrick, Marjorie R. Leek, Glen K. Martin, Hongzhe Li (Submitted), Effects of combined gentamicin and furosemide treatment on cochlear ribbon synapses.

Conference abstracts, papers and podium presentations

Liana Sargsyan, Alisa Hetrick, Yongchuan Chai, Hongzhe Li (2019), "The Response of Cochlear Microglia-Like Cells to Intratympanic LPS in Different Wildtype Strains", SoCal Hearing Research Conference, UC Irvine, CA.

Hongzhe Li, Liana Sargsyan, Alisa Hetrick, Glen Martin (2019), "Alternation of Synaptic-Ribbon Distribution after a Single-Dose Ototoxic Injury", The 2019 NCRAR Conference, Ototoxicity and Noise Damage: Translating Preclinical Findings to Audiological Management, Portland, OR.

Yongchuan Chai, Alisa Hetrick, Liana Sargsyan, Weiwei He, Timothy Jung, Hao Wu, Hongzhe Li (2020), "Intratympanic Lipopolysaccharide Induces Inflammatory Responses and Elevates Systemic Gentamicin Uptake in the Cochlea", 43rd Midwinter Research Meeting in Otolaryngology, San Jose, CA.

Liana Sargsyan, Alisa Hetrick, Yongchuan Chai, Hongzhe Li (2020), "The Response of Cochlear Microglia-Like Cells to Ototoxic Challenge in Different Strains of Wildtype Mice", 43rd Midwinter Research Meeting in Otolaryngology, San Jose, CA.

7. INVENTIONS, PATENTS AND LICENSES

Nothing to report.

8. REPORTABLE OUTCOMES

What opportunities for training and professional development has the project provided?

This research project provided opportunities for people with interest and motivation in biomedical research, including college students and international physicians. Liana Sargsyan, a European trained physician, and Weiqiang Yang, a full feathered doctor from Shenzhen, are both specialized in otology and worked in the PI's lab during the reporting period. They have both contributed to the project, accrued hands-on experience in cochlear dissection, and image acquisition *etc*. This experience will certainly provide positive impact on their upcoming career advancement.

What individuals have worked on the project?

Name: Hongzhe Li, PhD
Project Role: PI
Nearest person month worked: 6.0
Contribution to Project: Dr. Li has performed work in experimental design, staff training, tissue harvest and processing, confocal imaging, image acquisition and quantification, data analysis, documents, protocols, reports and manuscript preparation.

Name: Alisa Hetrick, BSc
Project Role: Research Technician
Nearest person month worked: 3.0
Contribution to Project: Ms. Hetrick has performed work in ABR and DPOAE recordings, noise and aminoglycoside exposures, and managed mouse cohort with genotyping procedures. She also assisted in acquiring lab equipment and consumables, and protocol development.

Name: Liana Sargsyan, MSc
Project Role: Research Associate
Nearest person month worked: 12.0
Contribution to Project: Ms. Sargsyan has performed work in animal treatment with aminoglycosides, cochlear microdissection, confocal microscopy, data analysis, and conference presentation, and manuscript preparation.

Name: Yongchuan Chai, MD, PhD
Project Role: Research Associate
Nearest person month worked: 3.0

Contribution to Project: Dr. Chai has performed work of intratympanic injections in mice, as well as cochlear microdissection, immunohistochemistry, confocal microscopy, data analysis and manuscript preparation.

Name: Weiqiang Yang, MD
Project Role: Research Associate
Nearest person month worked: 7.0
Contribution to Project: Dr. Yang has performed work of cochlear perfusion and intratympanic injections in mice, as well as cochlear microdissection, immunohistochemistry, confocal microscopy and data analysis.

How were the results disseminated to communities of interest?

Part of the content in this report has been presented at the following conferences

- SoCal Hearing Research Conference (2019), UC Irvine.
- NCRAR Conference (2019), Ototoxicity and Noise Damage: Translating Preclinical Findings to Audiological Management, Portland, OR.
- Midwinter meeting of Association for Research in Otolaryngology (2020), San Jose, CA.

9. OTHER ACHIEVEMENTS

a) Other general lab activities included personal recruitment and lab orientation, lab safety and compliance training, equipment acquisition and setup, and protocol selection or development *etc*.

b) During the last quarter of this annual reporting period, we did experience delays due to the Covid-19 pandemic. The Governor of the State of California issued an order in mid-March asking all non-essential worker to remain at home and practice social distancing. In seeking to comply with the order, my laboratory staff had been confined to essential duties such as animal care with reduced mouse cohorts, and the daily routine of conducting experiments had been significantly delayed. We took these precautions very seriously as my laboratory is located in a Veterans Affairs Medical Center, and so the immediate concern is for the welfare of the patient population. In response to the circumstance, the PI and senior lab members switched their focus onto data analysis and manuscript preparation.

10. REFERENCES

Not applicable.

11. APPENDICES

None.