Otoprotective Agents for Prevention of Acquired Hearing Loss in Humans

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Noise-Induced Hearing Loss
Webinar Series 2013

Acceptable Strategies for Prevention of Noise- and Music-Induced Hearing Loss
Brian J. Fligor, ScD

Tinnitus Assessment in Young Musicians
Frank Wartinger, AuD

Food for Thought: Nutrition and Noise
Christopher Spankovich, AuD, PhD, MPH

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Otoprotective Agents for Prevention of Acquired Hearing Loss in Humans

Colleen G. Le Prell, Ph.D.
Department of Speech, Language, and Hearing Sciences
University of Florida

Disclosure: Colleen Le Prell is a co-inventor on patents owned by the University of Michigan.
Acquired Hearing Loss

- Noise
- Ototoxic Drugs
  - Aminoglycoside Antibiotics
  - Cisplatin
  - Loop Diuretics
  - Salicylate
- Age
  - Age/Noise Interactions
- Chemical Solvents
- Increased oxidative stress (increased free radical production) implicated in all of the above

NIHL: The Clinical Problem

- Significant issue for military and personnel
- Quality of life issue for affected personnel
- Significant financial burden
- 1\textsuperscript{st}/2\textsuperscript{nd} most common occupational disease
  - Occupational noise adds to non-work exposure
- Multiple non-occupational exposure sources
  - Hunting, lawnmowers, power tools
  - concert/nightclubs, sporting events, MP3 player use
- Robust TTS may increase later age-related deficits
  - Mouse model (Kujawa and Liberman, 2006, 2009)
Sound is a Mechanical Stimulus that Causes a Mechanical Response


Strategies for Reducing NIHL

- Reduce Sound Level at it’s Source
- Reduce Exposure to the Source (decrease exposure time)
- Design Better Hearing Protection Devices
- Provide Better Education on Correct Use of HPDs
- Decrease Metabolic Stress That Induces Cell Death in the Inner Ear – Drugs or Dietary Supplements
Reactive Oxygen Species (ROS) Damage

- Free radicals essential for normal cell physiology, but in excess, they:
  - Damage cellular lipids, proteins, and DNA
  - Upregulate apoptotic pathways
  - Reactive Oxygen Species (ROS) and Reactive Nitrogen species (RNS)


Cochlear Cross-Section

Persistent RNS Confirmed Post-Noise

Nitrotyrosine Labeling, Adapted from Yamashita et al., *Brain Research. 1019(1-2):201-9*, 2004

Persistent ROS Confirmed Post-Noise

4-HNE Labeling, Adapted from Yamashita et al., *Brain Research. 1019(1-2):201-9*, 2004
Salicylate plus vitamin E reduces noise-induced hearing loss


Salicylate plus vitamin E reduces noise-induced outer hair cell death

Many Antioxidants Reduce NIHL

- N-acetylcysteine
  - Kopke et al., 2000, 2005 (w/salicylate)
  - Ohinata et al., 2003
  - Duan et al., 2004
  - Bielefeld et al., 2005; 2007
  - Coleman et al., 2007
  - Lorito et al., 2008
- Salicylate
  - w/NAC, Kopke et al., 2000
  - w/vitE, Yamashita et al., 2005
- resveratrol
  - Seidman et al., 2003
- allopurinol
- Cassandro et al., 2003
- R-phenylisopropyladenosine (R-PIA)
  - Hu et al., 1997
  - Hight et al., 2003
- SOD-polyethylene glycol
  - Seidman et al., 1993
- U74389F
  - Quirk et al., 1994

Animal Models of Otoprotection: The Problem

- Multiple Species
  - Guinea pigs, rats, chinchillas, mice

- Multiple Noise Exposures
  - Octave band noise: centered at 4 or 8 kHz, or 8-16 kHz
  - Exposures generally 4-6 hours at 105-120 dB SPL
  - Threshold shifts in control animals range from 20 to 50+ dB

- Multiple Treatment Paradigms
  - Onset of treatment ranges from several days pre-noise, to some period post-noise
  - Oral administration vs injected agents

- Difficult, if not impossible, to draw conclusions on relative efficacy across agents
Antioxidants Differ From Each Other

- **Mechanism of action**
  - Upregulate endogenous defense vs direct free radical scavenging
  - Specific free radicals scavenged
  - Prevention of excitotoxicity
- **Uptake into tissues, distribution, bioavailability**
- **Safety profile**
- **Method of delivery (oral, injected, round window)**

Translation to humans?

