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14. ABSTRACT

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). Both receptors actively participate in the process of cochlear inflammation, a condition resulting from exposure to moderate and intense noise stimulation. If the pathophysiological condition is quickly resolved, cochlear inflammation does not necessarily result in functional damage. However, the cochlea's permeability to circulating substances, especially, ototoxic aminoglycoside medications, is increased during an inflammatory episode, which can escalate the degree of ototoxic damage. Overall, we are getting very close to the goal of this project, to search countermeasures to prevent aminoglycoside-induced cochleotoxicity (and vestibulotoxicity) that can severely debilitate the recovery of military personnel, as well as civilians received aminoglycoside therapy with a history of acoustic insult.

15. SUBJECT TERMS

Noise trauma, combat injury, otoprotection, aminoglycoside antibiotic, bacterial infection, 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.

Table of Contents

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, by using mouse models with MET apparatus defects, including *Pcdh15*$^{3J/3J}$ (Ames waltzer) mice, *Myo7a*$^{8J/8J}$ (Shaker 1) mice; and *TrpV4*$^{-/-}$ and *P2X*$_2$$^{-/-}$ mice with channelopathies, compared to heterozygous littermates.

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. We will treat organ of Corti explants from *P2X*$_2$$^{-/-}$ and *TrpV4*$^{-/-}$ mice with aminoglycosides in presence and absence of curarine (to block MET channels), and compare its uptake from explants from littermate controls.

This is completed by the end of Year three at Loma Linda.

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

This is ongoing at Loma Linda.

What was accomplished under these goals?

1) Major activities

Due to lab relocation, the project was interrupted in *2015*, and resumed on *June 1st, 2016*, and re-budgeted. This is the third annual report after the relocation to VA Loma Linda Healthcare System, in Loma Linda, California. After resuming the project, we re-established mouse cohorts, acquired and calibrated instruments for auditory and physiological measurement in rodents. The PI's laboratory has been expanding with new members being recruited, trained in annual basis.

During this reporting period, we continued research activities using electrophysiology, tissue culture, and immunohistochemistry to conduct experiments proposed in this project. Beside the originally proposed molecular targets, such as TrpV1, we also identified a chemokine receptor, Darc, which elevates drug-induced ototoxicity in an inflammation-dependent fashion. The groundbreaking work is very relevant to the central aim of the present project. Research activities relating to Darc have been incorporated into the project and received regulatory approvals from the funding agency including ACURO at DoD.

2) Specific objectives

a) We continued to host mouse cohorts including *TrpV1* mice (#3770), *Darc* mice and wildtype *C57BL/6* mice (#0664) in the animal facility at Loma Linda. Breeding pairs were purchased from Jackson Laboratory (Bar Harbor, ME). We also initiated and worked with mouse strains including $P2X_2^{-/-}$ (#4603) and $TrpV4^{-/-}$ (#29582).

b) Using organotypic culture approach, we have evaluated aminoglycoside candidate channels, including TrpV4 and $P2X_2$ channels.

c) With noise exposure and intratympanic injection of LPS, we investigated the morphology and infiltration of cochlear macrophages. In addition, we studied LPS-induced cochlear uptake of ototoxic aminoglycosides, in the scope of inflammation-enhanced ototoxicity.

d) As our *TrpV1* mouse cohorts getting older, we tested the residual hearing in terms of age-related hearing loss in the background of *C57BL/6*, to increase our understanding of the immunomodulatory role of this multipotent cellular sensor.

4. KEY RESEARCH ACCOMPLISHMENTS

a) To fulfill Specific Aim 3 of the project, we first conducted *in vitro* experiments using cochlear explants from neonatal *C57BL/6* mice and started with pilot experiments by varying concentration of gentamicin-Texas Red (GTTR) and treatment temperature. Murine cochleae harvested from P3 was incubated with GTTR (0.5 μg/ml) for 2, 5, 10 and 20 min either at room temperature (~25°C) or in the incubator (37°C). GTTR enters sensory hair cells by 1) mechanoelectrical transduction (MET) channels, 2) endocytosis, and 3) candidate cation channels including $P2X_2$ and TrpV4 channels. Uptake by hair cells through MET channels are hypothetically less temperature-dependent compared to endocytosis. Our results indicated that GTTR uptake by hair cells was less dose-dependent at room temperature, and the uptake level was more equivalent with 5- or 10-min exposure time between room temperature and body temperature (37°C). We decided to select 10 min exposure time at 37°C to conduct future experiments.

b) Next, d-tubocurarine (DTC, MET blocker) at various concentration, including 30,100, 300, 600, and 1000 μM were added to successfully cultured cochlear explants. Highly effective blockade of MET channels, determined by negligible GTTR fluorescence signals, was observed at higher DTC concentration (600 and 1000 μM; Fig. 1).

