Pharmacologic Protection from Noise Induced Hearing Loss: (NIHL): Current Status

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Reasons for Developing Pharmacologic Otoprotectants for NIHL

- Even with maximal physical hearing protectors NIHL can occur (bone conduction of high intensity signals)
- Compliance with physical hearing protectors is imperfect
- Noise reduction at the source is not always feasible
- Noise exposure is sometimes unpredictable
Types of Otoprotectants

• Prophylactic Agents: Administered before and usually during noise exposure periods
• Pre-loading has not yet been tested
• Rescue Agents: First administered after noise exposure but before permanent NIHL has occurred
• Regeneration of Hair Cells for permanent NIHL is a different research area
Why Intersubject Variability in NIHL?

- Endogenous Factors:
  - Lipid Disorders
  - Diabetes
  - Reaction to Stress
  - Body Fat
  - Possible Pigmentation Factors
  - Genetic susceptibility and resistance
Why Intersubject Variability for NIHL?

- Exogenous Factors:
- Dietary differences among subjects (e.g., fat intake, type of fat intake, antioxidants in diet, protein, vitamins, minerals)
- Possible alcohol consumption differences
- Smoking
- Exercise and body fat
- Stress
- Use of Hearing Protectors
- Drugs/medications
Ways to Reduce NIHL?

• Diet (food and alcohol intake, vitamin and mineral intake)
• Exercise and reduction of body fat
• Not smoking
• Stress reduction?
• Hearing protectors
• Diabetes and lipid disorder prevention
When Could a Pharmacologic Agent Prevent/Reduce NIHL?

- When noise exposure exceeds physical hearing protection capability
- When NIHL is secondary to cochlear metabolic damage, not primarily cochlear mechanical damage
- When it can be administered before or within a few days after noise exposure
Reasons Some Agents Are Not Being Developed for the Clinic

- Administration Issues (ie only effective with round window or iv administration)
- Purpose is to elucidate mechanisms of NIHL
- Side Effects
- Not safe in humans (or dosing issues)
- Cost issues
- No patent coverage
Considerations for a Clinically Useful Pharmacologic NIHL Otoprotective Agent

- Oral administration preferable (& palatable)
- Safe (risk/benefit)
- Wide Therapeutic Index
- Minor or no side effects for all subjects
- Minor or no drug interactions
- Easy storage (temperature, volume, preparation, stable over time)
- Preferably Inexpensive
FDA Issues

• Currently no drug is approved by the FDA to treat or prevent noise induced hearing loss (or cisplatin or aminoglycoside induced hearing loss.)

• To obtain FDA approval for a new drug generally takes several years and over 1 billion dollars.

• Therefore patent protection is needed.
Why Focus on Anti-Oxidants for Hearing Protection?

• Many have good safety profiles.
• We know a lot about many of them, sometimes from the nutrition literature.
• Many can be given orally.
• Many of them are not foreign to the human system.
• Side effects are frequently minimal.
How Do Antioxidants Differ From Each Other?

• Mechanisms of action can be different
• They can act on different pathways and/or different cellular areas
• Uptake into tissues and distribution through the body can be different (BLB, BBB)
• Safety profiles can vary (by pt, by drug interactions) Not all work the same on all patients
• Therapeutic indices can vary
What is an Anti-Oxidant?

• A direct anti-oxidant is a compound that can freely donate an electron to stabilize the free radical.

• An indirect anti-oxidant promotes production of endogenous anti-oxidants such as glutathione, or enzymes with anti-oxidant actions (SOD, CAT, GR, GSH-Px)
What is a Free Radical?

• It is an atom, molecule or ion with an unpaired electron on its outer shell. The unpaired electron makes it highly reactive and thus potentially damaging to surrounding molecules.
Glutathione

- A tripeptide consisting of glutamate, cysteine and glycine
- Present in virtually all mammalian tissues and at lower levels in plasma
- Present in reduced (GSH) and oxidized (GSSG or glutathione disulfide) form
- In normal systems 99% of glutathione is in the GSH form
Glutathione

- Altered GSH homeostasis implicated in many disorders (e.g., Parkinson’s)
- Decreased GSH can increase therapeutic efficacy of some drugs and radiation but can increase side effects (i.e., can increase toxicity for all cells)
- Helps eliminate xenobiotics
- Important antioxidant pathway (reductant)
GSH Antioxidant Role

