White Paper

The Science and Research behind Soundbites

soundbites®

NEW STANDARD OF **CARE FOR HEARING**

Introduction

Soundbites is the result of three decades of biomedical research by a small team of University of Michigan Medical School neuroscientists led by Josef M. (Joe) Miller PhD (1937-2017).

This white paper provides a comprehensive overview of the Soundbites scientific R&D journey, divided into the topics below. Some of the content is technical, especially the section on the pathophysiology of SNHL, since the research findings were published in peer reviewed academic medical journals intended for doctors and research scientists, not for general audiences. All the references to the published research are included.

We are sharing this material because we believe it is important for doctors and the general public to have access to the complete Soundbites R&D story in one place.

Please contact doctors@soundbites.com or hello@soundbites.com with questions.

Contents

Cover The University of Michigan School ACEMg team, 2013. From right to left, Susan J. DeRemer, Josef M. Miller, Diane M. Prieskorn, unidentified postdoctoral fellow.

Patents ACEMg is covered by U.S. Patents for treating noise-induced hearing loss (7,951,845, 8,927,528, 8,338,398 and 9,889,156); age-related hearing loss (9,919,008); the hearing loss side effects of aminoglycoside antibiotics (8,338,397); hearing loss caused by certain genetic mutations (9,144,565); hearing loss from congenital cytomegalovirus (10,238,59); and for preventing and treating temporary and permanent tinnitus (9,770,433). All patents held by the University of Michigan and exclusively licensed to Soundbites Public Benefit Corporation. The Soundbites® registered trademark is exclusively licensed to Soundbites PBC.

1. Hearing loss is divided into three categories [1]

Cortical deafness (central hearing loss) is a rare condition caused by damage or disease in the central auditory pathway or in the auditory cortex of the brain.

Conductive Hearing Loss is caused by disorders or diseases that interfere with the transport of sound waves anywhere along the path from the opening of the ear canal through the outer ear to the tympanic membrane (the eardrum), through the tympanic cavity of the middle ear (between the tympanic membrane and the oval window) and into the inner ear.

Sensorineural Hearing Loss (SNHL) occurs within the inner ear (the Cochlea or Organ of Corti) and accounts for about 90% of reported hearing loss. It includes:

- Noise Induced Hearing Loss (NIHL);
- Age-Related Hearing Loss (ARHL or presbycusis);
- Hearing loss caused by the ototoxic side effects of prescription drugs including aminoglycoside antibiotics and several hundred others;
- Hearing loss caused by genetic mutations including GJB2/Connexin 26;
- Hearing loss from congenital cytomegalovirus (cCMV)

2. SNHL was the research motivation

The initial research motivation was to improve our understanding of the causes of SNHL and how it progresses.

Soundbites is known as ACEMg in peer reviewed medical literature. It was developed by a small team of University of Michigan Medical School neuroscientists led by Dr. Joe Miller. Their goal was to preserve normal hearing by understanding the root cause of SNHL. They focused on Noise Induced Hearing Loss (NIHL) because NIHL is the most common type of SNHL.

The research started in 1987. Translational research to get the ACEMg discovery from the lab into the market as a nutritional supplement for hearing preservation started in 2007.

The research was continuously supported by grants from the National Institute of Deafness and Other Communication Disorders (NIDCD) at the US National Institutes of Health (NIH) and the European Commission Directorate for Health Research and Innovation.

When lab research started, medical schools taught that hearing loss was caused by damage to the physical structures of the ear. Explosions and other types of extreme trauma can indeed literally break these delicate structures of the ear. The lab's research revealed that most people do not lose hearing that way.

3. Key finding: SNHL is a metabolic stress disorder

Research discoveries from 1987 to 1998 through empirical observations in animal models revealed a key finding: hearing loss is a metabolic stress disorder and the root cause of that disorder is excess inner ear reactive oxygen species (ROS) – free radicals, unstable oxygen molecules that are a byproduct of metabolism in the mitochondria. Mitochondria transform oxygen and sugar into energy that cells need in a process called mitochondrial respiration.