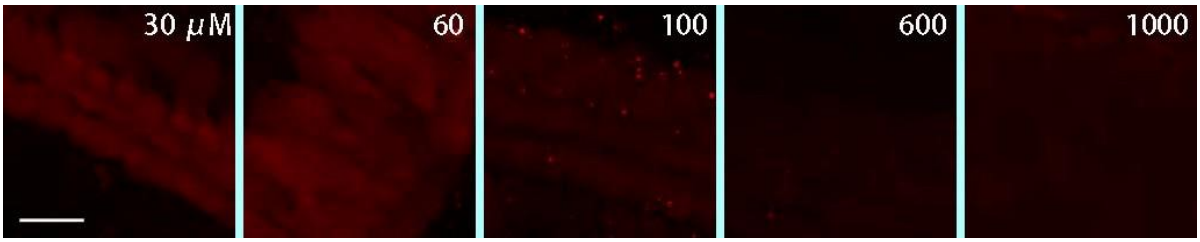

Figure 1. Uptake of fluorescence-tagged gentamicin by sensory hair cells is effectively blocked by d-tubocurarine in a dose-dependent manner, in organotypic cultures. *Red signals depict GTTR fluorescence indicating its uptake by hair cells, and scale bar = 20 μm.*

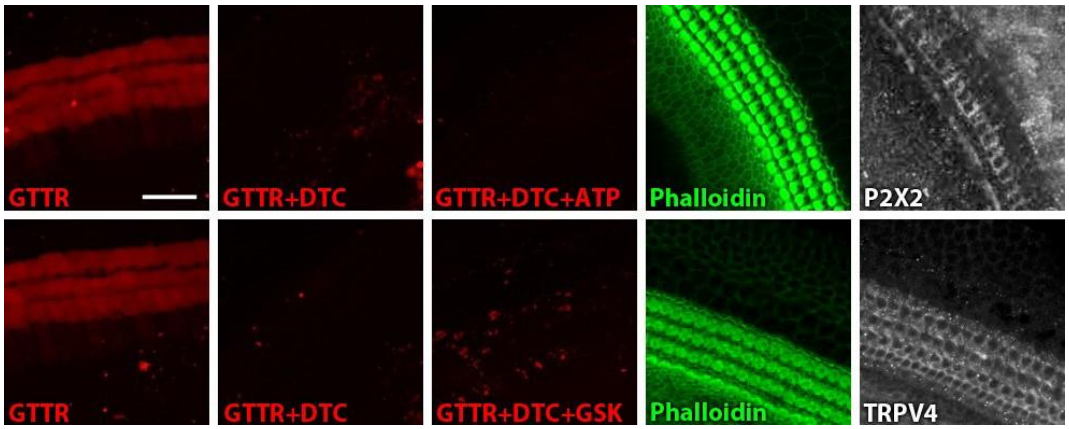

Figure 2. Uptake of fluorescence-tagged gentamicin by sensory hair cells is unaltered with the application of $P2X_2$ (top row) or TRPV4 activator (bottom row), under the treatment of MET blocker d-tubocurarine, in organotypic cultures*, and scale bar = 20 μm. Right panels indicate the immunolocalization of $P2X_2$ (top) or TRPV4 (bottom).*

c) We continued *in vitro* organotypic experiments using cochlear explants. Here, cochleae harvested from P3 *C57BL/6* mice were incubated with GTTR (0.5 µg/ml) for 10 min in the incubator (37°C) i) alone, ii) with MET channel blocker DTC, or iii) with both DTC and activator of aminoglycoside candidate channel of interest. When DTC was used to complete blockade of MET channels, it was added to the culture 5 min prior to the application of GTTR with or without the activator ATP (10-600 µM) or GSK1016790A (10-200 nM), which is the agonist of $P2X_2$ or TrpV4 respectively. The experiment was conducted with DTC at 600 or 1000 µM, at which MET-dependent GTTR uptake by hair cells was effectively blocked (Fig. 1). Unfortunately, neither activator demonstrated channel specific GTTR uptake. We suspect the lack of activator-induced GTTR uptake could be due to 1) non-specific inhibitory effect of DTC on $P2X_2$ or TrpV4, 2) minimum activator effect that is failed to reach system sensitivity, and/or 3) organotypic cultural environment does not properly represent the *in vivo* condition in adult animals.

