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13. SUPPLEMENTARY NOTES					
14. ABSTRACT The current study will identify D-methionine (D-met) dose and delayed time otoprotective response from steady state and impulse noise-induced hearing loss (NIHL). The third year was dedicated to completing data analysis from Year 2 and to further define maximum time delay for D-met post-noise rescue protection from permanent NIHL and to determine if additional D-met dosing for another 48 hours can improve post-noise rescue protection at the first rescue time with suboptimal protection. Groups of <i>Chinchillas laniger</i> (n = 10) received five intraperitoneal D-met (200 mg/kg/dose) every 12 hours beginning 28 or 32 hours after steady state or impulse. D-met otoprotection was assessed by auditory brainstem response (ABR) analysis and outer hair cell (OHC) quantification. ABR analyses identified 28 hour rescue as the first sub-optimal rescue dose time. Additional D-met administration at 28 hour rescue significantly recovered the lost protection in steady state noise-exposed animals. Histological cytochrome analysis is still in progress to confirm electrophysiological assessment.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusion.....	5
References.....	5
Appendices.....	N/A
Supporting Data.....	6

INTRODUCTION:

In military settings, noise exposure frequently exceeds the physical hearing protector capacity; resulting in Noise Induced Hearing Loss (NIHL). An oral antioxidant that could prevent permanent NIHL could allow more military personnel to be redeployed, to maintain the auditory capabilities needed in combat situations, and to keep our military personnel from having permanent disability. Currently, no pharmacologic protective agents exist in the clinical arena. We have developed an antioxidant, D-methionine (D-met), which has already shown promise in preventing NIHL in animals whether started before or after the noise exposure. In these studies we have performed dose timing studies in chinchillas to determine the latest time after noise exposure D-met may be administered to efficiently reduce impulse and steady state NIHL.

BODY:

Year 3 Purpose: To complete data analysis from Year 2 and to further define maximum time delay for D-met post-noise rescue protection from permanent NIHL and to determine if additional D-met dosing for another 48 hours can improve post-noise rescue protection at the first rescue time with suboptimal protection.

Methods: Two additional time delays for each exposure type will be selected for testing at intervals, determined from Year 2 experiments, between the last effective time delay and the first ineffective time delay for each stimulus using a time interval halving strategy. Then one group of animals for each noise exposure type will be tested at the first time delay providing less than maximal protection with an additional 48 hours of D-met administration to determine if post-noise rescue protection from permanent NIHL can be improved at that time delay by additional D-met administration. Auditory brainstem response thresholds (ABRs) will be measured at baseline, prior to any drug or noise exposure, and again 21 days after cessation of the noise exposure using tone-burst stimuli centered at the frequencies of 2, 4, 6, 8, 14 and 20 kHz.

Steady state noise exposure will comprise a 105 dB SPL narrow band of noise centered at 4 kHz. Impulse noise will comprise simulated M-16 weapon fire at 155 dB peSPL 150 impulses at 2/s.

After the 21 day post noise ABR, each animal will be sacrificed and the cochleae will be harvested for outer hair cell counts.

All data will be submitted to the FDA to obtain IND approval for clinical trials of D-met for protection and rescue from NIHL.

Results:

Year 3 Steady State and Impulse NIHL Studies:

Sixty of sixty of the scheduled animals were successfully tested for D-met protection from steady state or impulse NIHL.

We determined suboptimal D-met protection at 28 hours in the steady state and impulse noise-exposed groups (Figures 1 and 2, respectively). We also determined protection recovery in the 28 hour D-met rescue steady state group receiving 4 additional D-met doses (Figure 1). Impulse noise-exposed animals rescued at 28 hours and given additional D-met doses did not significantly reduce ABR threshold shifts compared to the 28 hour measured observed threshold shift reductions however more frequencies were significantly different compared to the saline control group (Figure 2).

ABR threshold analyses for Years 1, 2, and 3. Histological preparation and analysis required additional time. Thus, a one-year no cost extension has been approved to complete histological analysis.

KEY RESEARCH ACCOMPLISHMENTS:

- 30/30 of the animals scheduled for testing D-met time-delayed protection from impulse NIHL were tested using saline or 200 mg/kg/dose D-met at 28 and 32 hours and an additional group at 28 hours receiving 4 additional D-met injections.
- 30/30 of the animals scheduled for testing D-met time-delayed protection from steady state NIHL were tested with either saline or 200 mg/kg/dose D-met at 28 and 32 hours and an additional group at 28 hours receiving 4 additional D-met injections.
- ABR analysis identifies suboptimal D-met protection at 28 hours rescue time and protection recovery at 28 hours rescue time with 4 additional D-met injections in the steady state noise-exposed group.
- Cytocochleogram quantitative analysis of chinchilla cochlear hair cells is established.
- Developed an animal model for chinchilla cytochleogram quantitative analysis.
- Dissected, stained and prepared slides for Year 2. Year 3 dissection is progressing.
- A one-year no cost extension is approved to complete Year 3 histology.