Free radicals play a key role in diseases and disorders caused by a variety of environmental stresses. Free radicals are essential for normal cellular biochemistry, but excess free radicals lead to diseases and disorders in the ears and elsewhere in the body. They are, for example, important factors in sensorimotor disorders and neurologic diseases associated with the aging process.

Excess inner ear free radicals are the primary underlying trigger for all types of SNHL. Restricted cochlear blood flow is an additional key factor. Normally, the free radical byproducts of metabolism are neutralized and eliminated by antioxidant systems built into cells. Metabolic stress disorder happens when excess free radicals overwhelm cellular antioxidant systems, which creates oxidative stress.

The oxidative stress disrupts normal biochemical processes and leads to damage of mitochondrial DNA and the premature death of inner ear cells in the Organ of Corti. The death of these cells is experienced as hearing loss.

Inner ear oxidative stress can start outside or inside the body. For example, it can start with noise, or with aging, ototoxic drugs or certain hereditary genetic mutations or viruses.

Research by Dr. Miller and others demonstrated that high levels of noise can trigger formation of up to 40 times the normal amount of inner ear free radicals. [2]

This massive over-production of free radical chokes the inner ear blood supply, damages and destroys hearing cells and causes ischemia reperfusion injury, which is similar to having a heart attack or stroke in the inner ear.

4. Key finding: ACEMg blocks the root cause of SNHL

The discoveries were the starting point for the next research goal: eliminate the root cause of inner ear metabolic stress. That goal was reached in 2005 with the discovery of the high potency antioxidant-plus-vasodilator formula ACEMg, which was demonstrated to have the unexpected synergistic beneficial effect of maintaining normal auditory function when noise intensity increases by 30 dB, and to reduce by 75% both noise and drug (aminoglycoside)-induced inner ear pathology and hearing loss. The ACEMg formula was disclosed in the peer reviewed article "Free radical scavengers, vitamins A, C, and E, plus magnesium reduces noise trauma" (Le Prell et al., 2007) [3]

To be clear, human hearing cells do not grow back. ACEMg does not restore hearing or grow new hearing cells. However, ACEMg helps existing auditory cells to continue functioning normally when they would otherwise be injured by oxidative stress. It supplements inner ear antioxidant defense systems, maintaining normal cochlear blood flow and replenishing essential antioxidant nutrients.

5. Summary of key findings, 1987-1998

- Discovery of formation of free radicals in the inner ear following intense noise;
- Surprising quantification of a 40-fold increase in free radical formation in the inner ear following exposure to intense noise;
- Demonstration that modulation of endogenous antioxidant systems modulate the extent of noise-induced pathology (i.e., down-regulation of endogenous systems increases noise-induced pathology);

- Demonstration that additional exogenous antioxidants decrease noise-induced pathology;
- Identification of the source of free radicals reflecting noise-induced demand for energy resulting in increased free radical formation as part of the mitochondrial respiratory chain;
- Modeling of the biochemical cell death pathway from noise to free radical formation to cell death;
- Demonstrating that these findings were similar across a number of different rodent animal models and species;
- Discovery that this model is identical to that across organ systems demonstrating stress-induced free radical formation leading to pathology.

