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REVIEW ARTICLE

# HEARING LOSS: WHAT'S IN THE PIPELINE?

*G. Chen, E. Lobarinas, D. Ding and R. Salvi*

Center for Hearing and Deafness, University at Buffalo, Buffalo, New York, USA

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## SUMMARY

*Hearing loss due to aging, hereditary factors, noise exposure, ototoxic drugs and infection is a major healthcare problem. While the causative agents of hearing loss are diverse, many share common sequelae involving oxidative stress, generation of reactive oxygen and nitrogen species, depletion of antioxidant enzymes and signaling pathways leading to apoptosis or necrosis. During the past decades, a host of new strategies for preventing hearing loss have been evaluated. Animal studies have identified a variety of exogenous antioxidants or compounds that enhance antioxidant defenses, providing significant protection. An alternative therapeutic approach involves the use of small molecules to suppress downstream signaling pathways involved in apoptosis. Since many insults lead to inflammation, a third approach has focused on antiinflammatory drugs, some of which suppress the immune system. Finally, growth factors and neurotrophic factors represent a new method to protect and promote the survival of hair cells and neurons in the inner ear. While a great deal is now known about the efficacy of individual compounds, future efforts might benefit from a multifactorial approach involving therapeutic "cocktails" that optimize the degree of protection against age-related hearing loss and other ototraumatic insults.*

## INTRODUCTION

Intense noise, ototoxic agents and aging are all known to cause hearing loss. Approximately 28 million Americans suffer from hearing loss, making it one of the most prevalent health problems, especially among the elderly (1). In the vast majority of cases hearing loss results from death or dysfunction of sensory hair cells and spiral ganglion neurons in the inner ear due to a variety of ototraumatic insults or metabolic impairment. The delicate anatomical structures within the human inner ear make it very vulnerable to loud sound and toxic chemicals. Loud sounds produce enormous mechanical stress on the organ of Corti, which contains the sensory hair cells that convert mechanical motion into neural activity. The delicate stereocilia that reside at the apical surface of the sensory hair cells can be sheared off or damaged by intense acoustic overexposure. The high rate of metabolic activity in the sensory hair cells and neurons within the inner ear requires a steady and reliable blood supply. Drastic changes to blood flow or toxic chemicals taken up by the metabolically active cells of the inner ear can lead to disastrous and permanent damage. The sensory hair cells make synaptic contact with the peripheral afferent fibers of the spiral ganglion neurons, whose centrally projecting axons form the auditory nerve. Intense acoustic stimulation or ischemia can cause the excessive release of excitatory neurotransmitters, presumably glutamate, from the hair cells, leading to excitotoxic damage to the postsynaptic afferent nerve terminals, a process referred to as excitotoxicity. Additional factors such as infections of the inner ear or genetic disorders also contribute to hearing loss.

Over the past two decades, there has been a growing interest in identifying otoprotective compounds that could be administered systemically or directly to the inner ear. While a few agents are in the early stages of clinical trials, most are only at the preclinical stage of development involving animal experimentation. Some agents are primarily studied to gain insight into the underlying mechanisms of hearing loss, while others are aimed directly at the development of clinical treatments. Currently, there are no drugs approved by the FDA for the prevention of hearing loss (2). Therefore, this manuscript will attempt to critically review most of the therapeutic compounds reported to have otoprotective effects and to discuss their proposed protective mechanisms.

## FREE RADICALS

Under normal physiological conditions, approximately 0.2% of total oxygen consumption is converted into highly reactive oxygen species (ROS) that are toxic to cells (3, 4). Fortunately, most of these ROS are quickly eliminated by a variety of protective endogenous enzymatic and nonenzymatic actions that maintain a delicate balance between ROS production and ROS scavenging. However, this balance may be disrupted during periods of high stress when ROS production exceeds ROS inactivation, resulting in damage to proteins, RNA and DNA, which in turn leads to cell death. Direct supplementation with exogenous antioxidants or enhancing endogenous

**Correspondence:** Richard Salvi, PhD, Center for Hearing and Deafness, University at Buffalo, 137 Cary Hall, 3435 Main St., Buffalo, NY 14214, USA. E-mail: salvi@buffalo.edu.

antioxidant production may promote cell survival by restoring the balance between ROS production and scavenging. Cellular damage can also occur from a family of reactive nitrogen species (RNS) formed from superoxide ($O_2$•$^-$) and nitrous oxide (•NO) that results in peroxynitrite ($ONOO^-$). Peroxynitrite can lead to the production of other RNS that damage DNA, amino acids and lipids. Suppressing the production of RNS can protect cells from various forms of damage (5).

Stress conditions that result in high levels of intracellular calcium are also toxic to cells. For example, high levels of sound stimulation can lead to the release of large amounts of the excitatory neurotransmitter glutamate, which can activate postsynaptic NMDA receptors, causing excess influx of calcium into spiral ganglion neurons (SGNs) in the inner ear (6). Other pathological conditions can result in elevated intracellular calcium levels, which can activate proteases (e.g., calpains), leading to cell injury (7-9).

Otoprotective agents act at different stages to suppress calcium influx, prevent the activation of proteases or promote scavenging of RNS. Under conditions of high stress, cells can initiate apoptotic cell death cascades, resulting in systematic disassembly of the cell. Otoprotective agents can act downstream and suppress or block these cell death pathways, thereby promoting cell survival. Finally, growth factors and neurotrophic factors which can activate a broad range of cell signaling pathways have shown promise as otoprotective compounds.

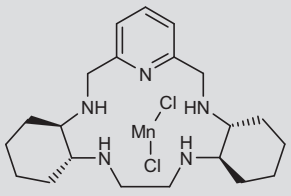
## OTOPROTECTIVE AGENTS AND MECHANISMS

### Preventing free radical production and accumulation

ROS are highly reactive due to the presence of unpaired valence shell electrons. ROS are a natural byproduct of normal oxygen metabolism. Chemical reactions occurring during the mitochondrial electron transfer chain (ETC) lead to low-level release of electrons from their normal path to molecular oxygen ($O_2$) to form superoxide anions (10). Superoxide dismutase (SOD) converts most of the highly toxic superoxide anions to $H_2O_2$ and $O_2$ (11). $H_2O_2$, which is also damaging, is detoxified by catalase to form $H_2O$ and $O_2$, or alternatively, reduced to hydroxyl anions and to highly reactive hydroxyl radicals (OH•). Cells contain an array of endogenous defenses against ROS, including antioxidant enzymes such SOD, catalase, glutathione peroxidase, thioredoxin reductase and other molecules (e.g., glutathione, thioredoxin, thiols, vitamin C and vitamin E). ROS levels can increase dramatically in the inner ear during environmental stress, as exemplified by exposure to intense noise or ototoxic compounds. During periods of extreme oxidative stress, antioxidant defenses are overwhelmed, resulting in significant cellular damage. Antioxidant compounds that protect the inner ear from oxidative stress are discussed below.