REPORTABLE OUTCOMES:

We refined our Year 2 D-met time response study. The Year 2 twenty-four-hour rescue remained the optimal dose rescue time. However, additional D-met administration recovers rescue from steady state NIHL (Figure 1). Additional D-met administration in the impulse noise-exposed animal groups was not as effective compared to the steady state noise-exposed animal groups (Figure 2). The results have just recently been statistically analyzed and have not yet been presented or published.

CONCLUSION:

We have identified protection loss when D-met administration begins 28 hours post steady state or impulse noise exposure. We have also identified protection recovery with additional D-met administration in the steady state noise-exposed groups. Additional D-met administration in the impulse NIHL model did not measure significant differences compared to the 28 hour rescue paradigm without additional D-met however more frequencies were significantly different compared to controls with additional D-met (Figure 2).

Thus, D-met protects from steady state or impulse NIHL via dose- and time- dependent mechanisms. Further, D-met protection can be dose-dependent via administrative extent or dose concentration.

REFERENCES:

Campbell, K., Claussen, A., Verhulst, S., Fox, D., Hughes, L. 2011. D-methionine (D-met) significantly rescues noise-induced hearing loss: timing studies. *Hear Res.* Dec: 282(1-2): 138-44.

APPENDICES: N/A

SUPPORTING DATA:

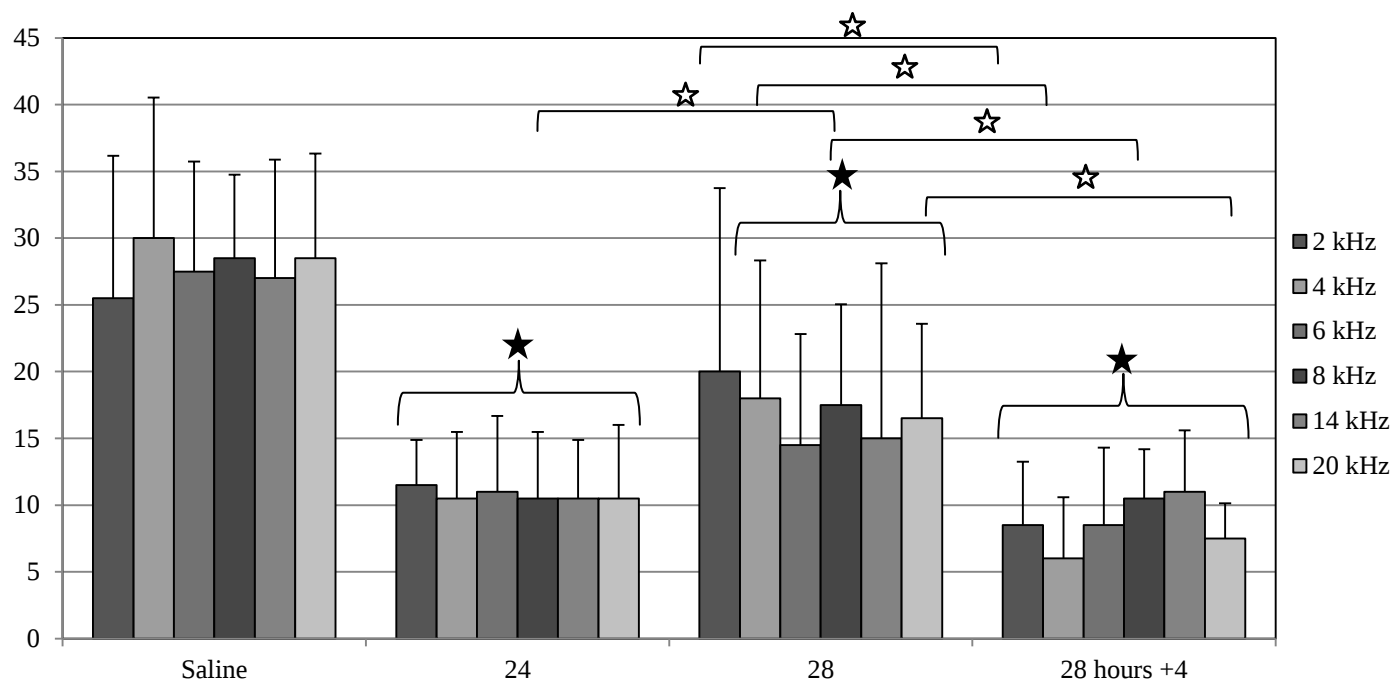


Figure 1. Steady state noise-induced ABR threshold shifts in chinchillas 21 days after a 105dB peSPL 6 hour noise exposure centered at 4 kHz ($n = 10$ per group). The 24 hour rescue group was referenced from the Year 2 study. D-met protection becomes sub-optimal when administration begins 28 hours post-noise exposure but recovers with 4 additional D-met doses. Black stars indicate significance difference at the 0.05 level compared to controls. White stars indicate significance at the 0.05 level compared to D-met-treated groups. $n = 10$ /group. Error bars are reported as standard deviation. Tukey's post-hoc follow up determined significance at $p \leq 0.05$.

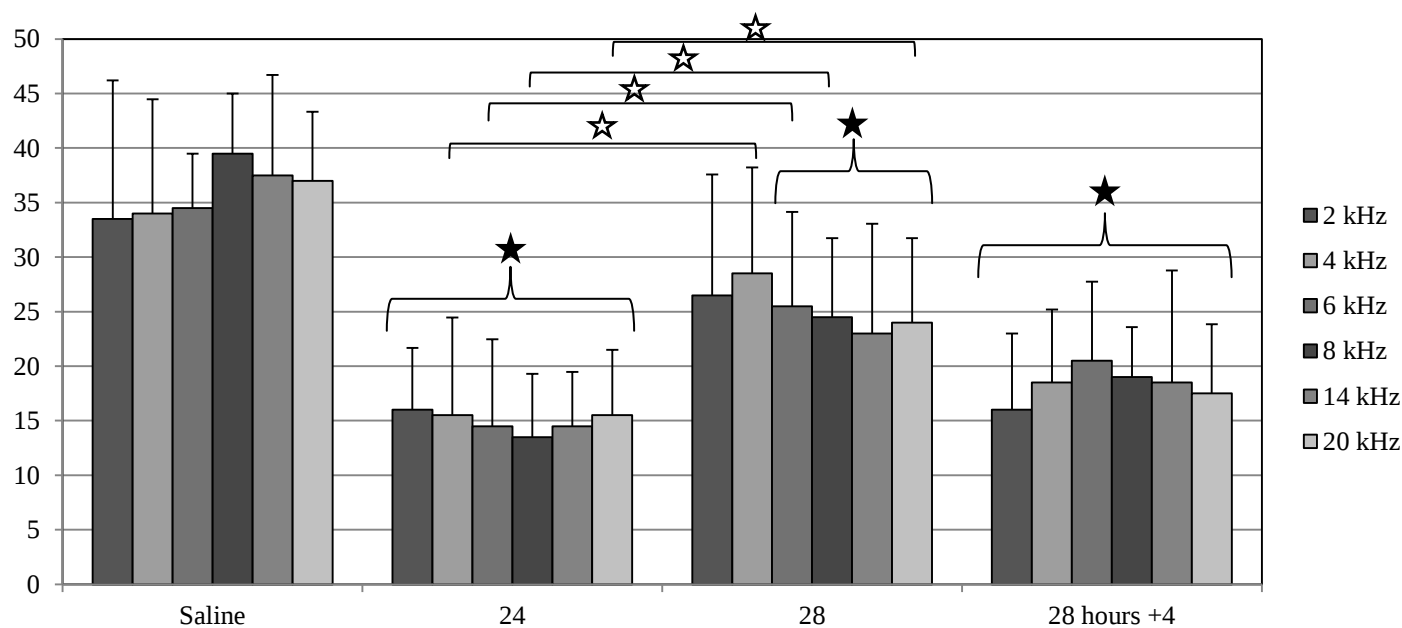


Figure 2. Impulse noise-induced ABR threshold shifts in chinchillas 21 days after simulated M-16 weapon fire at 155 dB peSPL for 150 impulses at 2/s ($n = 10$ per group). The 24 hour rescue group was referenced from the Year 2 study. D-met protection becomes sub-optimal when administration begins 28 hours post-noise exposure. Four additional D-met doses did not significantly improve protection compared to the 28 hour rescue group. Black stars indicate significance difference at the 0.05 level compared to controls. White stars indicate significance at the 0.05 level compared to D-met-treated groups. $n = 10$ /group. Error bars are reported as standard deviation. Tukey's post-hoc follow up determined significance at $p \leq 0.05$.