6. Selected peer reviewed research on free radicals and SNHL, 1998-2006

1998

- The antioxidant glutathione prevents noise-induced SNHL: "*Role of glutathione in protection against noise-induced hearing loss"* [4]
- Intense noise upregulated endogenous antioxidant systems: "*Influence of intense sound exposure on glutathione synthesis in the cochlea"* [5]
- Aging increases sensitivity to SNHL: "*Differential protective effects of neurotrophins in the attenuation of noise-induced hair cell loss*" [6]

1999

- A variety of interventions that reduced free radicals also prevented noise-induced SNHL: "*Attenuation of cochlear damage from noise trauma by an iron chelator, a free radical scavenger and glial cell line-derived neurotrophic factor in vivo*" [7]

2000

- Neurotrophins that have antioxidant properties reduce noise-induced SNHL: "*Glial cell line-derived neurotrophic factor has a dose dependent influence on noise-induced hearing loss in the guinea pig cochlea*" [8]
- Not all neurotrophins have similar properties: "*Differential protective effects of neurotrophins in the attenuation of noise-induced hair cell loss*" [9]
- Replicated and extended observation on the protective value of exogenous glutathione for noise-induced SNHL: "*Glutathione limits noise-induced hearing loss*" [10]
- Intense noise causes massive formation of free radicals in the inner ear. Identified the underlying mechanism for the decrease in blood flow: "*Intense noise induces formation of vasoactive lipid peroxidation products in the cochlea*" [11]

2003

- Showed that a variety of agents that prevent free radical induced lipid peroxidation prevent noise-induced SNHL: "*Protection from noise-induced lipid peroxidation and hair cell loss in the cochlea*" [12]
- Described pathway to cell death induced by noise: "*Pathways for protection from noise induced hearing loss*" [13]
- Demonstrated that 8-iso-prostaglandin F2 alpha is a key factor in noise-induced SNHL: "*8-iso-prostaglandin F2 alpha, a product of noise exposure, reduces inner ear blood flow*" [14]

2004

- Described new cell death pathway involved in noise-induced SNHL: "*Calcineurin activation contributes to noise-induced hearing loss*" [15]
- Showed that steroids could attenuate noise-induced SNHL: "*Direct inner ear infusion of Dexamethasone attenuates noise-induced trauma in guinea pig*" [16]
- Described yet another important pathway to cell death from intense noise: "*AIF and EndoG in noise-induced hearing loss*" [17]
- Showed that antioxidant treatment following the noise exposure ("*morning after pill*") could prevent noise-induced SNHL: "*Delayed production of free radicals following noise exposure*" [18]

2006

- Described mechanisms of noise-induced SNHL and biochemical pathways to cell death induced by noise: "*Mechanisms of Noise-Induced Hearing Loss Indicate Multiple Methods of Prevention*" [19]

7. Selected peer reviewed research on free radicals, SNHL and ACEMg, 2007 - 2019

2007

- Identified a new, unique micronutrient formulation of antioxidants-plus- vasodilator (ACEMg) that synergistically, effectively and dramatically prevented stress-induced cell death and protected the inner ear from NIHL: "*Free radical scavengers, vitamins A, C, and E, plus magnesium reduces noise trauma*" [20]
- Described additional important cell death pathways, elaborating the mechanisms involved with noise-induced SNHL: "*Creatine and tempol attenuates noise-induced hearing loss*" [21]

2008

- Described the genes involved with noise-induced SNHL: "Bcl-2 Genes Regulate Noise-Induced Hearing Loss" [22]

2009

- Demonstrated that antioxidant administration reduces inner ear ischemia perfusion injury in Sudden SNHL. "*Vitamin E and vitamin C in the treatment of idiopathic sudden sensorineural hearing loss*" [23]

2011

- Finding that in a small sample (N=8) of military officers undergoing "*urban warfare*" training, requiring firing a high powered rifle in an enclosed space which resulted in a temporary hearing loss, the loss was reduced by 50% when they had taken ACEMg prior to the exercise compared to the hearing loss when they had taken a placebo prior to the exercise. Demonstrated that the ACEMg antioxidant formulation given orally in humans increases plasma levels of the antioxidants: "*Increased vitamin plasma levels in Swedish military personnel treated with nutrients prior to automatic weapon training*" [24]