#### *Imisopasem manganese (M-40403)*

M-40403 is a synthetic manganese-containing SOD mimetic that has shown excellent efficacy in a variety of therapeutic applications. It reproduces the beneficial and highly selective action of natural SOD and is well suited as a therapeutic drug because it: 1) has a much lower molecular weight than native SODs and is able to permeate cell membranes; 2) is stable with a long half-life; and 3) does not trigger an immune response. When tested in vitro using cochlear organotypic cultures, M-40403 provided significant protection against hair cell death induced by gentamicin, an aminoglycoside antibiotic, and paraquat, a herbicide that generates superoxides (12, 13). However, M-40403 failed to protect against hair cell death induced by cisplatin, an ototoxic platinum-based antineoplastic agent (13).

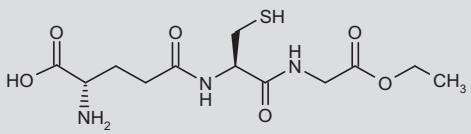



Imisopasem manganese

#### *Glutathione monoethyl ester*

Glutathione (GSH L-γ-glutamyl-L-cysteinyl-glycine), one of the most important antioxidants in the body, is synthesized intracellularly from three amino acids: glutamate, cysteine and glycine. The production of GSH is typically limited by the availability of cysteine, the sulfhydryl (thiol) group that serves as an electron donor and is responsible for its biological activity and ROS scavenging. Within the cell, GSH is oxidized by oxidants such as ROS and forms glutathione disulfide (GSSG). The oxidized form of glutathione is rapidly reduced by glutathione reductase. In fact, intracellular glutathione is found almost exclusively in its reduced form, GSH. The ratio of GSSG to GSH within tissue is often used as a measure of cellular oxidative stress.

Reducing intracochlear levels of glutathione by blocking its synthesis or maintaining a low-protein diet potentiates cochlear damage induced by intense noise or ototoxic agents (14-16). Conversely, oral supplementation of GSH significantly reduced gentamicin-induced hearing loss by 20-40 dB (17). Glutathione monoethyl ester (GSH-MEE) is a cell-permeable derivative of GSH that undergoes hydrolysis by intracellular esterases, thereby increasing the intracellular concentration of GSH. In a cisplatin ototoxicity protection study,




Glutathione monoethyl ester

treatment with GSH-MEE was more effective in preventing hearing loss than direct supplementation with GSH (18). Similarly, treatment with GSH-MEE reduced impulse noise-induced hearing loss by about 15 dB (19). GSH-MEE treatment in animals with low GSH levels induced by a low-protein diet reduced noise-induced hearing loss by 20-30 dB (15, 16). However, as a precaution, very high doses of GSH-MEE on their own may have toxic effects by inducing vasoconstriction and ischemia, a side effect of endogenous NO suppression (18, 19).

### *N-Acetyl-L-cysteine (L-NAC)*

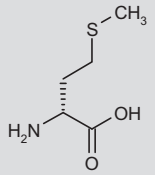
L-NAC (also acetylcysteine, *N*-acetylcysteine) is a cysteine (thiol-containing amino acid) derivative. It is used as a nutritional supplement and has passed stringent drug safety requirements by the U.S. Food and Drug Administration (FDA) for prescription use. L-NAC is used clinically as an antidote for acetaminophen overdose, which leads to depletion of hepatocyte GSH (20). Administration of L-NAC rapidly replenishes liver GSH. L-NAC is metabolized in the gut to cysteine and serves as a precursor for GSH synthesis. In addition, L-NAC is reported to be a direct scavenger of hydroxyl radicals, hydrogen peroxide and hypochlorous acid (21).

A number of studies report that L-NAC significantly reduces noise-induced hair cell loss and hearing loss by about 10-30 dB (22-30). L-NAC has also been found to reduce hearing loss and hair cell loss in vitro and in vivo due to cisplatin, carboplatin, gentamicin and styrene ototoxicity (31-35). However, other studies have reported that L-NAC fails to protect against noise-induced hearing loss (36-38). It is noteworthy that at least two of the studies that failed to show protective effects for L-NAC used less aggressive dosing regimens than those that found protection. For instance, in contrast to Bielefeld, who used an aggressive 10-dose regimen that included pre- and post-noise L-NAC treatments, Davis only used a single pretreatment dose. On the other hand, Hamernik, who also failed to find a protective effect of L-NAC, used noise exposures that were significantly longer (8 hours/day, 5 days) than other studies that found protection. Thus, the putative protective effects of L-NAC against noise-induced hearing loss are still unclear and may depend on the dosing regimen and the extent and intensity of the noise used to induce the hearing loss.

### *D-Methionine (D-Met)*

D-Met [also (*R*)-2-amino-4-(methylsulfanyl)butyric acid, D-2-amino-4-(methylthio)butanoic acid] is recommended by the World Health Organization (WHO) as an antidote for acetaminophen overdose, although in the U.S. L-NAC is more commonly used (39). D-Met amino acid exists in dietary protein and is particularly high in fermented proteins such as cheese and yogurt because fermentation transaminates the L- to the D-isomer (40). Methionine is reversibly oxidized and can serve as a free radical scavenger (41). D-Met increases intracellular glutathione levels (42, 43). The two major determinants of GSH synthesis are the availability of cysteine and the activity of GSH synthetase. The availability of cysteine is dependent on the membrane transport of three sulfur amino acids, cysteine, cystine and methionine, and the conversion of methionine to cysteine through the *trans*-sulfuration pathway. D-Met also acts as a sulfur-containing nucleophile and thus protects sulfur-containing enzymes and proteins.

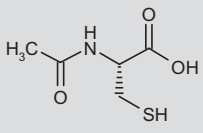
D-Met has been repeatedly shown to prevent hearing loss and hair cell loss induced by cisplatin, carboplatin (2, 44-49), aminoglycoside antibiotics (2, 50) and intense noise exposure (2, 51, 52). Cisplatin-induced hearing losses of up to 40 dB and noise-induced hearing loss of up to 20 dB were found to be completely prevented by D-Met treatment (2). While D-Met reduces the risk of cisplatin ototoxicity, some studies suggest that it does so by lowering the systemic levels of the drug, thereby potentially reducing its antitumor efficacy (53). However, other reports suggest that D-Met does not suppress the antitumor efficacy of cisplatin (54).



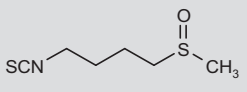

D-Methionine

### *Sulforaphane (SF)*

SF (also sulforaphane glucosinolate [SGS]) is classified as an isothiocyanate. SF, an organosulfur compound that exhibits anticancer, antidiabetic and antimicrobial properties, is present in cruciferous vegetables such as broccoli and cauliflower. The enzyme myrosinase transforms glucoraphanin, a glucosinolate, to sulforaphane upon




Acetylcysteine




Sulforaphane

oral consumption of these vegetables. As a naturally occurring isothiocyanate (55), it showed a protective effect against retinal degeneration in *Tub* mutant mice (56). The protective effects of SF treatment are related to enhanced thioredoxin (Trx) and thioredoxin reductase (TR) expression (56-60). Trx and TR, along with NADPH, comprise an important cellular redox system (61-63). Trx is characterized by a redox active site with the sequence of -Trp-Cys-Gly-Pro-Cys-Lys-. The two cysteine residues within the redox active center provide the sulfhydryl groups involved in the reducing activity. Trx is oxidized to Trx-S2 and is subsequently reduced to Trx-(SH)2 by TR in the presence of NADPH. The Trx/TR then serves as a system for free radical scavenging.