- For human clinical application, antioxidant agents must be safe for daily use.
- Long-term safety data for vitamins (7 yr high dose supplements with A, C, & E in AREDS)
- Extensive testing of Mg in animals and humans
- Prior studies show multiple days/weeks/months of pretreatment with single agents are effective; less benefit if initiated shortly before noise insult.
- Each agent scavenges different free radicals, and enter different parts of cells (lipid membranes, cytoplasm).
Vitamins plus magnesium reduce noise-induced hearing loss


Vitamins plus magnesium reduce OHC death

Oral Treatment Reduces NIHL in Mice: Dose-Dependent Effects

Adapted from Le Prell, C.G., Gagnon, P.M., Bennett, D.C., and Ohlemiller, K.K. 2011. Nutrient-enhanced diet reduces noise-induced damage to the inner ear and hearing loss. Translational Research, 158, 38-53.

Functional Protection Explained by Preservation of Cells in Lateral Wall

- Cell density in animals fed Diet B was equivalent to that in normal animals without history of noise exposure

Adapted from Le Prell, C.G., Gagnon, P.M., Bennett, D.C., and Ohlemiller, K.K. 2011. Nutrient-enhanced diet reduces noise-induced damage to the inner ear and hearing loss. Translational Research, 158, 38-53.
Early noise-induced deficits also reduced in Guinea Pigs


Translation to humans

- The Agents Must be Safe

<table>
<thead>
<tr>
<th>Daily Dose</th>
<th>Upper Limit</th>
<th>Percent of UL</th>
</tr>
</thead>
</table>
| **B-Carotene**  
(RDA=18 mg) | 18 mg\(^1\)  
20 mg (EU) | 36 mg (US)  
50 (US) |
| **Ascorbic Acid**  
(RDA=60 mg) | 500 mg | 2000 mg | 25 |
| **A-tocopherol**  
(RDA=15 mg) | 270 mg | 1000 mg | 27 |
| **Magnesium**  
(RDA=300-400 mg) | 315 mg | 350 mg | 90 |

\(^1\)Based on retinol activity equivalents

- AREDS study provides 7-year safety data for the vitamins
Human Trial Design Considerations

- Most pre-clinical studies measure reduction of PTS
- Most human trials to date assessed reduction of TTS
- Clinical relevance of reduced PTS is clear
  - why use TTS models?
- Shorter duration, reduced cost, decreased attrition
- Subject safety: PTS not expected in any subjects
- Value of TTS trial hinges on assumption that reduced TTS provides “proof of concept” (predictive value) for reduced PTS
- Most (if not all) agents that have reduced TTS (in animals) have also reduced PTS (in animals)
- Confirmatory data in PTS trials will be required
  - key issue is access to populations in which the extent, prevalence, variability, and rate of change are documented

“Failed” TTS studies

- Swedish military weapons trial
  - TTS not reliably induced during weapons training (Le Prell et al., 2011, ACEMg; Lindblad et al., 2011, NAC)
- Nightclub study
  - Variable noise exposure across exposure dates (Kramer et al., 2006, NAC)
- Occupational Noise Study
  - Failure to measure robust post-shift TTS in workers enrolled as subjects (Lin et al., 2010, NAC)
Swedish soldiers exposed to automatic weapons fire

- Two rounds Ksp58 weapon fire, 20 shots/round
  - 2 training periods per subject, ~3 months apart
- Max SPLs 164 - 166 dB SPL; SPLs under hearing protectors 135-154 dB SPL
- 31 subjects

Vitamin concentrations increased with 2 days of treatments

- 18 mg beta-carotene, 500 mg vitamin C, 270 mg vitamin E, 315 mg Magnesium per day, delivered in two half doses
- Samples taken prior to first treatment and 2 hours after final treatment (Mean ± S.D., n=9)

No reliable hearing changes during nutrient or placebo

- Military upgraded protectors
- Improved weapons
- Subjects may have used hearing protection more carefully
  - Hawthorne Effect


Protection of most vulnerable subjects?