2014

- Showed that elevated intake of the micronutrients ACEMg reduced the risk of hearing loss: "*Antioxidant vitamins and magnesium and the risk of hearing loss in the US general population*" [25]
- Showed that ACEMg slowed progression of deafness for a boy with GJB2/Connexin 26 mutations: "*ACEMg supplementation ameliorates progressive Connexin 26 hearing loss in a child*" [26]

2015

- Showed that ongoing administration of ACEMg delayed the onset and reduced the level of age-related hearing loss (ARHL). "*Synergistic effects of free radical scavengers and cochlear vasodilators: a new otoprotective strategy for age-related hearing loss*" [27]
- Showed that ACEMg can be beneficial for reducing hearing loss side effect of aminoglycosides: "*Assessment of Nutrient Supplement to Reduce Gentamicin-Induced Ototoxicity*" [28]
- Described potential causes and therapeutic interventions to balance cell damage and survival pathways associated with presbycusis: "*Mechanisms of sensorineural cell damage, death and survival in the cochlea*" [29]

2016

- Showed that an appropriate conditioning noise exposure may reduce a subsequent noise-induced threshold shift: "*Toughening Effect in Wistar Rats: Enhanced Auditory Brainstem Responses Are Related to Calretinin and Nitric Oxide Synthase Upregulation*" [30]
- Further to the 2014 Green et al Connexin 26 case study: "*ACEMg Diet Supplement Modifies Progression of Hereditary Deafness*" [31]
- Edited collection of articles examining the state of free radical biology and its impact on otology, laryngology, and head and neck function, published as Free Radicals in ENT Pathology, Josef Miller, Colleen G. Le Prell, Leonard Rybak, Editors.

2019

- Mitochondria-Targeted Antioxidants for Treatment of Hearing Loss: A Systematic Review [32]

  From the abstract: Mitochondrial dysfunction is associated with the etiologies of sensorineural hearing loss, such as age-related hearing loss, noise- and ototoxic drug-induced hearing loss, as well as hearing loss due to mitochondrial gene mutation. Mitochondria are the main sources of reactive oxygen species (ROS) and ROS-induced oxidative stress is involved in cochlear damage. Moreover, the release of ROS causes further damage to mitochondrial components. Antioxidants are thought to counteract the deleterious effects of ROS and thus, may be effective for the treatment of oxidative stress-related diseases. The administration of mitochondria-targeted antioxidants is one of the drug delivery systems targeted to mitochondria. Mitochondria-targeted antioxidants are expected to help in the prevention and/or treatment of diseases associated with mitochondrial dysfunction.

8. The pathophysiology of SNHL

Growing knowledge of the role of free radical biology in other diseases and pathologies lend credence to their important role in SNHL. Free radical formation associated with environmental stress from intense noise or sound energy, drugs, aging and trauma play a key role in hearing loss and cell death in the inner ear, as free radicals function as triggers to upregulate necrotic and apoptotic pathways to cell death. Specifically, free radical formation defines a final common cell death pathway inducing pathology from a large variety of etiological factors. Moreover, for the ear and hearing, these factors are potentiated by reduced organ circulation, which is a dramatic feature of noise-induced stress and a significant feature of the aging process.

Free radicals play a key role in pathology from a variety of environmental stress factors. While free radicals are essential for normal cellular biochemistry, in excess free radicals lead to pathology and are important factors in sensorimotor disorders, as well as other neurologic diseases associated with the aging process. Free radicals function as triggers to upregulate necrotic and apoptotic pathways to cell death. They may be either generated as part of the metabolic processes (Ames et al, 1993) of the cell or frequently as side effects of environmental stress factors, such as visible light (Agarwal et al.,1993; Oleinick and Evans, 1998 [33]) solar and ionizing radiation (Godar and Lucas, 1995; Godar, 1999; [34] Zhao, et al.; 2007), cigarette smoke (Aoshiba et al. 2001 [35]) hyper- and hypoxia (Budinger et al., 2002; Wang et al., 2003), drugs (Forge and Schacht, 2000; [36] Rybak and Ramkumar, 2007 [37]), or intense noise exposure (Ohinata et al., 2003; [38] Le Prell et al., 2007 [3]). Models detailing the pathways to cell death initiated by free radicals have been developed, across a broad range of pathologies in the peripheral and central nervous systems; and importantly the enhanced production of free radicals secondary to cell metabolism and environmental stress agents with aging has been well documented (for review see: Ryter et al., 2007 [39]).