- Essential component of antioxidant defenses
- Protects cells from oxidative damage by donating a hydrogen atom from the thiol group of the cysteine residue
- Hydrogen atom can be donated to most carbon, nitrogen, or oxygen centered radicals
Cellular GSH

- Most cellular GSH is in the cytosol
- Only 10-20% of GSH is in the mitochondria
Enzyme

- Complex protein substance produced in living cells
- Causes or accelerates other chemical reactions in an organism
- Is not altered itself
- Enzymes are organic catalysts
Two enzymes you should know

• Superoxide Dismutase: Converts $O_2^-$, the superoxide radical anion, into oxygen ($O_2$) and hydrogen peroxide ($H_2O_2$).

• Catalase: Converts hydrogen peroxide ($H_2O_2$) into water ($H_2O$) and oxygen ($O_2$).
Two More Enzymes You should Know

- Glutathione Peroxidase oxidizes GSH to GSSG
- Glutathione Reductase reduces GSSG to GSH
Natural Otoprotective Agent

• Axelsson 1975: Pop/rock musicians had lower than predicted levels of hearing loss
• Liking the music?
• Continuous contraction of stapedius muscle/
• Efferent system does not have a major protective effect (Liberman and Gao 1995)
Moderate alcohol consumption

• Moderate alcohol consumption (less than 4 drinks per day) inversely correlates with the odds of having LF or HF NIHL (Popelka et al 2000)

• Heavy alcohol consumption increased the odds of HFHL
Putative Protective/Recue Agents for NIHL

- GSH Prodrugs
- Magnesium
- NOS Inhibitors
- Cell Death Inhibitors
- Free Radical Scavengers/Antioxidants
- Antioxidant enzyme protectants/upregulators
- Combinations?
Why Not Just Administer Glutathione Directly?

• Intracellular GSH is probably a major factor in cochlear protection
• However the liver metabolizes GSH and it is not readily taken up into cells (Meister 1991)
• GSH esters may produce toxicities (Levy et al 1993)
• RW application effective but not practical (Hight et al 2000)
Neurotrophic Factors

- Some neurotrophic factors show good NIHL protection (e.g., glial cell line-derived neurotrophic factor Ylikoski et al. 1998, Shoji et al. 2000) but may be secondary to anti-oxidant properties.
- Neurotrophic factors without anti-oxidant properties (brain derived neurotrophic factor, fibroblast growth factor) may not protect (Miller and Altschuler 2000, Shoji et al. 2000).
- Oral availability and safety may also be issues.
Dietary Supplements

• Several NIHL otoprotective agents are also micronutrients:
  • Mg: fish, almonds, spinach, shrimp, bran
  • D-met: cheese, yogurt
  • NAC: brussel sprouts
  • Resveratrol: red wine
  • Selenium: Brazil nuts, N. Dak and S.Dak grown foods, prime component of ebselen
  • Alcohol: 2-4 drinks per day
Antioxidant therapies
Approaching Clinical Trials

- Ebselen
- N-Acetylcycteine (NAC)
- D-Methionine (D-MET)
- ACE Mg
- Salicylate (as concomitant agent)

Agents have good safety profile and oral bioavailability
Chinchilla model

- 105 dB SPL noise band centered at 4kHz
- D-met or ALCAR administered at 200mg/kg ip, NAC at 325mg/kg plus salicylate
- Administered every 12 hours starting 48 hours prior to noise and 1 hour prior to the noise and then twice per day for 2 days following noise exposure
N-acetylcysteine (NAC): putative mechanisms

• L-NAC is a free radical scavenger
• Neuroprotectant
• GSH precursor: provides cystolic but not mitochondrial GSH
Ebselen
A Catalyst for Hearing Loss Treatment
Eric D. Lynch, PhD

4010 Stone Way N Suite 120
Seattle, WA
Ebselen (SPI-1005)—How does it work?

- Small molecule mimic of glutathione peroxidase (GPx)
  - Glutathione Pathway—ROS/RNS neutralization
  - GPx catalytic activity
Otoprotection across frequencies
Continuous 4 hr noise exposure
4-16 kHz noise at 113 dBSPL
4 mg/kg SPI-1005, ABR at 9 wks post noise,
n=8 (3 & 14d), n=6 (7d), SEM shown
Cytocochleogram analysis
3 wks post noise
Dietary Micronutrients

