Pharmacologic manipulation of the labyrinth with novel and traditional agents delivered to the inner ear

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Abstract
We describe the methodology and rationale behind the delivery of therapeutic medicines to the inner ear. The inner ear has long been impervious to pharmacologic manipulation. This is most likely the result of a protective mechanism called the blood-labyrinth barrier, whose function closely resembles that of the blood-brain barrier. This protective barrier impedes the clinician's ability to treat inner ear diseases with systemically administered medications. Since 1935, otolaryngologists have attempted to manipulate the inner ear with transtympanically injected medicines. Success has varied widely, but medicinal ablation of vestibular function can be achieved in this manner. Unfortunately, the auditory system is also at great risk from any medicine that is delivered to the inner ear via the middle ear. Over the past 10 years, significant improvements in drug delivery have allowed for more "titratable" treatment, which has reduced (but not eliminated) the risk of permanent hearing loss. In this article, we discuss both novel and time-tested methods of delivering medicines to the inner ear. We also review the classes of medications that alter inner ear function and the attendant risks of such treatments.

Introduction
The ability to locally or directly treat inner ear diseases has eluded the scientist and clinician for years. It is well known that the inner ear is isolated, physically and anatomically, from the rest of the body's systems. Therefore, although systemically administered medications used to treat otologic abnormalities might have a desired effect on the inner ear, their application can be limited by potentially adverse effects on the inner ear. Such limitations have been seen in the use of diuretics for Ménière's disease, anxiolytics for tinnitus, and steroids for autoimmune inner ear disease. For example, diuretics are used to reduce the overall fluid volume to the inner ear as part of the management of Ménière's syndrome. However, their primary effect is a reduction of fluid systemically, with probably only a very slight fluid reduction in the inner ear. One of the side effects of some diuretics is that they lower potassium levels. It would be much better and perhaps more target-specific if we had a diuretic that worked only on the inner ear without causing systemic side effects.

When given systemically, some medications have a profound effect on the inner ear. For example, aspirin in high doses causes tinnitus, and certain aminoglycosides, when given systemically, pass into the inner ear and can cause permanent balance and auditory disturbances. Likewise, the systemic use of cisplatin for its antitumor activity can cause significant hearing loss. Therefore, it would be most beneficial if we could develop means to use novel medicines and novel delivery techniques to both treat the labyrinth and protect the inner ear from harm. Perhaps if the ear could be treated with a protective agent (e.g., methionine), hearing could be spared without reducing or eliminating the anticancer effects of a chemotherapeutic regimen. A poignant example can be found in China, where the leading cause of hearing loss (60% of all cases) is ototoxicity from aminoglycosides that are used routinely for the treatment of bacterial infections. Sha and Schacht have shown that iron chelators and aspirin can ameliorate the otoxic effects of the aminoglycosides without reducing their antibacterial properties.

Perturbations in oxygenation, blood supply, nutrient delivery, and waste elimination in the inner ear can all have deleterious effects on otologic function. Interest-
ingly, even minute changes in inner ear dynamics, regulatory mechanisms, and homeostasis can cause tinnitus, a severe balance disturbance, and/or a complete hearing loss. Tinnitus alone affects 90 million people worldwide, hearing loss affects more than 30 million Americans, and more than 50% of patients older than 65 years have experienced some difficulty with balance.3

Although the theme of this article might suggest that the delivery of medicines via a local route to the inner ear to treat specific otopathologies is new, this is not the case. There is a significant history of such delivery that dates back to 1935, when Bárány reported his treatment of Ménière’s disease patients with middle ear delivery of lidocaine.4 Later, Schuknecht reported his use of streptomycin,5 and Sakata et al published their results with steroids.6 Many others have reported direct transtympanic treatment of the inner ear for a number of different otologic disorders.6,19

The rationale for treating inner ear disorders directly seems to be logical. For years, ophthalmologists had a significant advantage in the management and treatment of ocular diseases merely by applying appropriate drops to the eye. But in light of the presence of the blood-labyrinth barrier, the nonselectivity of some drugs (systemic drugs typically do not go directly to the inner ear), and the tympanic membrane, otolaryngologists cannot always use the particular medications we would prefer to use.