Given knowledge of the biochemical pathways to cell death, there are a number of sites along any of these pathways that could block cell death; for example, by blocking upregulation of one of the caspases or by inserting Bcl-2 genes into the mitochondrial membrane, or perhaps blocking release of cytochrome c. However, these are each parallel pathways to cell death and if one is blocked others may take its place.

Free radical formation is associated with environmental stress from intense noise, drugs, aging and trauma play a key role in hearing loss and cell death in the inner ear. Moreover, for the ear and hearing, these factors are potentiated by reduced organ circulation (Miller, et al., 2003; [14] Le Prell et al., 2007 [40]), which are dramatic features of SNHL. Evidence on the key role of free radicals in cell death in these many fields provided strong theoretical and empirical support for the likelihood of their importance in hearing impairment.

The mitochondria are the source of cellular energy for normal homeostasis and function. In the process of their normal function, mitochondria produce both molecular oxygen and partially reduced forms of oxygen, which become reactive oxygen species (ROS), the pathway to the production of free radicals.

Reactive nitrogen species (RNS) is another pathway to the production of free radicals. Both ROS and RNS act similarly to trigger necrotic or apoptotic cell death pathways (Figure 1).

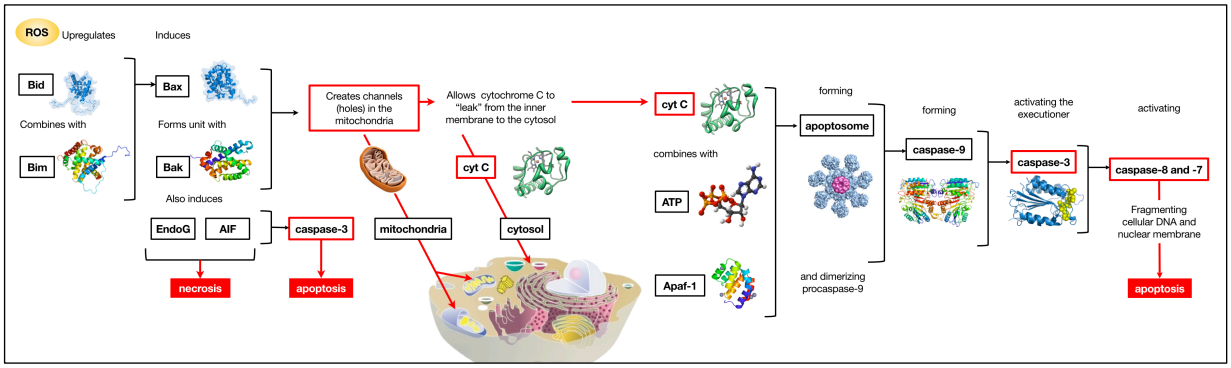

Figure 1. The genetic cascade of ROS-induced cell death

The electron transport chain of mitochondria ends with cytochrome c and an oxidase-dependent tetravalent reduction of oxygen to form water. In this process redox carriers leak additional electrons to oxygen generating free radicals. Some free radicals are essential for normal cellular processes. If there are excess free radicals, built-in antioxidant systems reduce or scavenge the excess, detoxifying the cell.

However, with cellular stress, such as intense noise or direct surgical trauma, there is an increase demand for energy to maintain a greater level of metabolic activity required of the cell under stress. The ROS-initiated cascade can lead to necrosis or apoptosis; and induces mitochondrial dysfunction, disrupting the cell's respiratory chain, creating more ROS leading to apoptotic cell death.