<table>
<thead>
<tr>
<th>ID</th>
<th>Max TTS Placebo Arm</th>
<th>Max TTS Nutrient Arm</th>
<th>difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1058</td>
<td>14</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>1002</td>
<td>10</td>
<td>-2</td>
<td>12</td>
</tr>
<tr>
<td>1007</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>1057</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>1062</td>
<td>8</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>1010</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

- 6 subjects ≥8 dB TTS in placebo arm (3, 4, or 6kHz)
- 5 of those 6 subjects: TTS_{ACEMg} < TTS_{placebo}
  - 2 of 5 subjects: 12 dB difference
  - 3 of 5 subjects: 2-4 dB difference
- 6th subject: TTS_{ACEMg} = TTS_{placebo}

“Successful” TTS studies

- Laboratory noise models
  - broad-band noise (Attias et al., 2004; magnesium), narrow-band noise (Quaranta et al., 2004; vitamin B12) noise, and pure-tones (Quaranta et al., 2012; alpha-lipoic acid) are unpleasant to listen to
- Alternative TTS models based on music player studies?
  - If subjects select listening level, selected levels vary
    - Small sample sizes for any given level
    - TTS limited to subset of subjects
    - Lee et al., 1985; Pugsley et al., 1993; Hellstrom et al., 1998.
- No reliable TTS in recent studies (level set by investigator)
  - Krishnamurti & Grandjean, 2003; Bhagat & Davis, 2008; Keppler et al., 2010
- New model developed specifically for otoprotection studies
  - Le Prell et al., 2012

4-hour music exposure induces level-dependent TTS

4 hr exposure to 100-dBA music (in-ear level) temporarily depresses OAE amplitude

- F2=6 kHz, pre and post music
- Mean ± S.E. for the 12 subjects exposed to 100-dB (A) music.


Power Analysis

- Predicted TTS for placebo group in clinical trials is TTS as measured in the pilot studies
  - 4.0 ± 3.4 dB shift measured with 98-dBA (in-ear) x 4 hrs
  - 6.3 ± 3.9 dB shift measured with 100-dBA (in-ear) x 4 hrs

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Predicted TTS (dB)</th>
<th>Std dev (dB)</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo Treated</td>
<td></td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>TTS at 4 kHz</td>
<td>4.0 dB</td>
<td>3.4</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>6.3 dB</td>
<td>3.9</td>
<td>40</td>
</tr>
</tbody>
</table>

- New studies can be powered to detect 50% reduction in TTS using only 20 subjects per dose; increasing subject numbers increases power for detecting smaller effects
- Using this model to assess both ACEMg (NCT00808470) and Ebselen (NCT01444846)
University of Florida iPod® study

- Randomized placebo-controlled, double-blind, between-subjects design

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- Increased oxidative stress (increased free radical production) implicated in all of the above
**Drug-Induced Cell Death: Simple Model**

- Toxin enters cell
- Free radical formation increases
- Other pathways activated (JNK/pro-inflammatory cytokines)
- Apoptotic cell death initiated

From: *Anatomy and Physiology of Hearing for Audiologists.*

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**Aminoglycoside Antibiotics**

- Progressive, permanent hearing loss after aminoglycoside treatment, beginning at high frequencies
- In animal subjects:
  - Hair cell death, beginning in base; hearing loss, beginning in high frequencies
  - Free radicals after kanamycin (Jiang et al, 2005) and gentamicin (Takumida et al. 1999; Heinrich et al., 2008; Choung et al., 2009; Jeong et al., 2010)
  - Pro-inflammatory cytokines (TNF-α, IL-1β and TNF-receptor type 1) increased after gentamicin (Bas et al., 2012)
  - Antioxidant system depleted after amikacin (Klemens et al., 2003)