Many delivery techniques are available to treat the inner ear. The three procedures that are most often used all involve transtympanic drug delivery; the three tools used are a tuberculin syringe and a 27-gauge or smaller-gauge needle, the round window microcatheter (Round Window m-Cath; Durect Corp.; Cupertino, Calif.), and the Silverstein MicroWick (Micromedics; Eagan, Minn.).

Prior to undertaking a specific intervention, of course, it is critical to understand the pathophysiology of the particular disease that is being treated. The mechanisms of many common otologic disorders, while understood in part, have not been completely elucidated. Specifically, Ménière’s disease has long been thought to occur as a result of endolymphatic hydrops, but the true cause of this pathology is still open to speculation. While we have reasonable medical and surgical options for the treatment of the vertigo associated with Ménière’s disease, the efficacy of our choices for the management of associated hearing loss, aural fullness, and tinnitus pales in comparison. These latter symptoms are often just as disconcerting to the patient as is the vertigo.

Some scientific evidence and a moderate amount of clinical and anecdotal evidence support the use of steroids applied directly to the middle ear cavity and round window membrane for the management of hearing loss, aural fullness, in perhaps tinnitus in selected patients.6,8,13,16,21-25 Exploratory tympanotomy allows us to directly perfuse the inner ear as well as directly explore the round window membrane. It is important that adhesions surrounding the round window membrane niche are removed to ensure that fluid transfer through the membrane is not impeded. Doing so will eliminate one potential cause of treatment failure. Even so, this idea is certainly controversial, particularly with respect to the use of transtympanic gentamicin without exploring the middle ear to remove round window adhesions. Transtympanic gentamicin appears to alleviate vertigo in as many as 85% of patients.26,27 Could the treatment failures be attributable to the fact that medicine was eliminated too quickly via the eustachian tube? Or could they be attributable to a lack of direct access to the round window membrane as a result of pre-existing adhesions? Clearly, many questions remain to be answered, but some of the variability in outcomes could be controlled if clinicians and investigators had more standardized treatment paradigms.

Many otologic disorders might respond to direct pharmacologic manipulation of the labyrinth. They include (but are not limited to) sudden sensorineural hearing loss, noise-induced hearing loss, ischemia-induced hearing loss, virus-induced hearing loss, autoimmune inner ear disease, tinnitus of the peripheral type, congenital or acquired hearing loss (using gene vectors), and balance disturbances that are peripheral in origin.

Many medications can be used to treat the inner ear, and they have been discussed extensively in several articles. The most frequently used intratympanic medications are gentamicin and steroids. Less-used options are antioxidants, growth factors, antivirals, diuretics, vasodilators, antisense oligonucleotide agents, gene vectors, and others.6,8,13,16,18,25,35-39

In this article, we provide a general discussion of some of the more commonly used medications as well as some novel compounds that might have a role in the pharmacologic manipulation of the inner ear. We also provide a rationale for their use.

Antibiotics

The aminoglycoside antibiotics are routinely used to treat aerobic gram-negative bacterial infections. As a result, their ototoxic side effects have been well documented in the literature and in everyday practice.40-44 In attempts to treat Ménière’s disease, otolaryngologists have used the aminoglycosides to partially ablate vestibular function for vertigo relief while attempting to maintain cochlear function. Beginning in 1957, the use of streptomycin administered directly to the middle ear resulted in an overall alleviation of vertigo, but hearing loss was evident in all patients.2 Subsequent studies with gentamicin showed significant improvements in relieving vertigo (>84%) and much less treatment-related hearing loss (<58%), presumably because gentamicin differentially destroys the
endolymph-secreting dark cells rather than the cochlear hair cells involved in hearing. Intratympanic administration of gentamicin to optimize the control of vertigo and minimize hearing loss in patients with Ménière’s disease has proved to be fairly successful.

Many suggested methods and treatment protocols have been described. Protocols that limit the amount and rate of administration have proved to be the most successful. In 1999, Minor wrote that the best time to discontinue treatment is when spontaneous nystagmus, head-shaking nystagmus, or head-thrust signs are present; vertigo was controlled in 91% of patients, and profound hearing loss occurred in only 3%. A year later, Kaplan et al reported that intratympanic administration of gentamicin resulted in complete control of vertigo in 84.4% of patients and substantial control in another 9.3%. At the 2-year follow-up, hearing had improved in one-fourth of patients and worsened in one-fourth; the remaining half were unchanged. Thomsen et al delivered gentamicin to the inner ear via a round window microcatheter and found that vertigo was controlled in 81% of patients; however, 22% of patients experienced significant hearing loss, possibly related to the flow rate. Finally, Hoffer et al achieved vertigo control rates of 90% without hearing loss in more than 40 patients with Ménière’s disease who were treated via the round window membrane niche microcatheter delivery technique.