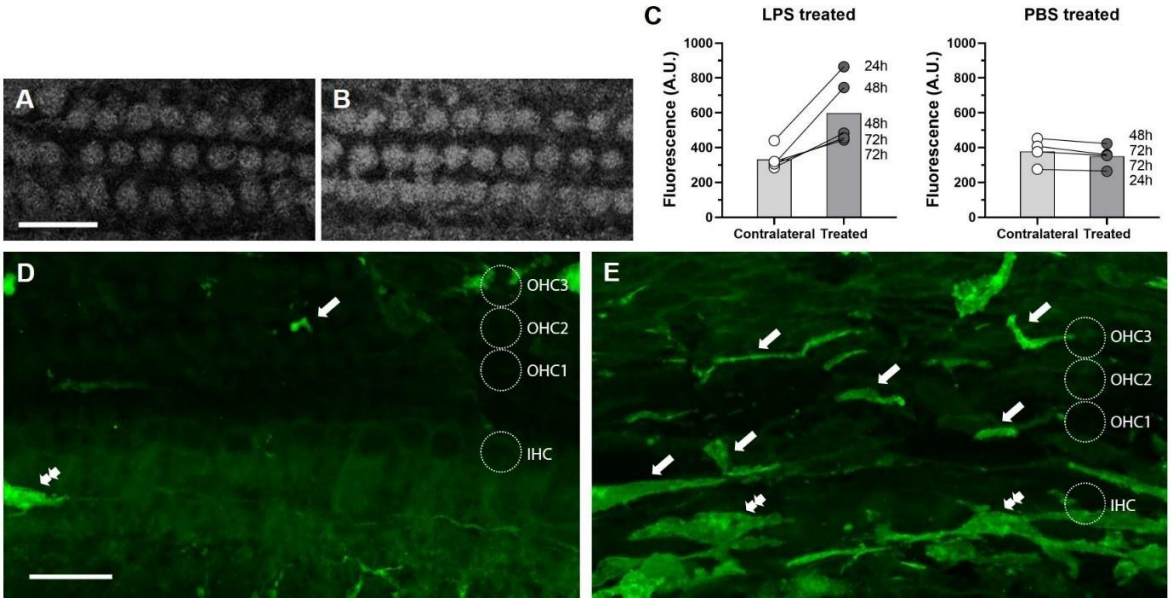

Figure 3. Intratympanic injection of LPS enhanced hair cell uptake of systemically administered GTTR and macrophage infiltration to the cochlea's basilar membrane. ***A****: Baseline uptake of GTTR (2 mg/kg bw, ip, at 1 h) by OHCs in the contralateral ear of an LPS treated subject.* ***B****: Elevated GTTR fluorescence in the OHCs from the LPS-treated ear 48 hours after the treatment (it, ~10 µl, 1 mg/ml). Scale bar = 20 µm.* ***C****: LPS treatment enhanced OHC uptake of GTTR in the middle cochlear coil of the treated ears compared to the untreated contralateral ears from all tested mice (n=5, p=0.017, paired t-test), while PBS treatment did not (n=4). Systemic GTTR was administered to the mice at 24, 48 and 72 hours after the LPS/PBS treatment. The effect of the time point of post-LPS/PBS treatment was not evaluated, given the limited animal number per time point. A.U. = arbitrary unit. LPS batches were not identical among tested animals, nor the laser power for confocal imaging to quantify GTTR fluorescence, thus, the fluorescent values were not intended to be compared within each group.* ***D****: Anti-Iba1 labeling revealed a limited number of macrophages along the basilar membrane (BM) in the contralateral ear of it-LPS treatment. Scale bar=20 µm.* ***E****: Increased number of anti-Iba1-identified macrophages were recruited to the BM. These macrophages were either located below OHCs and Deiter's cells (arrow), or located in the osseous spiral lamina, basolateral to inner hair cells (IHCs, double arrows), and appeared in the activated form that lacks fine processes and orientated along the BM.*

d) To examine if $P2X_2$ or TrpV4 channels are expressed in P3 cochleae, we performed immunolabeling with correspondent antibodies. We did observe positive immunosignals for both channel proteins (Fig. 2, rightmost panels). The signals were primarily immuno-localized in the supporting cells, though we could not rule out membranous immune-localization in the hair cells, especially for TRPV4. Also note that the lateral membrane of the immature hair cells was not directly bathed in the Corti-lymph/perilymph, instead, the cell body is wrapped by supporting cells, mainly Deiters' cells. This configuration may block quick GTTR access (and activator access) to the candidate channel even if they are properly located and functional.