In response to that demand mitochondria produce more energy (ATP) and with that generate excess free radicals. With intense noise exposure, a remarkable 40-fold increase in free radical formation in the tissues of the inner ear has been quantified (Ohinata el al., 2000).[10] Such vast amounts of free radicals overwhelm the endogenous antioxidant system and initiate processes that damage the cell. While mitochondrial activity likely accounts for the major increase in free radical formation with noise, additional sources include excitotoxic events in the hyper excited cochlear nerve, and ischemia/reperfusion, or reperfusion injury, which occurs with intense noise exposure in the inner ear.

Excess free radicals may cause cell death by initiating lipid peroxidation of nuclear and cell membranes, destroying the integrity of the cell and leading to necrotic cell death (necrosis), the likely the path to cell death in the presence of extreme concentrations of free radicals. In the presence of less extreme excess, free radical cell death is likely by apoptotic mechanisms. Thus free radicals may up regulate genetic pathways that lead to programmed, apoptotic, cell death via a number of biochemical pathways.

Oxidative stress first initiates an influx of calcium leading to calcium-dependent calcineurin/calpain activation, initiating dephosphorylation of NFAT and activation of the Bcl-2 family

gene Bad. Bad causes release of cytochrome c, activation of caspases 9 and 3, and cell death. Second, a caspase-2 dependent pathway to cell death can be triggered by free radical-induced DNA damage. Third, caspase-independent pathways to cell death include release of AIF and EndoG from the mitochondria. Translocation of EndoG to the cell nucleus results in chromatin condensation (prophase) and high-molecular mass-chromatin fragmentation and cell death. Fourth, receptor-mediated cell death is initiated with ligation of death receptors on the surface of the cell, forming a death inducible signaling complex, which activates pro-caspase-8. Caspase-8 activates caspase-3, leading directly to cell death, and/or cleaves the gene Bid, resulting in translocation and insertion of the Bax-Bak complex into the mitochondrial membrane and release of cytochrome c, in turn activating caspases 9 and 3, and cell death. The caspase-2 dependent pathway differs from the caspase-8 and caspase-9 dependent pathways in that pro-caspase-2 is activated by DNA damage. Upregulation of a number of these pathways have been demonstrated in laboratories in the noise-stressed inner ear, and the efficacy of interventions that block them have been demonstrated (Minami et al., 2004 [15] Yamashita et al., 2004,[17] Le Prell et al., 2007,[40] Minami et al., 2007,[21] and Yamashita et al., 2008 [22]).

This model of the biochemistry of free radical initiated cell death is entirely consistent with similar models of free radical initiated pathology in the cardiovascular system, brain and stress induced cell death from the many etiologies mentioned above.

9. Solving the problem – Pharmacological research on SNHL preventive care interventions

The finding that up-regulated reactive oxygen species (ROS) in the cochlea triggers SNHL by apoptotic cell death has driven interest in a new opportunity to use antioxidants as a pharmacological intervention for hearing preservation. ACEMg is among those candidates, and the first to reach the market.

Other candidates include D-methionine [41, 42, 43]; LN Acetylcysteine (L-NAC) [44] and N Acetylcysteine (NAC) [45]; Ebselen (novel GPx1 mimic) [46]; Salicylate with L-NAC [47] and Salicylate with trolox (Vitamin E) [48]; resveratrol [49]; allopurinol [50]; and R-phenylisopropyladenosine (R-PIA).[51] Available evidence suggests none of these compounds are currently under investigation.