Corticosteroids
Corticosteroids are commonly used in the management of several inner ear disorders, including sudden sensorineural hearing loss from idiopathic, vascular, viral, or traumatic causes; Ménière’s syndrome and disease; autoimmune inner ear disease; and certain vestibulopathies. The activity of steroids varies widely, but these agents primarily affect carbohydrate, lipid, and protein metabolism by interacting with receptors in target tissues that affect the expression of regulatory genes.

Some authors have suggested that steroids might be harmful to the inner ear and other tissues. In view of these reports and the fact that many clinicians were finding that steroids had some beneficial effects, the lead author’s (M.D.S.) laboratory initiated an animal study to investigate the effects of transtympanic steroids on cochlear blood flow, auditory threshold sensitivity, and histology. We transtympanically injected both dexamethasone and methylprednisolone once a week for 8 consecutive weeks. A separate group of animals was used to assess changes in cochlear blood flow. We found a statistically significant 29% increase in cochlear blood flow within 30 seconds of steroid application to the round window membrane. No change from baseline auditory thresholds was observed, and no difference was seen between treated ears and opposite ears injected with saline. Furthermore, no histologic differences were observed between the treatment and control ears.

Typically, the administration of steroids for inner ear disorders has been undertaken via the systemic route. However, in view of the blood-labyrinth barrier, there is a valid concern that adequate inner ear drug levels might not be achieved through this method. Studies have demonstrated that different administration routes result in a significant degree of inconsistency in inner ear steroid levels. For example, delivery via the middle ear cavity route has been reported to result in significantly higher perilymphatic drug levels than does delivery via other routes.

Even though steroids are a valid therapeutic option for several inner ear disorders, their use is controversial because there have been several anecdotal reports that patients lost additional hearing following the delivery of steroids by catheter. Questions surrounding this issue are numerous, and consideration should be given to the possibility that this additional hearing loss might have been secondary to the progression of the disease itself, to the actions of carrier molecules in the steroid preparation, to infection (bacterial or viral), to trauma to the round window causing a fistula, and to the side effects of anesthesia. In general, additional harm is not likely, but patients certainly need to be warned of all potential adverse side effects that can result from the direct delivery of steroids to the middle ear and round window.

Lidocaine and dexamethasone combination
Sakata et al treated 168 tinnitus patients (220 ears) with transtympanic injections of lidocaine and dexamethasone. Tinnitus was completely eliminated or considerably ameliorated in 185 ears (84.1%). In another study, Itoh and Sakata treated 136 Ménière’s disease patients with a combination of lidocaine and dexamethasone by perfusion. Of this group, 113 (83.1%) experienced immediate relief of aural fullness and dizziness; at 1 year, relief persisted in 94 patients (69.1%).

In 2000, Shea and Ge reported that 35 of 50 ears (70.0%) that had intractable tinnitus achieved relief within 1 month of treatment with lidocaine, dexamethasone, and hyaluronidase perfusion of the round window membrane niche in addition to intravenous lidocaine. Relief was maintained in 20 of 26 ears (76.9%) that were tested at 3 months and in 10 of 12 (83.3%) that were tested at 1 year. However, these results were met with some skepticism because Shea and Ge also used conventional oral antidepressant medications and provided counseling, which might have had an effect on their findings—that is, we cannot be certain which of the interventions led to the improvement. Additional studies of lidocaine and combined lidocaine/dexamethasone/hyaluronidase are warranted.
Neuroprotection and glutamate antagonists

Neuroprotection is a process by which neuronal function is shielded from injury or is restored following injury. The two major causes of neuronal injury are ischemia (induced by trauma, hemorrhage, etc.) and neurodegenerative disease. In the inner ear, neuronal injury manifests as hearing loss, vertigo, and tinnitus.