e) It is known that intensive noise exposure elevates cochlear immune activities, manifested by increased number of tissue specific macrophages in the cochlea. Thus, 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 Trp1 in the cochlea and increased uptake of circulating aminoglycosides (GTTR as tracer) by hair cells. 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 did observe increased number of Iba1+ cells in many cochlear locations, including the spiral ligament, the basilar membrane (Fig. 3E), the spiral limbus and the spiral lamina, but not in the stria vascularis (SV; Fig. 4).

f) The Iba1+ macrophages in the SV are considered perivascular macrophages, essential for the integraty of the blood labyrinth barrier. In order to understand their behavior after prior noise exposure, we studied Iba1+ labeling in the SV after 2-hour intensive OBN expsoure (112 dB SPL, 8-16 dB). Suprisingly, the number of Iba1+ cells in the SV is modulated with a tendency of reduction instead of increment. The reduction is alarmingly significant 24 hours

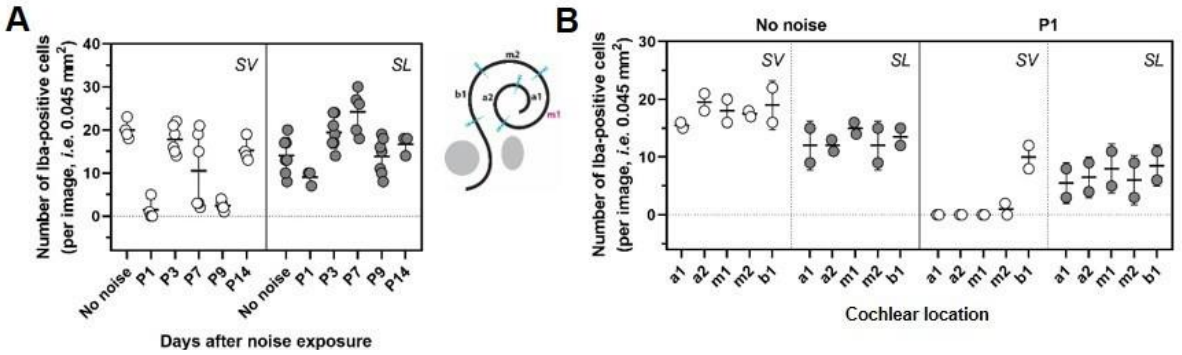

Figure 4. Intense noise exposure (112 dB SPL, OBN) modulated Iba1-positive strial macrophages. ***A****: A significant reduction of Iba1-positive macrophages in the stria vascularis (SV) occurred 1 and 9 days after the noise exposure ($p<0.0001$, unpaired t-tests, n = 3-5 per time point). Iba1-positive macrophages located laterally to the SV, in the spiral ligament (SL) were also examined. The number of these macrophages was also modulated, though to a lesser degree. The middle cochlea coil (m1) was sampled here, as indicated in the diagraph. Mean and s.d. are depicted in whiskers.* ***B****: The density of macrophages in the SL was generally lower than that in the SV along the entire length of the cochlea. However, the tendency was reversed one day after noise exposure.*