Treatment with sulforaphane has been found to prevent *Tub*-related decreases in Trx and TR expression and *Tub*-related increases in caspase-3 expression at both the mRNA and protein levels in the cochlea (57). *Tub*-related hair cell loss was significantly less in SF-treated mice than in control animals. Sulforaphane therefore appears to be an effective compound for suppressing a specific form of genetic hearing loss, but further work is needed to determine if it is effective in preventing hearing loss from aging, noise or ototoxic drugs.

### *Coenzyme $Q_{10}$ ($CoQ_{10}$)*

$CoQ_{10}$ (also ubiquinone, ubidecarenone, coenzyme Q, CoQ, Q10, Q) is a mobile electron carrier in the mitochondrial electron transfer chain (ETC) that is mainly responsible for the production of ATP. $CoQ_{10}$ carries electrons from the ETC complex-I/complex-II to complex-III. The $CoQ_{10}$ is then reduced to its active ubiquinol form and serves as an effective antioxidant that prevents lipid peroxidation and mitochondrial damage (64). Aging and certain chronic diseases cause a decrease in the levels of $CoQ_{10}$, leading to decreased cellular function and metabolism. Natural $CoQ_{10}$ is practically insoluble in water and thus exhibits poor bioavailability. A water-soluble coenzyme $Q_{10}$ formulation (Q-TER®) is available with a suitable carrier and bioactivator (65). The resulting composite is approximately 200 times more soluble in water than $CoQ_{10}$ and retains its antioxidant capacity (66).

Supplementation with $CoQ_{10}$, especially Q-TER®, has been reported to reduce noise-induced hearing loss by about 30-40 dB in animals (67) and to decrease age-related hearing loss by about 3-5 dB in humans (68). Others have found that long-term dietary supplement with $CoQ_{10}$ greatly reduced hearing loss and cochlear pathology in C-57 mice that bear age-related hearing loss genes.

Unfortunately under certain circumstances, $CoQ_{10}$ may become a pro-oxidant. These circumstances are typically associated with hypoxia or lack of oxygen. In cases of shock, heart attack, stroke or poor circulation, $CoQ_{10}$ auto-oxidizes and unleashes massive amounts of free radicals that damage delicate tissues.

### *Idebenone*

Idebenone, a synthetic analogue of $CoQ_{10}$, is a relatively safe and potent antioxidant. However, unlike $CoQ_{10}$, idebenone suppresses free radicals and continues to promote ATP production under hypoxic conditions. This may make idebenone a useful supplement for individuals at risk for the conditions associated with poor circulation noted above. Idebenone has been reported to have beneficial effects in patients with mitochondria-related diseases (69). Hearing loss prevention studies with idebenone have thus far been limited to noise exposure. In these studies, idebenone was shown to significantly reduce hearing loss in noise-exposed guinea pigs and decrease hair cell loss and the number of apoptotic-labeled cells (70, 71).

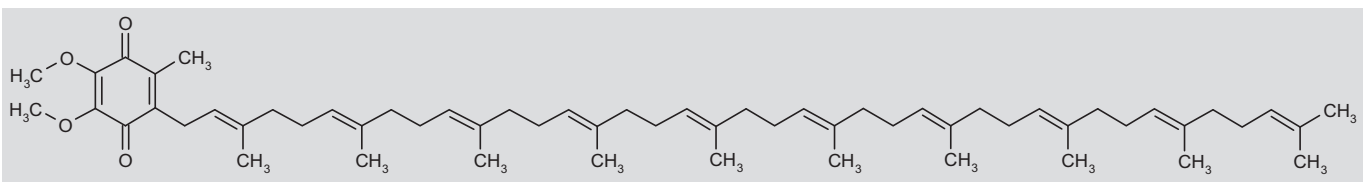
### *Phenyl N-tert-butylnitrone (PBN)*

PBN has been used as a spin-trapping agent in free radical research (72), and more recent studies have shown that PBN can prevent oxidative stress in vitro and in vivo, including restoration of age-related changes in the brain (73) and reduction in mortality-associated endotoxin shock (74, 75). Its protective effect on hearing loss has also been explored. Application of PBN, its analogue POBN (α-[4-pyridyl-1-oxide]-*N*-*tert*-butylnitrone) or its metabolite 4-hydroxyphenyl-*N*-*tert*-butylnitrone significantly reduced noise-induced hearing loss by approximately 30 dB (76-79) and reduced hearing loss induced by carbon monoxide (80). PBN also attenuated loss of cochlear function induced by local application of aminoglycoside antibiotics and systemic administration of lead acetate and tetraethyl lead (81, 82).

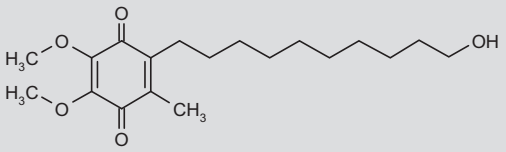
### *Aspirin (acetylsalicylic acid) and salicylate*

Aspirin (acetylsalicylic acid), synthesized in the 1860s, is one of the most widely used antipyretic, analgesic and antiinflammatory drugs. Salicylate, the active component of aspirin, acts as a potent antioxidant that can inactivate the hydroxyl and superoxide radical (83-87).

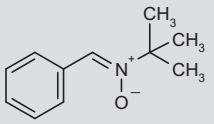
It has long been known that very high doses of salicylate can cause reversible hearing loss and tinnitus (88). This may be due in part to its ability to block outer hair cell (OHC) electromotility by binding to prestin, the OHC motor protein (89, 90). On the other hand, more recent studies indicate that lower doses of salicylate can protect




Coenzyme $Q_{10}$



Idebenone



PBN

against a variety of ototraumatic insults. Administration of salicylate during cisplatin treatment attenuated the cisplatin-induced hearing loss by as much as 15 dB, greatly reduced the amount of hair cell loss, and also protected against nephrotoxicity (91). In a more recent study, however, salicylate failed to prevent cisplatin-induced loss of distortion product otoacoustic emissions (DPOAE) even though salicylate reduced the amount of OHC damage (92). Salicylate also attenuated gentamicin-induced hearing loss in animals by more than 40 dB and greatly reduced the amount of hair cell damage, without affecting the antibacterial action of gentamicin (93). Similarly, aspirin was found to attenuate gentamicin-induced ototoxicity in humans (94, 95). The combined applications of salicylate plus Trolox, a water-soluble analogue of vitamin E, and salicylate plus L-NAC showed a protective effect against noise-induced hearing loss; the hearing loss reductions ranged from 20 to 30 dB (22, 96, 97). In contrast, when just salicylate was administered during noise exposure, four studies found that salicylate offered no protection against noise-induced hearing loss or hair cell loss (98-101). It is possible that salicylate may acts as a pro-oxidant under some circumstances (102). Indeed, it exacerbated noise-induced hearing loss in an early observation in humans (103) and animals (104). In addition, one recent study in rats showed a permanent auditory functional loss after chronic high-dose salicylate treatment (105).