Ischemia in the central and peripheral nervous systems damages neurons in several ways. The oxidative stress that is associated with ischemia produces a variety of damaging reactive oxygen species (ROS), which include hydrogen peroxide, the superoxide anion, and the hydroxyl radical. The accumulation of ROS promotes the expression of intercellular adhesion molecules and subsequent neutrophil-endothelial cell adhesion. A cascade of inflammatory events ensues, which ultimately results in edema, vascular insufficiency, and cell death. The reduction of intracellular energy and neuronal depolarization that accompanies ischemia also disrupts calcium homeostasis. The accumulation of intracellular calcium leads to dendritic and cellular edema and ultimately neuronal death.

Furthermore, neuronal depolarization leads to an accumulation of extracellular glutamate (a major excitatory neurotransmitter in the central nervous system) and inner ear). Excess glutamate release from depolarized presynaptic neurons, together with a diminution in the uptake mechanisms of glia and neurons, results in increases in synaptic glutamate and pathologic stimulation of N-methyl-D-aspartate (NMDA) receptors. Excessive stimulation of glutamate receptors leads to neuronal injury (i.e., glutamate neuroexcitotoxicity) by either direct or indirect activation of receptors on the postsynaptic neuron. This leads to an opening of gated channels that allows an influx of sodium, potassium, and calcium. This alteration in intracellular ion concentrations exacerbates dendritic and cellular edema and hastens neuronal death. A breakdown in calcium homeostasis, excessive glutamate activation, oxidative stress, and free-radical production also play significant roles in the age-associated neuropathologic processes that lead to neuronal death.

Inner ear ischemia can be caused by exposure to intense noise. Studies using intravital microscopy, laser Doppler flowmetry, and microcast techniques have demonstrated reduced cochlear blood flow, decreased red blood cell velocity, capillary constriction, and increased vascular permeability during noise exposure. The role of ROS in promoting cellular death in the inner ear and the protective effects of antioxidants and ROS scavengers in attenuating ischemia/reperfusion-induced and noise-induced cochlear damage have been substantiated in many animal studies.

Studies have indicated that glutamate excitotoxicity occurs in the cochlea under pathologic conditions, such as noise trauma and ischemia. At least three receptor types are generally accepted as being responsible for glutamate excitotoxicity—those selectively activated by NMDA, quisqualate, and kainate. The latter two are non-NMDA receptors. Excitotoxicity appears to be primarily dependent on NMDA and kainate receptor activation.

Experimental work has demonstrated that monosodium L-glutamate administered to neonatal rats is toxic to the auditory system and produces a high-frequency hearing loss. The primary peripheral target appears to be the neurons in the spiral ganglion. The lead author’s laboratory studied the effects of a broad-spectrum glutamate receptor antagonist, kynurenic acid (KYNA), on glutamate- and noise-induced trauma in guinea pigs. KYNA is a tryptophan metabolite that has selective activity against NMDA, quisqualate, and kainate receptors in the central nervous system. We obtained baseline levels of compound action potentials and cochlear microphonic thresholds in the guinea pigs, and then randomly assigned them to one of three groups:

- Group I received a vehicle control (1.5 M of sodium chloride) applied to the round window membrane, followed by 110 dB of wide-band noise for 90 minutes.
- Group II received 5 mM of KYNA, followed by the same noise exposure.
- Group III received 5 mM of KYNA without noise.

We then measured postdrug and postnoise compound action potentials and cochlear microphonic thresholds. We found that noise exposure caused a moderate and temporary threshold shift of 30 to 40 dB across the frequencies tested (3, 6, 9, 12, and 18 kHz); the highest temporary threshold shift (40 dB) occurred at 9 kHz. Animals that received KYNA prior to noise exposure (group II) demonstrated significant protection against noise-induced damage, as reflected by their minimal temporary threshold shift (range: 5.4 to 8.4 dB) at 3, 6, 9, 12, and 18 kHz (p<0.001). Animals that received KYNA without noise (group III) experienced no change in hearing thresholds. These findings suggest that antagonizing non-NMDA glutamate receptors attenuates a noise-induced temporary threshold shift. These data further support the idea that glutamate excitotoxicity might play a direct role in the generation of acoustic trauma and an indirect role in the production of tinnitus.