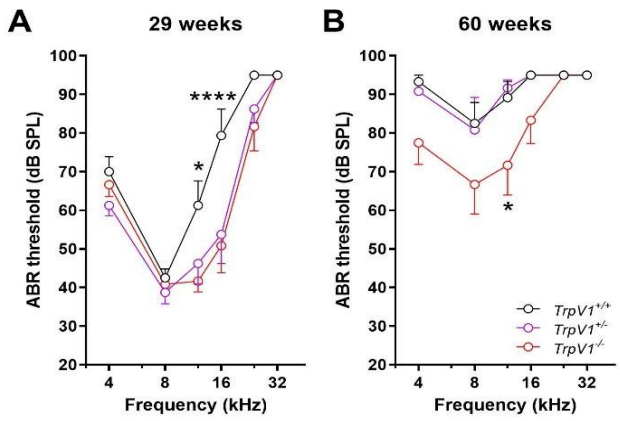

Figure 5. TrpV1 deficiency slowed down the age-related hearing loss in mice with C57BL/6 background. A*: Both TrpV1$^{+/-}$ and TrpV1$^{-/-}$ littermate mice from homo-to-heterozygous breeding setting exhibited reduced sloping hearing loss from the higher frequency region, compared to C57BL/6 lab control at age of 29 weeks, n=6-8, error bars are SEM, *p<0.05, ****p<0.0001. **B****: TrpV1$^{-/-}$ mice from hetero-to-heterozygous breeding setting exhibited reduced hearing loss compared to TrpV1$^{+/+}$ and TrpV1$^{+/-}$ littermates at age of 60 weeks, n=6.*

after noise exposure (Fig. 4A) towards the apex (Fig. 4B). We'd like to further confirm this observation and investigate the functional significance of this SV specific redution.

g) Since intratympanic LPS clearly increased the macrophage infiltration to the basilar membrane region and osseous spiral limbus region, we asked the question whether this classic innate immune event is correlated to a modification of uptake of aminoglycosides in the cochlea. We measured hair cell uptake of GTTR which was intrapearitoneally administered to mice 1-3 days after LPS inoculation. Evidently, the GTTR fluorescence was significantly higher in the LPS-treated ears compared to the contralateral untreated ears (Fig. 3A-C). This observation suggests that intratympanic (or transtympanic) LPS inoculation in mice, an animal model of otitis media, activates the inflammatory activities in the cochlea and potentially exacebates aminolgycoside-induced ototoxicity. Using this observation, we have submitted a grant proposal to DoD through a JWMRP mechanism to continue this line of research, aiming to develop therapeutics to allievate inflammation-dependent ototoxicity.

h) In addition to intratympanic-LPS-enhanced cochlear uptake of aminoglycosides, we also found that systemic-LPS enhanced aminoglycoside uptake, which is reduced in *TrpV1* KO mice, and systemic-LPS-enhanced aminoglycoside-induced ototoxicity is greatly reduced in *TrpV1* KO mice. This observation has been accepted for publication (Jiang et al., 2019), so that the pertinent data and results won't be reiterated here. *TrpV1* KO mice are "youthful", exhibit improved glucose tolerance and insulin sensitivity, and extended longevity (Riera et al., 2014). In parallel, we found *TrpV1* deficiency led to better hearing sensitivity in aged mice, given their *C57BL/6* background is a common age-related hearing loss model (see Fig. 5).

5. CONCLUSION

In the past few years of the project, we have made considerable progress in understanding the functions of TRP channels in the cochlea, especially TRPV1 channels. In the neural system, TRPV1 channel is recognized as an inflammation detector, a key player responsible for many neuroinflammatory events. In addition, it is known that intensive noise exposure elevates cochlear immune activities, manifested by increased number of tissue specific macrophages in the cochlea. Thus, we rationalized that the prior noise trauma induces cochlear immune activities including TRPV1 channel activation which subsequently enhances aminoglycoside ototoxicity.

Candidate aminoglycoside channels (*e.g.* TrpV1) and their regulating components in the inner ear, control hearing sensitivity in a characteristic fashion. In addition, our data suggest the neurotransmission between the inner hair cell and the spiral ganglion neuron is another major ototoxic target. It is already known that noise exposure does modify this neural transmission adversely, with the potential complication factor, inflammation. How prior noise exposure and aminoglycoside-induced ototoxicity interplay at this pivotal functional region is open question in auditory research, and needless to say, very relevant to personals in military settings.

Organotypic culture using cochlear explants to study ototoxicity has been adopted by many research labs. However, our recent observation suggests the data achieved from this approach should be interpreted with great caution, given tissue's developmental stage and artificial cultural environment. Our data from organotypic experiments were negative in evaluation of aminoglycoside candidate channels, TrpV4 and $P2X_2$. However, this doesn't necessarily imply our hypothesis is faulty. We will use other approaches, such as cochlear perfusion techniques proposed in Aim 4, to *in vivo* test the same hypothesis originally raised in the present project.