### *Vitamin E*

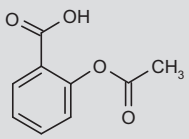
Vitamin E (also Alpha E, Amino-Opti-E, Aquasol E, Aquavite-E, Centrum Singles-Vitamin E, E Pherol, E-400 Clear, Nutr-E-Sol) is a fat-soluble antioxidant that blocks the production of ROS and RNS formed when fat undergoes oxidation. The term vitamin E describes a family of eight antioxidants: four tocopherols (alpha, beta, gamma and delta) and four tocotrienols (alpha, beta, gamma and delta). alpha-Tocopherol is the only form of vitamin E that is actively maintained in the human body; therefore, it is the form of vitamin E found in the largest quantities in blood and tissue. Because alpha-tocopherol has the greatest nutritional significance, it is the only form that is listed under the Recommended Dietary Allowance (RDA) for vitamins.

Vitamin E application has been shown to attenuate cisplatin-induced hearing loss by approximately 15-40 dB (106, 107). One study found that vitamin E alone provided some protection against noise-induced hearing loss (71). However, another study showed that a significant protective effect against noise-induced hearing loss and hair cell loss occurred only when vitamin E was applied in combination with magnesium (108). The combined application of vitamin E and magnesium attenuated noise-induced hearing loss by about 20 dB. Interestingly, treatment with vitamin E plus vitamin C in patients with sudden hearing loss improved hearing recovery after therapy with steroids (109).

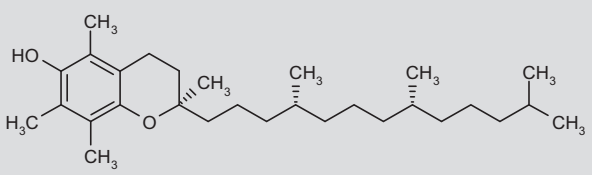
### *Vitamin C*

Vitamin C (also L-ascorbic acid, L-ascorbate) is a six-carbon lactone that is synthesized from glucose in the liver of most mammalian species, but not in humans, who lack the synthesizing enzyme gulonolactone oxidase. Therefore, humans generally obtain their vitamin C from the consumption of fruits and vegetables. Vitamin C is an electron donor, donating two electrons from a double bond, thereby preventing other compounds from being oxidized (110).

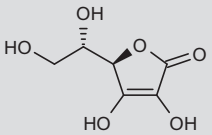
Treatment of noise-exposed rabbits with vitamin C reduced lipid peroxidation and oxidative damage to proteins and prevented the decline of free radical scavengers in blood (111). In addition, vitamin C partially reduced the decline in transient otoacoustic emission amplitudes. Guinea pigs, like humans, cannot produce vitamin C (110) and thus provide a relevant model for studying the protective effect of vitamin C on noise-induced hearing loss. When guinea pigs were fed with either a low or high dose of vitamin C and exposed to



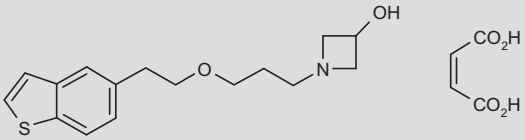
Acetylsalicylic acid



alpha-Tocopherol




Vitamin C




T-817MA

intense noise, the hearing loss in the group receiving the high dose of vitamin C was about 10 dB lower than that in the control group or low-dose vitamin C group (112, 113). While the preceding animal studies are encouraging, a survey in humans found no significant association between vitamin C intake and risk of hearing loss (114). While some animal studies suggest that high doses of vitamin C may protect against noise-induced hearing loss, further work is needed to determine the effectiveness of this line of treatment in humans.

### *Amifostine hydrate (WR-2721)*

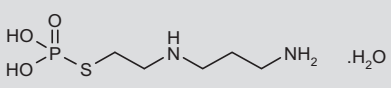
Amifostine is an organic thiophosphate that has been extensively used as a chemical radioprotector in cancer radiotherapy and chemotherapy (115, 116). Amifostine is dephosphorylated to the active metabolite WR-1065, an oxygen free radical scavenger which prevents the formation of platinum–DNA adducts (117). Its protective effect may also be attributed to modulation of GSH (118).

Amifostine is probably the only otoprotective agent that has reached clinical practice thus far. Unfortunately, in two randomized trials, the otoprotective effect of amifostine was not significant (119). However, other studies suggest that amifostine provides significant otoprotection against radiation and chemotherapeutic agents (120, 121). Amifostine provided a dose-dependent rescue from cisplatin ototoxicity in hamsters. No protection was observed at the low dose of 18 mg/kg, moderate protection was seen at 40 mg/kg, and nearly complete protection occurred at 80 and 400 mg/kg (122, 123). However, doses of 40 mg/kg or higher caused neurotoxicity and prolonged auditory brainstem response (ABR) interpeak latencies (122).

### *T-817MA*

T-817MA is a neuroprotective and neurotrophic compound developed for the treatment of neurodegenerative disorders such as Alzheimer's disease. It has been found to attenuate $H_2O_2$-induced neuronal cell death in cortical neuron cultures by preventing $H_2O_2$-induced decreases in GSH. In addition to preventing oxidative stress, T-817MA has also been found to have neurotrophic effects, including promotion of neurite outgrowth in hippocampal slice cultures (124).

Given that T-817MA suppresses oxidative stress and has neurotrophic effects, this agent could exert protective effects against a variety of ototraumatic agents. Guinea pigs treated with T-817MA before and after noise exposure had significantly less auditory brainstem response threshold shifts and less hair cell loss than untreated controls; threshold shifts were reduced by approximately 20 dB and hair cell loss was reduced by 50% (125). While these results are encouraging, the noise-induced hearing loss findings need to be replicated and the compound's efficacy assessed with other ototraumatic agents.




Amifostine hydrate

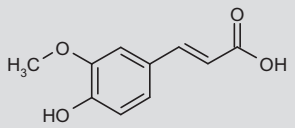
### *Sodium thiosulfate (STS)*

STS ($Na_2S_2O_3$) is a reactive thiol used clinically as an antidote to cyanide or nitroprusside poisoning (126, 127). Thiosulfate acts as a donor of sulfur to cyanide, promoting the formation of thiocyanate, which can be excreted. Most thiols are electrophilic and are thought to act as free radical scavengers.

STS has been evaluated as an otoprotective agent in platinum-based chemotherapy. Significant otoprotection against cisplatin-induced hearing loss and hair cell loss was observed after systemic STS administration (35, 123, 128). STS has also been reported to prevent carboplatin ototoxicity (129, 130). The protection afforded by STS was most effective when administered at the same time and or shortly after cisplatin treatment; its efficacy largely disappeared when administered 12 hours post-cisplatin (35, 128). The protection afforded by STS against cisplatin toxicity may be due to covalent binding of STS to platinum, thereby producing an inactive complex which suppresses its antitumor efficacy (131, 132). To maintain the antitumor efficacy of cisplatin while providing maximal otoprotection, STS can be perfused into the cochlea. Local administration of STS completely prevented cisplatin-induced hearing loss and reduced the loss of more than 90% of OHCs and cochlear inner hair cells (IHCs) destined to die (133). STS was also evaluated as an otoprotective agent against hearing loss and hair cell loss induced by combined exposure to noise plus acrylonitrile. However, STS failed to provide any protection under these conditions of oxidative stress (134).