The hypothesis that subjective tinnitus is primarily caused by glutamate excitotoxicity at the synapses between inner hair cells and their afferents in the cochlea has been confirmed. Furthermore, the ionotropic glutamate receptors—NMDA and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)—have been identified on the afferent neurons of inner hair cells, and...
Nystatin, USP
For Extemporaneous Preparation
of Oral Suspension

DESCRIPTION
Nystatin USP is an antifungal antibiotic obtained from Streptomyces noursei. It is known to be a mixture, but its composition has not been completely elucidated. Nystatin A is closely related to amphotericin B. Each is a macrocyclic polyether containing a ketonic ring, an all-trans polyene system, and a mycosamine (3-amino-3-deoxy-mannose) moiety. Nystatin A has a molecular formula of C_{47}H_{74}NO_{17} and a molecular weight of 926.11.

Nystatin A

Nystatin USP is a ready-to-use, non-sterile powder for oral administration which contains no excipients or preservatives. It is available in containers of 50 million, 150 million, 500 million, and 2 billion units. Each mg contains a minimum of 5,000 units.

CLINICAL PHARMACOLOGY
Nystatin probably acts by binding to sterols in the cell membrane of the fungus, with a resultant change in membrane permeability allowing leakage of intracellular components. It is absorbed very sparingly following oral administration, with no detectable blood levels when given in the recommended doses. Most of the orally administered nystatin is passed unchanged in the stool.

INDICATIONS FOR USAGE
For the treatment of intestinal and oral cavity infections caused by Candida (Monilia) albicans.

CONTRAINDICATIONS
Hypersensitivity to the drug.

ADVERSE REACTIONS
Large oral doses of nystatin have occasionally produced diarrhea, gastrointestinal distress, and possible irritation of the stomach that may result in nausea and vomiting.

DOSEAGE AND ADMINISTRATION
General
Adults and older children: Add 1/8 teaspoonful (approximately 500,000 units) of Nystatin USP to about 1/2 cup of water and stir well. One-eighth teaspoonful of Nystatin USP is equivalent to the recommended dose for adults and children of Nystatin Oral Suspension (4 to 8 mL, or 400,000 to 600,000 units). This product contains no preservatives and therefore should be used immediately after mixing and should not be stored. It is designed for extemporaneous preparation of a single dose at a time.

Infections of the oral cavity caused by Candida (Monilia) albicans:
Infants: 200,000 units four times daily.
Children and adults: 400,000 to 600,000 units four times daily (one-half dose in each side of mouth). NOTE: Limited clinical studies in premature and low birth-weight infants indicate that the 100,000 units four times daily is effective. Local treatment should be continued at least 48 hours after the oral candidiasis has disappeared and cultures returned to normal. It is recommended that the drug be retained in the mouth as long as possible before swallowing.

Intestinal candidiasis (moniliasis):
Usual dosage: 500,000 to 1 million units (approximately 1/8 to 1/4 teaspoonful) three times daily. Treatment should generally be continued for at least 48 hours after clinical cure to prevent relapse.

HOW SUPPLIED
Nystatin USP is supplied in containers of 50 million, 150 million, 500 million, and 2 billion units.

Product Code  Size  Approx. Weight
(NDC)   (Units)     (grams)
0574-0404-05  50 million  8.3 - 10
0574-0404-15  150 million  25 - 30
0574-0404-50  500 million  83 - 100
0574-0404-02  2 billion  333 - 400


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glutamate has been found to function as the fast excitatory neurotransmitter in these synapses.91

Using micro-iontophoretic techniques, several authors have demonstrated that glutamate antagonists can protect the afferent neurons of the inner hair cells from neuroexcitotoxicity.92-94

These studies have prepared the landscape for further efforts to assess the ability of specific glutamate antagonists and NMDA channel blockers to protect against noise-induced hearing loss and to serve as a possible treatment for peripheral tinnitus. Three such drugs under investigation are memantine, caroverine, and magnesium.