• Beta-carotene, Vitamins C and E, Magnesium
  – Beta-carotene: scavenges singlet oxygen, prevents lipid peroxidation
  – Vitamin E: reduces peroxyl radicals in lipid layer
  – Vitamin C: scavenges free radicals in aqueous phase
  – Magnesium: reduces noise-induced vasoconstriction, blocks NMDA receptors, prevents calcium influx and neural excitotoxicity

• Patent pending, University of Michigan
  – Inventors: Josef Miller, Colleen Le Prell, Jochen Schacht, Diane Prieskorn

• Option to license by OtoMedicine, Inc.

• Human trials beginning in 2008
Antioxidants plus magnesium reduce noise-induced hearing loss: additive effects

2.1 mg/kg beta-carotene, p.o., 71.4 mg/kg L-threoscorbic acid, s.c., 26 mg/kg trolox, s.c.); magnesium sulfate, 2.85 mmol/kg, equivalent to 343 mg/kg, s.c.; 1 hour pre and 5 days post Mean ± S. E., Le Prell et al., *Free Rad. Med. Biol.*, 42,1454-1463.
Protection is greatest in the base of the cochlea
Human Clinical Trials: 2008-2013

• Temporary Threshold Shift Model
  – Swedish soldiers exposed to automatic weapons fire
  – US students listening to music with insert earphones

• Permanent Threshold Shift Model
  – NATO soldiers at Spanish airbase
  – Employees at Spanish stamping factory
Human Trials

- Safe dosing limits well-characterized

<table>
<thead>
<tr>
<th></th>
<th>US RDA</th>
<th>Upper Limit</th>
<th>Percent of UL</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-Carotene</td>
<td>1.5 mg/5000 IU (18 mg(^1))</td>
<td>3 mg/10,000 IU (36 mg(^1))</td>
<td>50% (18 mg) 90% of EU UL</td>
</tr>
<tr>
<td>Vitamin C (Ascorbic Acid)</td>
<td>60 mg</td>
<td>2000 mg</td>
<td>25% (500 mg)</td>
</tr>
<tr>
<td>Vitamin E ((\alpha)-tocopherol)</td>
<td>15mg</td>
<td>1000 mg</td>
<td>27% (270 mg)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>300-400 mg</td>
<td>350 mg</td>
<td>90% (315 mg)</td>
</tr>
</tbody>
</table>

\(^1\) Based on retinol activity equivalents
EU Upper limit=20 mg
Procedures to be used

• Pure-tone Audiometry
  – Conventional and Extended High Frequencies

• Distortion Product Otoacoustic Emissions
  – Input-Output Functions

• Tinnitus Surveys
Key Steps To Date: Funding

• Funding has been obtained from the NIH
  – Submitted Translational Research Application, Reviewed by Neurology Institute Clinical Trials section, with ad hoc ENT participation

• Parent grant awarded to UM (PI: Josef Miller); Subcontracts to each trial site and other partner institutions
Key Steps To Date: MOP, DSMB, TSB

• Data Safety Monitoring Board (DSMB) assembled by NIH after review of MOP
• Submitted revised MOP for review by DSMB, Dec. 2007
• Submitted 700+ page “Trial Site Binder (TSB)” for NIH Review, Jan. 2008
• Meeting of study team members with NIH and DSMB took place in Jan. 2007
Key Steps To Date: Additional Administrative Steps

• IRB applications at UM and at each trial site must match, and both must be approved
  – Swedish military trial approved both at UM and by Swedish regulatory committee
• Subcontracts with trial sites under negotiation
• Initial site visits scheduled to occur prior to trial onset
Conclusions

- Antioxidants attenuate NIHL
  - Across species
  - Across agents
  - Across dose schedules
  - Across noise insults

- We can effectively reduce NIHL even with treatments that follow the noise exposure

- It is indeed time to evaluate antioxidant prevention of NIHL in human trials
Contraindications for ACE Mg

• Vitamin A and its precursors (Beta Carotene) should not be administered to smokers
• Magnesium has laxative properties and may not be appropriate for subjects with gastrointestinal disorders.
Current Status of D-met Research