### *Ferulic acid (FA)*

FA is an antioxidant found naturally in plants such as oats, brown rice, whole wheat, peanuts, apples and pineapples. It is a phenolic compound with free radical scavenging properties largely mediated



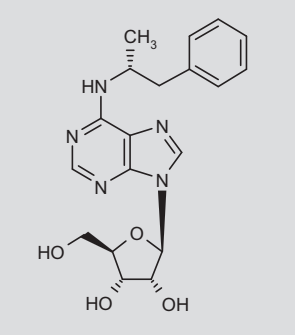
Ferulic acid

by its capability to form resonance-stabilized phenoxyl radicals. FA has been used as a neuroprotectant to treat neurodegenerative disorders (135). Recently, FA was found to significantly attenuate noise-induced hearing loss (10 dB reduction) and hair cell loss in guinea pigs. This protective effect was accompanied by a significant decline in apoptotic signaling and oxidative stress in the cochlea and an upregulation of the cytoprotective enzyme heme oxygenase 1 (HO-1) (136). While these results are encouraging, further work is needed to evaluate the full range of its otoprotective effects under other ototraumatic conditions and in humans.

### *R-Phenylisopropyladenosine (R-PIA)*

Adenosine receptors ($A_1$, $A_{2A}$, $A_{2B}$ and $A_3$) are expressed in numerous tissues, including the cochlea (137); however, their function varies with the type of tissue in which they are expressed. Adenosine $A_1$ and possibly $A_3$ receptors appear to be involved in cytoprotection (138). Activation of the $A_3$ receptor has been found to lead to an increase in activity of SOD, catalase and glutathione peroxidase (139). However, adenosine $A_2$ receptors appear to potentiate ototoxicity (140). Application of cisplatin to the round window of the chinchilla resulted in an upregulation of the adenosine $A_1$ receptor in the cochlea (141).

*R*-PIA is a selective adenosine $A_1$ receptor agonist. Application of *R*-PIA to the chinchilla round window resulted in significant increases in cochlear SOD, glutathione peroxidase (138) and GSH (19). These data suggest that *R*-PIA activation of adenosine $A_1$ receptors may promote antioxidant defenses and the scavenging of free radicals in response to oxidative stress. Indeed, round window application of *R*-PIA significantly reduced noise-induced threshold shift by 10-20 dB, DPOAE loss and hair cell loss (19, 142). In addition, round window application of *R*-PIA reduced the cisplatin-induced threshold shift by about 35 dB. Another adenosine $A_1$ receptor agonist, 2-chloro-*N*-cyclopentyladenosine (CCPA), also provided significant protection against cisplatin ototoxicity (140). While *R*-PIA and other adenosine $A_1$ receptor agonists appear to be effective otoprotective compounds, local application of these agents to the round window limits their widespread clinical use.



*R*-Phenylisopropyladenosine

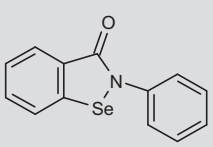
## PREVENTING NITROGEN FREE RADICAL PRODUCTION

Nitric oxide (NO) is an important messenger molecule involved in many physiological and pathological processes. NO is a weak radical produced by nitric oxide synthase (NOS). Sustained NO production under stress by the inducible isoform of the enzyme may result in direct tissue toxicity. As noted above, if NO and superoxide anion ($O_2^-$) are simultaneously produced, they rapidly react with each other, yielding a highly oxidizing peroxynitrite anion ($ONOO^-$), an RNS which is very toxic. The activation of NOS is associated with an increase in calcium influx, regulated to some extent by NMDA receptors and extracellular magnesium. Several compounds that suppress RNS and calcium influx have been evaluated for their otoprotective efficacy, as discussed below.

### Ebselen

Ebselen (also PZ-51, DR-3305) is a glutathione peroxidase mimetic and can also react with peroxynitrite anions ($ONOO^-$) (143). Glutathione peroxidase is the general name of an enzyme family with peroxidase activity. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides and to reduce free hydrogen peroxide to water ($2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$). $ONOO^-$ is an unstable structural isomer of nitrate ($NO_3^-$) and can damage a wide array of intracellular molecules, including DNA and proteins. $ONOO^-$ can be reduced to $ONO^-$ when ebselen is oxidized to ebselen Se-oxide (144). Ebselen has also been reported to inhibit the activity of NOS (145).

Treatment with ebselen showed a significant protective effect against acoustic overstimulation, reducing the degree of hearing loss by about 10 dB and decreasing the amount of hair cell loss (146, 147). Ebselen also protected against cisplatin-induced damage in cochlear organotypic cultures (148), and in vivo, ebselen attenuated cisplatin-induced hearing loss by 20-30 dB and prevented the cisplatin-induced declines in glutathione and other antioxidant enzymes (149, 150). Ebselen also attenuated cochlear damage



Ebselen

induced by local application of gentamicin to the inner ear (151). Taken together, these animal data indicate that ebselen is a useful otoprotective compound, but further studies are needed to assess its clinical efficacy in humans.

### L-NAME (*N*-nitro-L-arginine methyl ester)

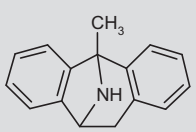
L-NAME competitively inhibits all isoforms of NOS. As described above, NO can have both beneficial and detrimental functions. Under stress, inducible NOS (iNOS) produces large amounts of NO, leading to production of $ONOO^-$ and resulting in cellular damage (152). Therefore, inhibition of iNOS may protect against cellular injury under high stress conditions.

Production of iNOS and increased levels of NO have been observed in cochlear perilymph after intense noise exposure (153). Application of L-NAME significantly reduced the noise-induced levels of cochlear NO and attenuated noise-induced hair cell loss and auditory threshold shift by 10-15 dB (154, 155). In contrast, L-NAME provided only a slight degree of protection against the gentamicin-induced high-frequency (31.5 kHz) threshold shift, and offered no protection at other frequencies (156). L-NAME also failed to provide protection against endotoxin-induced sensorineural hearing loss (157). Thus far, the data suggest that L-NAME is mainly otoprotective against noise-induced hearing. Further animal studies are warranted to better gauge the effectiveness of L-NAME and its potential for clinical use.

### NMDA receptor antagonists

NMDA receptor antagonists are discussed here because activation of the NMDA receptor leads to the production of NO. Cochlear IHCs release glutamate, the putative neurotransmitter that activates the afferent dendrites of type I SGNs. NMDA receptors are expressed on spiral ganglion neurons along with AMPA and kainate receptors. Glutamate activation of AMPA and kainate receptors triggers rapid excitatory neurotransmission by promoting the influx of $Na^+$. In the resting state, NMDA receptors are normally blocked by $Mg^{2+}$ and unresponsive to glutamate; however, during depolarization Mg is released, allowing calcium to enter the neuron. NMDA receptors are believed to play a major role in activity-dependent synaptic plasticity mediated by calcium entry. However, overactivation of NMDA receptors causes excessive calcium entry, initiating a series of cytoplasmic and nuclear processes that promote cell death, a process referred to as excitotoxicity (158). Upon binding to calmodulin, calcium activates NOS to produce NO. Excess calcium activates proteolytic enzymes and endonucleases, leading to the degradation of proteins and DNA.

**Dizocilpine** (MK-801) is a selective, voltage-dependent, noncompetitive antagonist of the NMDA receptor. Dizocilpine exerts its therapeutic effects by binding to a site within the open NMDA ion channel, thereby preventing the influx of calcium during periods of intense depolarization (159-161). Glutamate excitotoxicity, which leads to swelling of afferent synapses, has been observed in the cochlea after intense noise exposure (6, 162), and animals treated with dizocilpine showed significantly less noise-induced permanent threshold shift and fewer vacuoles in afferent nerve fibers than untreated, noise-exposed controls (163-165).