Memantine. Memantine (1-amino-3,5-dimethyladamantane) is a low-affinity, noncompetitive NMDA receptor blocker.95-96 It has been used in Europe for more than 10 years for the treatment of Parkinson’s disease97 and dementia.98,99 Several authors have noted its protective effect against glutamate neuroexcitotoxicity and hypoxia.100-103 More recently, studies have demonstrated memantine’s selective NMDA antagonism in the mammalian cochlea.92,103 Additionally, Oestreich et al proposed the use of glutamate antagonists such as memantine in treating inner ear disorders and recommended that they be delivered locally to the cochlea in order to maintain an effective therapeutic drug level and avoid systemic side effects.106

The safety and tolerability of memantine have been clearly demonstrated throughout its use in treating neurologic disorders in Germany.107,108 No serious treatment-related adverse effects have been recognized. In several double-blind, placebo-controlled trials of memantine as a treatment for primary, vascular, and Alzheimer’s-related dementias, only mild and transient side effects were encountered.95,100,109,110

Caroverine. Caroverine, a quinoxaline derivative, acts as a competitive AMPA antagonist, and at higher concentrations it noncompetitively blocks NMDA receptors at the glycine site in the cochlea.106 The neuroprotective effects of caroverine were confirmed in a study by Ehrenberger and Felix, who demonstrated that caroverine can depress the activity of glutamate receptors in the cochlea of guinea pigs.94 Caroverine is currently available in some countries (e.g., Austria) as a spasmyloytic drug. Its safety and tolerability have been clearly demonstrated in clinical studies of alcohol withdrawal syndrome and hypoxia.111-113

The efficacy of caroverine for the treatment of tinnitus was demonstrated in a single-blind, placebo-controlled clinical study by Denk et al.10 They found that approximately 63% of patients who were treated with intravenous caroverine reported a significant improvement immediately following infusion; the effect was still present after 1 week in 48% of these patients. Although no severe
adverse effects were identified, a few patients did experience mild and transient side effects, which included a bad taste, vertigo, headache, a "hot head" sensation, and additional noise. However, Saletu et al reported that caroverine might not have any therapeutic effect on tinnitus beyond that seen with placebo.111 Clearly, more clinical studies need to be conducted to resolve this conflict.

Magnesium. Extracellular magnesium plays an important role in maintaining membrane polarization. Through its effect on calcium channels, magnesium can reduce the influx of calcium that leads to cell damage.114,116 In the central nervous system, magnesium blocks the calcium-dependent release of glutamate117 and postsynaptically blocks NMDA receptors.79 Moreover, extracellular magnesium can improve inner ear microcirculation.118 Because magnesium concentration in the perilymph decreases significantly after intense noise exposure,119 researchers have studied its protective effects in preventing noise-induced hearing loss. For example, Attias et al conducted a double-blind, placebo-controlled study and found that a group of patients who had been given oral magnesium supplements displayed a significantly lower incidence of noise-induced permanent threshold shifts than did the controls.120 No significant side effects were identified.

In 1998, a highly motivated patient of the lead author elected to undergo catheter-delivered magnesium sulfate infusion to the round window. She had had right-sided tinnitus for 10 years, and it had become worse after she attended a football game 2 years earlier. Her tinnitus was severe and had not responded to aggressive medical intervention, including counseling, anxiolytic and oral steroid treatment, masking, and tinnitus retraining therapy. Within 60 seconds of the infusion of a dilute solution of magnesium sulfate via the round window microcatheter, the patient reported a complete resolution of her tinnitus. This effect persisted until the flow of solution was discontinued 48 hours later; at that point, her tinnitus returned within minutes.

Calpains

The calpains are a family of calcium-activated, neutral cysteine proteases. The two most common isozymes—mu-calpain (calpain I) and m-calpain (calpain II)—are ubiquitously distributed throughout the body. The mu-calpain is activated by micromolar concentrations (1 to 20 μM) of intracellular Ca++, the m-calpain is activated at higher concentrations (250 to 750 μM) of this ion.

Structurally, calpain contains a large calcium-dependent catalytic subunit (80 kD) and a smaller regulatory subunit (30 kD). The catalytic subunit contains four complexes that can bind to calcium. Calpain exhibits relative selectivity for proteolysis of a subset of cellular proteins. Normal substrates of calpain include cytoskele-

tal proteins, membrane proteins, transcription factors, calmodulin-binding proteins, and enzymes that are involved in signal transduction.121,122 Because some of the most preferred substrates are structural proteins, it has been hypothesized that extensive calpain activity can lead to a loss of structural and membrane integrity, reducing the cell's ability to maintain homeostasis.