- FDA previously approved our Investigational New Drug Application for D-met protection from radiation induced oral mucositis.
- Phase I Clinical Trial Data has been submitted for publication.
- Phase II clinical trials (India) results for protection from cisplatin induced ototoxicity and radiation induced oral mucositis are being prepared for publication.
- In discussions with military for NIHL protection.
- More bench work also needed.
Patents Issued

• Southern Illinois University School of Medicine
• Kathleen C.M. Campbell, PhD Inventor
• 5 US patents and 34 foreign patents issued
• Others in prosecution
Other findings

- D-met can be administered directly to the round window and still protect against systemic or topical cisplatin-induced ototoxicity
- D-met protects against carboplatin-induced ototoxicity
- D-met protects against aminoglycoside-induced ototoxicity
- Some patients receive all 3 drugs
- D-met also can protect against NIHL
D-methionine: putative mechanisms

• Unlike most amino acids, methionine is reversibly oxidized (Vogt 1995) and thus may serve as a free radical scavenger
• Methionine can provide cysteine, a precursor for glutathione (GSH) synthesis
• Can increase mitochondrial GSH levels and can prevent the efflux of GSH from injured cell
• May protect antioxidant enzyme levels
• D-met well tolerated even at high dosages
D-methionine: putative mechanisms (continued)

• At least for noise improves/protects GSH/GSSG ratio
• Preferable pharmacokinetics to L-met for protection
• Has been previously used in humans and animals
• Part of normal nutrition (particularly present in fermented proteins)
• Methionine part of protein and needed for protein formation
Met HC protection
D-met Post-Noise Rescue
Mitchell, Meech, Campbell

- D-met can be administered 1 hour after noise exposure and provide protection from permanent NIHL.
- Does not provide significant against TTS but only PTS.
- Methods: 6 hour: 105dB SPL 4kHz octave band noise, 200 mg/kg D-met 1 hour after exposure and 2 days BID.
- With 10 animals per group, significant protection at 2, 4, 6 & 8 kHz
D-met rescue from NIHL

D-Met Rescue From Noise-Induced Hearing Loss

ABR Threshold Shift From Baseline To 21 Days Post

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2kHz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4kHz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6kHz</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8kHz</td>
<td></td>
<td></td>
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</tbody>
</table>

- Control
- Treated

Graph showing ABR threshold shift from baseline to 21 days post.
D-met Rescue from Noise-Induced Hearing Loss: Post Administration Intervals

Kathleen Campbell, PhD, Robert Meech, MA, Deb Larsen, MA, Diana Mitchell, MD, Larry Hughes, PhD
Clinical Question: How long can D-met be delayed and still prevent permanent NIHL?

• Unexpected high level noise exposure can occur in:
  • Certain professions such as emergency workers, miners, military personnel
  • Recreational exposure such as concerts, power equipment
  • Air bag deployment, alarm systems
Methods:

- Study in progress
- 105 dB SPL 4 kHz NB for 6 hours
- Chinchillas Laniger (male 3 year old)
- 200 mg/kg D-met initially started at either 1, 3, 5 or 7 hours after noise cessation with 4 additional BID doses 12 hours apart
- 5 animals per group for 1, 3, 5 hour groups and saline control, 3 per group at 7 hours
- ABR thresholds and outer hair cell counts at 21 days
ABR Threshold Shift on Post-Noise Exposure Day 21: 2000 Hz

- Saline Control (n=5)
- D-met 3 hour (n=10)
- D-met 5 hour (n=8)
- D-met 7 hour (n=10)
ABR Threshold Shift on Post-Noise Exposure Day
21: 8000 Hz

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>ABR Threshold Shift (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Control</td>
<td>5</td>
<td>15.0 ± 5.0</td>
</tr>
<tr>
<td>D-met 3 hour (n=10)</td>
<td></td>
<td>0.0 ± 2.0</td>
</tr>
<tr>
<td>D-met 5 hour (n=8)</td>
<td></td>
<td>0.0 ± 2.0</td>
</tr>
<tr>
<td>D-met 7 hour (n=10)</td>
<td></td>
<td>0.0 ± 2.0</td>
</tr>
</tbody>
</table>
Percentage Outer Hair Cell Present on Post-Noise Exposure Day 21: 2000 Hz Region