Dizocilpine

Carbamethionine, an NMDA antagonist, and caroverine, an NMDA antagonist that also blocks AMPA receptors and N-type calcium channels, provided significant protection against acoustic trauma, attenuating permanent threshold shift on the order of 15-20 dB and reducing hair cell loss (51, 166). Dizocilpine was also effective in reducing acute hearing loss induced by carbon monoxide (167) and permanent hearing loss and cochlear pathologies induced by aminoglycoside antibiotics (168). Taken together, these animal studies suggest that dizocilpine and other NMDA antagonists might be effective otoprotective agents; however, dizocilpine has potentially serious side effects that have limited its clinical use in humans. High doses of dizocilpine can induce neuronal degeneration by AMPA/kainate-mediated excitotoxicity (169, 170).

### Magnesium ($Mg^{2+}$)

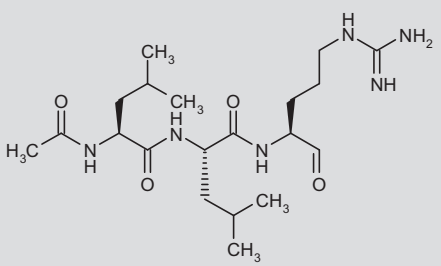
Magnesium, after potassium, is the second most abundant intracellular cation, and plays an important role in maintaining cellular electrolyte balance. Magnesium also participates in many metabolic processes essential for life, acting as a metallic cofactor in more than 300 enzymatic reactions, including those responsible for energy metabolism, fatty acid metabolism, protein synthesis and neuromuscular contraction/relaxation (171). It also functions as a transmembrane and intracellular modulator of other ions, such as potassium and calcium (172). Magnesium is discussed in this section because of its role in blocking NMDA receptors and calcium channels, thereby reducing excessive calcium influx, which may lead to the production of RNS.

In studies of susceptibility to noise-induced hearing loss, magnesium deficiency increases susceptibility to noise trauma (173). Rats fed a magnesium-deficient diet were more susceptible to noise-induced hearing loss than rats fed a magnesium-rich diet. Moreover, the magnitude of noise-induced hearing loss was inversely related to magnesium levels in the cochlear perilymph (174). In guinea pigs exposed to impulse noise, those treated with a high dietary intake of magnesium had 10- to 35-dB less hearing loss than those with a low (suboptimal) magnesium intake. Noise exposure caused a significant decline in cochlear blood flow and oxygen partial pressure in the low magnesium group, but not in the high magnesium group (175, 176). In a double-blind, placebo-controlled study with soldiers exposed to rifle fire (impulse noise), personnel given a magnesium supplement had less frequent and less severe noise-induced permanent threshold shifts than those receiving placebo. Moreover, the amount of permanent threshold shift was negatively correlated with magnesium levels in red blood cells (177). In a follow-up double-blind study of noise-induced temporary threshold shifts, human subjects were given placebo, magnesium or no drug. The temporary threshold shifts and otoacoustic emission reductions were lower with magnesium treatment versus placebo or no drug (178). Taken

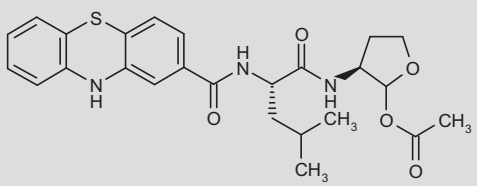
together, these results indicate that low blood levels or low dietary intake of magnesium may be a risk factor for noise-induced hearing loss, while magnesium supplementation may provide significant otoprotection.

### Leupeptin, BN-82270 and MDL-28170

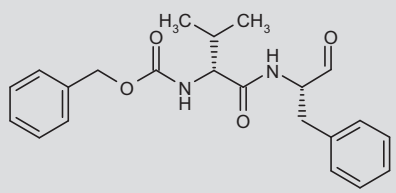
In addition to increasing RNS, the excessive influx of calcium may also upregulate calpains, calcium-activated proteases that promote the breakdown of cytoskeletal and membrane proteins (8). Noise trauma increases the expression of calpains in the cochlea, thereby implicating calpains in noise-induced hearing loss (179). Intracochlear infusion of leupeptin, a calpain inhibitor, significantly reduced noise-induced hair cell loss. BN-82270, an inhibitor of both calpains and lipid peroxidation, also provided significant protection against noise-induced hearing loss when infused into the cochlea or applied to the round window (180). Interestingly, BN-82270 continued to protect against noise-induced hearing loss when applied to the round window up to 24 hours post-exposure. In cochlear organotypic cultures damaged by gentamicin, leupeptin also protected hair cells from gentamicin ototoxicity, with a high dose of leupeptin providing complete protection (9). MDL-28170, a cell-permeable calpain inhibitor, also provided significant protection against gentamicin-induced hair cell loss in cochlear cultures (181). However, leupeptin and other calpain inhibitors failed to protect hair cells and spiral ganglion neurons against the ototoxic effects of cisplatin and carboplatin (179, 182). While calpain inhibitors appear to be effective in suppressing cochlear damage from noise and gentamicin when applied to the inner ear, systemic and clinical use of these compounds may be limited by their ability to cross the blood–ear barrier.



Leupeptin



BN-82270



MDL-28170

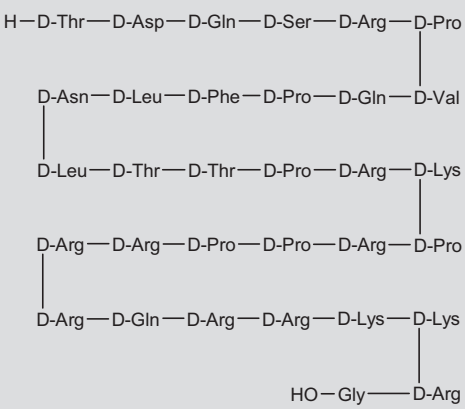
## BLOCKING CELL DEATH PATHWAYS

Under stressful conditions, a broad range of intertwined cell signaling pathways are activated to promote either cell survival or cell death. A number of compounds have been used to suppress cell death cascades or activate prosurvival pathways in attempt to protect the ear from a variety of ototraumatic insults.

### D-JNKI-1

D-JNKI-1 (also, AM-111, XG-102) is a synthetic, cell-permeable peptide that blocks the stress-activated protein kinase JNK signaling pathway, which leads to apoptotic cell death. D-JNKI-1 inhibits all three isoforms of JNK (JNK1, 2 and 3). In animal models of acoustic trauma, intracochlear or systemic treatment with D-JNKI-1 provided significant protection against noise-induced permanent threshold shift by up to 40 dB and greatly reduced hair cell loss (183-186). In a follow-up phase I/II clinical trial of 11 patients with acute acoustic trauma from firecracker noise, D-JNKI-1 was applied intratympanically within 24 hours following exposure. Assessment included recovery of audiometric thresholds and otoacoustic emissions (184). Based on clinical experience and using an exponential model of hearing loss recovery, it was claimed that D-JNKI-1 had a beneficial effect; however, the lack of a placebo control group seriously undermines any firm conclusions regarding its efficacy in humans.