10. The ACEMg/Soundbites solution

In 2007, the antioxidant-plus-vasodilator formulation of β-carotene (converted in the body to vitamin A), ascorbic acid (vitamin C), trolox (vitamin E) and the vasodilator magnesium became known in the peer reviewed medical literature as ACEMg with the publication of the peer reviewed paper disclosing the formula and demonstrating its unexpected synergistic beneficial effect of maintaining normal auditory function when noise intensity increases by 30 dB, and to reduce inner ear pathology for noise by 75% (Le Prell et al, 2007).[3]

Subsequently, an urban warfare training clinical study demonstrated temporary hearing loss was reduced by 50% in participants in the pre-treated cohort compared to the control group (Le Prell et al, 2011) [52]. Elevated intake of the micronutrients in the antioxidant/vasodilator

formula were demonstrated to reduce the risk of hearing loss in the US general population (Choi et al, 2014) [53]. The formula was demonstrated to slow the progression of deafness for a boy with GJB2/Connexin 26 mutations (Thatcher et al, 2013) [54]; to delay the onset and reduce the severity of age-related hearing loss (Alvarado et al, 2015) [55]; to be beneficial for reducing hearing loss side effect of aminoglycosides (Le Prell et al, 2012 and 2015); [56] to modify the progression of hereditary deafness (Green et al, 2016) [57]; and to modify the progression of hearing loss caused by congenital cytomegalovirus (cCMV), the most common type of infectious sensorineural hearing loss. [71]

The primary antioxidant action of β-carotene (metabolized to vitamin A in vivo) is to scavenge singlet oxygen. Because singlet oxygen reacts with lipids to form lipid hydroperoxides, the removal of singlet oxygen prevents lipid peroxidation (for review, see Schafer et al. 2002 [58]).

Vitamin E, present in the lipid compartments of cells, is a donor antioxidant that removes free radicals from the lipid compartments, also reacting with and reducing peroxyl radicals, inhibiting the spread of lipid peroxidation.

Vitamin C detoxifies by scavenging oxygen radicals in the aqueous phase (Niki 1987a [59]; Niki 1987b [60]). Vitamin C also blocks lipid peroxidation by free radicals that 'escape' neutralization by antioxidant vitamins A and E (for review, see Evans, Halliwell 1999 [61]).

In most tissues, increased metabolism increases blood flow, which provides additional oxygen. However, intense stimulation of hair cells reduces blood vessel diameter and red blood cell velocity and thus decreases cochlear blood flow (Miller et al. 2002 [62]; for review, see Le Prell et al. 2007 [63]). Reduced cochlear blood flow has significant implications for metabolic homeostasis, as cellular metabolism clearly depends on adequate supply of oxygen and nutrients as well as efficient elimination of waste products (e.g., Miller et al. 1996).

Vasoconstriction in the cochlea is caused by the release of a powerful vasoconstrictor, the isoprostane 8-iso prostaglandin F2 alpha. In addition, the reduction in blood flow is followed by a rebound effect – an 'overshoot' – causing reperfusion injury-induced formation of additional free radicals, which synergistically add to those formed previously. Supplemental magnesium reduces vasoconstriction and reperfusion injury, which attenuates SNHL. (for review, see Le Prell et al. 2007 [63]).

In addition to well-known effects of magnesium on blood flow, other biochemical mechanisms may further contribute to the protective effects of Mg. Magnesium modulates calcium channel permeability, influx of calcium into cochlear hair cells, and glutamate release (Cevette et al. 2003 [64], Gunther et al. 1989 [65];), each of which may reduce SNHL. Magnesium is also a NMDA-receptor antagonist. That the NMDA-receptor antagonist MK-801 reduces the effects of noise, ischemia, or ototoxic drugs (Janssen 1992 [66]; Basile et al 1996 [67]; Duan et al. 2000 [68]; Konig et al 2003 [69]; Ohinata et al 2003 [70A]), suggests another potential protective mechanism for Mg.

Hence, each of the components in the antioxidant/vasodilator formula ACEMg provides a necessary mechanism of action, which, when combined, results in a prophylactic compound that blocks the initiating biological events that trigger SNHL.

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