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage Outer Hair Cell Present</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Control (n=5)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>D-met 3 hour (n=10)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>D-met 5 hour (n=8)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>D-met 7 hour (n=10)</td>
<td>100</td>
<td></td>
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</table>
Percentage Outer Hair Cell Present on Post-Noise Exposure Day 21: 4000 Hz Region

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage Outer Hair Cell Present (± SEM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline Control (n=5)</td>
<td>60.0 ± 10.0</td>
<td></td>
</tr>
<tr>
<td>D-met 3 hour (n=10)</td>
<td>100.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>D-met 5 hour (n=8)</td>
<td>100.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>D-met 7 hour (n=10)</td>
<td>100.0 ± 0.0</td>
<td></td>
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</tbody>
</table>
Percentage Outer Hair Cell Present on Post-Noise Exposure Day 21: 6000 Hz Region

- Saline Control (n=5)
- D-met 3 hour (n=10)
- D-met 5 hour (n=8)
- D-met 7 hour (n=10)
Percentage Outer Hair Cell Present on Post-Noise Exposure Day 21: 8000 Hz Region

- Saline Control (n=5)
- D-met 3 hour (n=10)
- D-met 5 hour (n=8)
- D-met 7 hour (n=10)
Conclusions:

• Post-noise D-met administration, at least within 5 hours following the noise, may protect against permanent ABR threshold shift and OHC loss.

• Further research needed to explore maximum allowable time delay, various D-met dosing strategies, and efficacy for different noise exposures.

• Further research needed on D-met mechanisms of protection for not only NIHL, but aminoglycoside and cisplatin otoprotection.
Methionine (met) Safety Issues

• Met is a micronutrient and thus not alien to the human system
• Methionine comprises 26 mg/g high quality protein in the diet
• Has been used in human studies with no side effects (Kaji et al 1987, Kies et al 1975, Stegink et al 1986)
Met: Safety Issues (cont)

- World Health Organization lists methionine as essential drug for treating acetaminophen overdose (2.5 gm doses at 4 hr intervals for a total of 10 gm over 12 hours)
- Monteagudo 1986 methionine “remarkably free of side effects” including nausea and vomiting
- DiRocco et al 1998 20gm/day safe for adults even for chronic administration
Advantage of D-met

- Although L-met would be safe even at high levels, D-met is even safer.
- D-met has no apparent toxicity unless converted to the L isomer at high levels.
- Toxicity can occur secondary to high dose L-met or racemate in the presence of a very low protein diet (animal studies).
Advantage of D Met

• In humans, 60-70% of D-met is excreted without conversion (Printen et al 1979, Baker 1994)

• In humans D-met results in higher plasma levels than L-met

• D-met has longer half life and enhanced bioavailability (Borg and Walstrom 1989)
Over the counter (OTC) Methionine

• For decades OTC oral Met has been used to reduce urinary odor and associated dermatitis

• Recommended dosing is 200-400 mg orally 3-4 times per day
Kluge, Meech, and Campbell

- Effect of D-met on GSH/GSSG with various duration noise exposures
Design-Details

- 36 total animals
- 18 animals had intraperitoneal (IP) D-methionine injections (Experimental group)
- 18 animals had IP saline injections (Control group)
- Injections were twice-a-day for two days prior to exposure and on the morning of the exposure (total five injections per animal)
- The concentration of D-methionine was 200mg/kg
Noise Exposure

• The noise exposure was 105 dB SPL 4kHz NB noise
• There were three chinchillas in each noise exposure group
• The noise durations were:
  – None (15 min in booth with no sound on)
  – 30 min
  – 2 hr
  – 4hr
  – 6hr
  – 8hr
GSH, GSSG and Protein Determinations

- GSH and GSSG were determined by HPLC analysis
- Protein concentration was determined by spectrophotometry
- GSH and GSSG levels were standardized to the protein concentration in each sample
Next step?

- Clinical trials to compare efficacy and side effects for cisplatin, carboplatin, aminoglycoside and noise otoprotection and for radiation induced oral mucositis
- More work on mechanisms
- Hopefully more than one agent will be FDA approved for all of these applications in the not too distant future.
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Discussion and Questions