In cochlear cultures and in animals treated with ototoxic aminoglycoside antibiotics, local application of D-JNKI-1 prevented nearly all hair cell death and provided significant protection against hearing




D-JNKI-1

loss (20 dB) (185, 187). D-JNKI-1 also suppressed the mechanically-induced hearing loss and hair cell loss associated with the insertion of a cochlear implant into the inner ear (188) and experimentally induced labyrinthitis and semicircular canal injury (189, 190).

### Pifithrin-alpha (PFT)

PFT is a cell-permeable small molecule that inhibits p53, a tumor suppressor protein that is strongly upregulated by cell stress and DNA damage (191). Since cisplatin induces considerable cell stress and DNA damage, it was thought that cisplatin would upregulate the expression of p53, resulting in cochlear apoptosis. When cisplatin was applied to cochlear organotypic cultures, it significantly upregulated p53 expression, activated both caspase-1 and caspase-3, and caused significant hair cell death. Application of PFT blocked the cisplatin-induced expression of p53, prevented the upregulation of caspase-1 and caspase-3, and provided significant protection against cisplatin-induced hair cell death (192). Since other ototraumatic insults may increase the expression of p53 in the inner ear (193), further studies regarding the protective effects of PFT are warranted.

### Minocycline hydrochloride

Minocycline is a broad-spectrum, lipid-soluble tetracycline antibiotic that crosses the blood–brain barrier more effectively than other tetracycline derivatives. In addition to being an antibiotic, minocycline has neuroprotective and antiinflammatory properties (194-196). Minocycline exerts its protective effects by inhibiting the opening of mitochondrial permeability transition pores, blocking cytochrome c release and suppressing the activation of downstream caspases (195, 197).

In a novel approach, minocycline was used to suppress the ototoxic effect of another antibiotic, gentamicin. When minocycline was applied to cochlear organotypic cultures, it inhibited gentamicin-induced cytochrome c release, caspase activation and MAP kinase p38 activity (198-200). In addition, minocycline greatly reduced gentamicin-induced hair cell loss in the culture. Since minocycline is a broad-spectrum antibiotic, one might assume that it could be used in conjunction with aminoglycoside antibiotics to suppress life-threatening bacterial infections, with the added advantage that minocycline would reduce the ototoxic side effects.

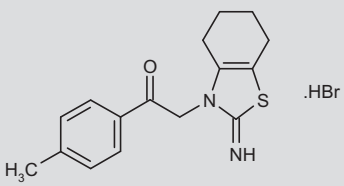
## OTHER OTOPROTECTIVE AGENTS

### Src inhibitors

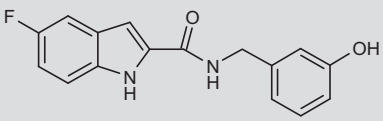
The Src family of kinases is composed of nine structurally related, membrane-associated, non-receptor tyrosine-protein kinases. Src kinases are overexpressed in a variety of human tumors and play an important role in tumor growth. Src activation in cultured epithelial cells downregulates E-cadherin, disrupting intercellular connections, which can lead to cell death by anoikis (201). Src also plays an important role in upregulating the activity of NMDA receptors (202, 203). Several Src kinase inhibitors (**KX1-004**, KX1-005 and KX1-174) have been used to prevent noise-induced hearing loss. Among these, KX1-004 offered the greatest protection against acoustic trauma, attenuating hearing loss by approximately 20 dB and reducing the amount of hair cell loss (27, 204).

### Acetyl L-carnitine (ALCAR)

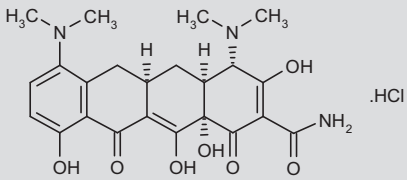
ALCAR (also levocarnitine acetyl hydrochloride) is the acetylated ester of the amino acid L-carnitine. ALCAR is present in mitochondria and helps maintain mitochondrial energy production. Both ALCAR and L-carnitine are absorbed into the bloodstream efficiently and are effective at carrying fatty acids across the membrane into mitochondria, where fats are oxidized to produce energy in the form of adenosine-5′-triphosphate (ATP) (205, 206). The acetyl group of



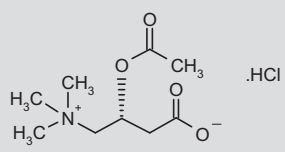

Pifithrin-alpha




KX1-004




Minocycline hydrochloride




Levocarnitine acetyl hydrochloride

ALCAR is used to form acetyl-CoA, the most important intermediary in the generation of energy from amino acids, fats and carbohydrates. Therefore, ALCAR serves as an energy reservoir of acetyl groups and both ALCAR and carnitine help improve energy production.

A major consequence of aging is deterioration of the energy-producing components of cells, resulting in reduced cellular metabolic activity or oxygen consumption, and eventually cell death. ALCAR supplementation in aged rats increases cellular oxygen consumption and significantly reverses age-associated decline of mitochondrial membrane potentials. However, the oxidant production in ALCAR-treated rats was 30% higher than in untreated aged rats. Cellular glutathione levels were also found to be lower in ALCAR-treated animals, indicating that ALCAR supplementation increased oxidative stress (207). When evaluated in the context of hearing loss, ALCAR supplementation was shown to prevent noise-induced hearing loss by 10-30 dB (23, 30, 51). However, when aging rats were treated systemically with ALCAR, it failed to slow the progression of age-related hearing loss (208).

### Growth factors and neurotrophic factors

Naturally occurring growth factors stimulate cellular growth, proliferation and differentiation. Some are critical for normal cochlear development, but largely disappear in the developed cochlea. Interestingly, the activity of some growth factors, such as epidermal growth factor (EGF) and fibroblast growth factors (FGFs) may be reactivated in the adult cochlea after noise trauma, indicating their possible involvement in hearing recovery after trauma (209-211). Neurotrophic factors contribute to the survival, development and function of neurons. Some neurotrophins, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), are critical for the development of inner ear neuronal innervation (212). Lack of NT-3 appears to contribute to the loss of SGNs and lack of BDNF reduces the innervation of outer hair cells. Growth factors and neurotrophins may promote survival and inhibit apoptosis.

Cochlear trauma caused by intense noise exposure, ototoxic agents and aging often triggers apoptotic hair cell death, leading to permanent hearing loss (33, 213-217). Agents that protect against apoptosis may prevent the death of auditory hair cells and SGNs, and thus prevent hearing loss. Cochlear implant surgery is currently the therapy of choice for profoundly deaf patients. However, the effectiveness of cochlear implants depends on the integrity of the auditory SGNs. Interventions that prevent the degeneration of SGNs would be of therapeutic significance and lead to increased benefits of cochlear implants. Deafferentation resulting from hair cell degeneration leads to the loss of neurotrophic factors, which can increase free radical formation and upregulate cell death signaling pathways. Type I SGNs appear to require NT-3 for their survival, whereas type II neurons appear to depend on BDNF (218). Infusion of BDNF into inner ear fluids significantly increases the population of surviving SGNs following deafening and increases the efficacy of electrical stimulation (219, 220). Other neurotrophic factors, such as ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF), also prevented degeneration of SGNs following deafening (219-221).