For recent reviews:
Antioxidants reduce aminoglycoside ototoxicity

- D-Methionine
  - Sha et al., 2000 (Gentamicin)
  - Cambell et al., 2007 (Amikacin)
- Ebselen/Selenium
  - Takumida et al., 1999 (Gentamicin)
- N-acetylcysteine
  - Bertolaso et al., 2001 (Gentamicin)
  - Maniu et al., 2011 (Gentamicin)
- Sodium Salicylate
  - Sha & Schacht, 1999 (Gentamicin)
  - Jiang et al., 2005 (Kanamycin)
  - Sha et al., 2006 (Gentamicin, protection shown in humans)
  - Mazurek et al., 2012 (Gentamicin, but NOT neomycin)
- Vitamin E
  - Fetoni et al., 2003, 2004 (Gentamicin)
  - Kharkeli et al 2007: (Gentamicin, NO protection shown in humans)
- Vitamin C
  - Bertolaso et al., 2001 (Gentamicin)
- Co-enzyme Q
  - Fetoni et al., 2012 (Gentamicin)
- Ginkgo Biloba
  - Yang et al., 20121 (Gentamicin)

www.clinicaltrials.gov


Gentamicin ototoxicity reduced

- 140 mg/kg/day x 14 days; animals maintained on standard or supplemented diet
- Hearing loss greater in controls
- Hearing loss more variable in experimental (treated) animals
- Animals with most hearing loss were the animals that lost the most weight
  - They were not consuming the supplemented chow

Partial protection of function and structure

- Hearing loss greater in controls
- Cell death greater in controls

Cisplatin Ototoxicity

- Progressive, permanent hearing loss after cisplatin, beginning in high frequencies
- In animal models:
  - Hair cell death, beginning in base; hearing loss, beginning at high frequencies
  - Free radicals (O₂, H₂O₂, OH) produced
  - Biomarkers related to free radical production (4-HNE) and lipid peroxidation (malondialdehyde) observed
  - Antioxidant system depleted (reduced glutathione, glutathione peroxidase, SOD, catalase)
- Work by Schacht, Rybak, Campbell, and others

For recent reviews:
Antioxidants reduce cisplatin ototoxicity

- D-Methionine
  - Kopke et al., 1997; Campbell et al., 1996, 1999; Korver et al., 2002; Campbell et al., 2003, 2007; Lonito et al., 2011
  - Preliminary presentations of human data suggest benefit
- Ebselen/Selenium
  - Kopke et al., 1997; Rybak et al., 2000; Kim et al., 2009
- Ebselen plus Allopurinol
  - Lynch et al. 2005;
- N-acetylcysteine
  - Feghali et al., 2001; Thomas Dickey et al 2004
- Sodium Salicylate
  - Li et al. 2002; Minami et al. 2004; Hyppolito et al., 2006
- Vitamin E
  - Kalkanis et al., 2004; Sergi et al., 2004; Fettine et al., 2004; Paksoy et al., 2011; Tokgöz et al., 2012
- Vitamin C
  - Tokgöz et al., 2012
- Amifostine
  - Meta-analysis of 4 completed trials did not reveal statistically significant benefit

www.clinicaltrials.gov


What CAN we say now?

HFPTA related to dietary quality

- Choose a healthy diet!
- Higher (better) HEI score related to lower (better) HFPTA
  - Good: 81-100
  - Intermediate: 51-80
  - Poor:<51

Take Home Message

- We can reduce hearing loss induced by noise, drugs, other chemicals, and perhaps aging, in animal models
- We urgently need new drugs and/or other agents
- No “silver bullet”
  - Different therapeutics likely more effective for some insults than others
  - Different people likely to need different treatments
- With human clinical data, new interventions can become possible

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