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). Both receptors actively participate in the process of cochlear inflammation, a level-dependent condition resulting from exposure to moderate and intense noise stimulation. If the pathophysiological condition is quickly resolved, cochlear inflammation does not necessarily result in functional damage. However, the cochlea's permeability to circulating substances, especially, ototoxic aminoglycoside medications, is increased during an inflammatory episode, which can escalate the degree of ototoxic damage. Overall, we are getting very close to the goal of this project, to search countermeasures to prevent aminoglycoside-induced cochleotoxicity (as well as vestibulotoxicity and nephrotoxicity) that can severely debilitate the recovery of military personnel, as well as civilians received aminoglycoside therapy with a history of (or likely ongoing) acoustic insult.

6. PUBLICATIONS, ABSTRACTS AND PRESENTATIONS

Peer reviewed publication

Meiyan Jiang, Hongzhe Li, Anastasiya Johnson, Takatoshi Karasawa, Yuan Zhang, William B. Meier, Farshid Taghizadeh, Allan Kachelmeier, Peter S. Steyger (2019). Inflammation upregulates cochlear expression of TRPV1 to potentiate drug-induced hearing loss. Science Advances.

Conference abstracts, papers and podium presentations

Liana Sargsyan, Alisa Hetrick, Weiwei He, Glen Martin, Hongzhe Li (2018), "Ribbon synapse distribution correlated with aminoglycoside-induced hearing deficit", SoCal Hearing Research Conference, USC, Los Angeles, CA.

Hongzhe Li, Liana Sargsyan, Alisa Hetrick, Bouchra Edderkaoui (2018), "Alleviated Cochlear Damage in an Inflammation Suppressing Model", Joint Meeting 176th Meeting Acoustical Society of America and 2018 Acoustics Week in Canada Canadian Acoustical Association, Victoria, British Columbia, Canada.

Meiyan Jiang, Hongzhe Li, William Meier, Anastasiya Johnson, Yuan Zhang, Farshid Taghizadeh, Allan Kachelmeier, Peter Steyger (2019), "Upregulation of Transient Receptor Potential Vanilloid 1 (TRPV1) Potentiates Aminoglycoside-Induced Hearing Loss", 42nd Midwinter Research Meeting in Otolaryngology, Baltimore, MD.

Weiwei He, Alisa Hetrick, Liana Sargsyan, Yu Sun, Hongzhe Li (2019), "Modulation of strial macrophages after noise exposure", 42th Midwinter Research Meeting in Otolaryngology, Baltimore, MD.

Liana Sargsyan, Alisa Hetrick, Weiwei He, Glen Martin, Hongzhe Li (2019), "Distribution of ribbon synapses correlated with hearing decline after a single dose of ototoxic injury", 42th Midwinter Research Meeting in Otolaryngology, Baltimore, MD.

"The enigmatic TRPV1 in inflammation-enhanced ototoxicity", Symposium of Advanced Hearing Research, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China, April 2019.

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. For instance, Weiwei He, a medical student from Tongji Medical College, Huazhong University of Science and Technology, has involved in this project since spring 2018. Liana Sargsyan, a European trained physician, and Yongchuan Chai, an attending doctor from Shanghai, are both specialized in otology and joined the PI's lab in 2017 and 2018 respectively. 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: 6.0
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 cochlear microdissection, confocal microscopy and data analysis.

Name: Weiwei He, MSc
Project Role: Research Assistant
Nearest person month worked: 9.0

Contribution to Project: Ms. He has performed work of organ culture with cochlear explants, as well as cochlear microdissection, confocal microscopy and molecular biology such as western blot.

Name: Yongchuan Chai, MD, PhD
Project Role: Research Associate
Nearest person month worked: 9.0
Contribution to Project: Dr. Chai has performed work of 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 published at the SoCal Hearing Research Conference, USC in 2018 and the Midwinter meeting of Association for Research in Otolaryngology in 2019, Baltimore, MD.

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) Institutional IACUC protocol #1150 was renewed and approved on 09/20/2018, and forwarded to the DoD/ACURO.

c) Based on our observation of cochlear inflammation enhanced otoxicity, a grant proposal has been prepared and submitted to DoD/JWMRP, aiming to continue this line of research, and to develop therapeutics, and eventually allievate inflammation-dependent auditory deficits.

10. REFERENCES

Not applicable.

11. APPENDICES