Noise trauma can trigger death of both hair cells and SGNs by activating apoptotic signaling pathways. Application of acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), EGF or neurotrophic factors such as NT-3, glial cell line-derived neurotrophic factor (GDNF) alone or in combination with other otoprotective agents has been found to protect against noise-induced hearing loss (154, 210, 211, 222-226). Treatment with different growth factors or neurotrophic factors attenuated noise-induced threshold shift by 10-30 dB (223-225). Aminoglycoside ototoxicity can also be suppressed by hepatocyte growth factor (HGF), nerve growth factor (NGF) or NT-3 (165, 227-229). BDNF also provided significant protection against several ototoxic agents (157, 230) and promoted the survival of SGNs following deafening (219, 220).

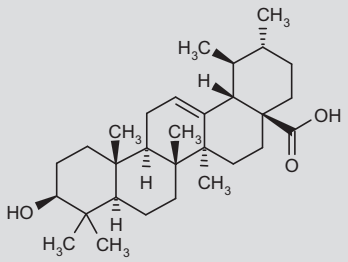
### Corticosteroids

Corticosteroids are divided into glucocorticoids and mineralocorticoids. While glucocorticoids are predominantly involved in carbohydrate, fat and protein metabolism, mineralocorticoids are primarily involved in regulating electrolyte and water balance through their effect on ion transport in epithelial cells of renal tubules, resulting in retention of sodium and loss of potassium. Corticosteroids also act on the immune system by blocking the production of substances that trigger allergic and inflammatory reactions, such as prostaglandins.

Several glucocorticoids have been used alone or in combination to treat patients with sudden idiopathic hearing loss (231-234). In general, these treatments appear to be more effective in immune-mediated hearing loss (235). In recent years, there has been increasing interest in treating sudden hearing loss in humans by means of local steroid delivery via the middle ear or round window. A comprehensive overview of the outcomes can be found in a recent review (236). In animals, prednisolone, a glucocorticoid, and aldosterone, a mineralocorticoid, have been shown to prevent ongoing hearing loss due to autoimmune disease. Without treatment, 80% of the animals lost auditory sensitivity progressively. After treatment with prednisolone or aldosterone, more than 90% of animals retained or showed improved sensitivity (237, 238). Round window application of dexamethasone attenuated the ischemia-induced cochlear dysfunction by 10-15% (239). In addition, intratympanic injection of dexamethasone also significantly reduced the threshold losses and DPOAE reductions induced by systemic cisplatin treatment (107, 240). On the other hand, systemic dexamethasone treatment did not provide any protection against noise-induced hearing loss or hair cell loss (241). Taken together, these results suggest that corticosteroid treatments may be beneficial under some circumstances.

### Ursolic acid

Ursolic acid, a pentacyclic triterpene acid, is present in many plants. The fruits of *Cornus officinalis* have been used in traditional Chinese medicine for the treatment of inner ear diseases such as tinnitus and hearing loss (242). A bioassay-guided fractionation of the methanol extract of *Cornus* fruits resulted in the isolation of ursolic acid as its major active component. Treatment with ursolic acid significantly attenuated hydrogen peroxide-induced decreases in catalase and glutathione peroxidase activity in HEI-OC1 cells, an immortalized cell line derived from the inner ear. Although there are no in vivo data to establish its efficacy, ursolic acid may be a potential otoprotective agent.




Ursolic acid

## SYNOPSIS

In this review, more than 29 different compounds in 4 different categories have been found to protect against 1 or more ototraumatic insults. Many of these compounds exert their protective effects by reducing oxidative stress, upregulating antioxidant defenses, reducing inflammatory responses or suppressing cell death signaling pathways. When these otoprotective compounds are administered individually the hearing protective effects generally range from 5 to 20 dB. Given the magnitude of the protective effects, the variability of the measurement techniques, the different modes and magnitude of trauma and species difference, it is difficult to clearly specify which of the compounds evaluated thus far is the most efficacious. Moreover, additional studies are needed to identifying the optimal dose and duration of treatment to maximize each drug's otoprotective effects. Since most ototraumatic insults are likely to involve multiple forms of stress and cell death signaling pathways, future efforts should be directed at developing "otoprotective cocktails" designed to enhance antioxidant defenses, reduce oxidative stress and inflammation, and to suppress one or more cell death signaling cascades.

Another largely unexplored but important factor to consider is when to apply and when to stop a particular form of treatment. In the case of ototoxic drugs, when the time of insult is known, optimal preventive therapy can begin prior to, during and following the administration of drugs such as cisplatin and gentamicin. However, other ototraumatic insults, such as impulse noise exposure and sudden hearing loss, occur unexpectedly. In such cases, therapy should begin as soon as possible and continue for several weeks or more to prevent the degeneration of hair cells, neurons and supporting cells in the inner ear. In the case of acute trauma, such as sudden hearing loss, trauma during cochlear implant surgery or exposure to impulse noise or ototoxicity, another decision that must be considered is the route of drug administration. Intracochlear delivery of otoprotective drugs would seem to be the optimal approach for hybrid cochlear implant surgery (243, 244). Likewise, intratympanic delivery of otoprotective compounds in cases of unilateral sudden hearing loss or acoustic trauma might be more effective than systemic therapy, since the drugs are delivered directly to the site of injury at higher concentrations. On the other hand, regulating the drug concentrations reaching the inner ear may be extremely difficult with this method. Improved drug delivery to the inner ear over a longer period of time may be facilitated with a round window catheter; however, the costs and benefits of this more invasive approach remain to be determined (245-247).

At the other end of the spectrum, human age-related hearing loss develops slowly over decades. The same is true for long-term exposure to occupational or recreational noise. The progressive nature of age-related hearing loss and occupational noise exposure would likely benefit most from daily, low-cost nutritional supplements or drugs that can be taken orally, assuming of course that these compounds do not have any negative long-term side effects.

Since sensory hair cells are essential for converting sound to neural activity, most otoprotective efforts have focused on preventing hair cell degeneration while largely ignoring afferent neurons, which transmit acoustic information to the central nervous system. Some forms of hearing impairment resulting from aging or exposure to noise or ototoxic drugs may preferentially damage the SGN (248).

While much of the preceding discussion has focused on preventing cellular degeneration, some forms of hearing impairment may result from functional impairments in apparently structurally intact cells. Reinvigorating these dysfunctional auditory cells may restore hearing. For example, chronic salicylate treatment was found to prevent age-related loss of DPOAE, which reflects OHC electromotility. Unfortunately, this treatment did not prevent the age-related hearing loss (105, 249). Growth factors are known to play an important role in the development of auditory hair cells, but they generally decline with age or adulthood. Interestingly, some growth factors are upregulated in the adult cochlea after noise trauma (209-211), indicating that growth factors may be involved in attempts to repair injured auditory cells.

While many compounds have shown significant otoprotective effects in animal models, performing the necessary clinical trials to show that they are also effective and safe in humans will prove challenging. Clinical studies aimed at identifying drugs to prevent age-related hearing loss in humans are impractical because of the slow progression of the disease. Clinical studies to test the efficacy of drugs to prevent noise-induced hearing loss could be performed in a relatively short time span; however, many ethical challenges come into play when exposing humans to noise or withholding treatment from the placebo or control groups. Similar considerations apply to studies of ototoxicity in humans.

## DISCLOSURES

The authors state no conflicts of interest.

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