Although much is known about the structural and enzymologic properties of mu-calpain and m-calpain, information on their physiologic roles is limited. The major technical obstacle to calpain research is the difficulty in identifying the physiologically relevant substrates from among the tens of thousands of proteins in the cell and the lack of sensitivity and specificity in detecting the in vivo proteolysis of these substrates in spatial terms. However, the physiologic and pathologic roles of calpain have been implied by several investigators.123,124 Previous studies have shown that calpains play a harmful role in a variety of pathologic states. Calpain is believed to be strongly related to certain brain pathologies (e.g., ischemia,125,126 traumatic brain injury,122 and Alzheimer's disease127), multiple sclerosis,128 toxic and anoxic injury to hepatocytes,129 oxidative stress in endothelial cells,130 spinal cord injury,131 human renal cell carcinomas,132 and calpain-mediated apoptosis.133

Several calpain antagonists are neuroprotective in vitro and in vivo during ischemia and in brain, spinal cord, and peripheral nerve injury.134-136 Two studies showed that the oral administration of leupetin, a potent calpain inhibitor, promoted neuronal muscle recovery after median nerve transection and repair.137,138 Moreover, prolonged administration of leupetin did not cause any adverse reactions. Immunofluorescent staining and Western blot analysis revealed that the continuous delivery of leupetin directly into the inner ear significantly reduced the amount of hearing loss experienced by chinchillas who had been exposed to noise. Calpains have been identified in the cochlea, and they are active during ischemic injury to cochlear tissues139 and during noise-induced hearing loss.140

Calpain inhibitors have been shown to significantly protect spiral ganglion neurons from damage by hypoxia and neurotrophin-withdrawal-induced apoptosis.141 Salvi et al showed that leupetin caused as much as a 60% reduction in outer hair cell loss from acoustic overstimulation (an octave-band noise centered at 4.0 kHz for 48 hr) and significantly reduced the amount of hearing loss during recovery.142 In addition, no deleterious effects on auditory function as measured by auditory brainstem-evoked response testing were observed when leupetin was continuously administered for 4 days to the scala tympani of chinchillas.143,144 In an animal study at the lead author's institution, we found that 1 mg/ml of leupetin could be safely infused into the round window membrane for 8 weeks.122 Leupetin had no effect on cochlear

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blood flow, auditory sensitivity, or cochlear histology.

Long-term studies have suggested that leupeptin is safe when administered to primates orally or via intramuscular injection; toxicologic testing has indicated that it does not adversely effect hematologic, clotting, or plasma complement component C3 profiles over a 6-month period.\textsuperscript{125,140}

Collectively, these data might provide the basis for future leupeptin clinical trials aimed at achieving tinnitus control and preventing noise-induced hearing loss. The use of leupeptin to treat tinnitus and noise-induced hearing loss has not yet been attempted in humans.

**Antioxidants**

The primary function of antioxidants is to scavenge ROS and thus reduce the toxic effects of oxygen. ROS contain an unpaired number of electrons, which makes them chemically reactive and extremely toxic to subcellular and cellular structures. It has been speculated that ROS are involved in more than 100 clinical conditions.\textsuperscript{145} They are produced in vivo during mitochondrial respiration as well as by auto-oxidation of chemical and biologic molecules. ROS are also environmental contaminants and can be formed by ionizing and ultraviolet radiation. Typically, the effects of these molecules and their activation is deleterious to the cell and tissue involved. Some of these molecules have the ability to upregulate adhesion receptors, increase vascular permeability, damage DNA and tissues, impair endothelial function,\textsuperscript{64,142} and possibly contribute to hearing loss, balance disturbance, and tinnitus.

Many intrinsic enzymes protect cells from oxidative damage. They include superoxide dismutase,\textsuperscript{143} glutathione peroxidase,\textsuperscript{144} glutathione transferase,\textsuperscript{145} and catalase. Additionally, antioxidant mechanisms require the action of a variety of small molecules in the human diet, such as vitamin E (tocopherol) and vitamin C, which trap radicals in lipid- and water-soluble membranes and reduce oxidative stress.\textsuperscript{146}

Experimentally and clinically, it is well known that ROS are primarily generated as a byproduct of oxidative phosphorylation and ischemia/reperfusion or prolonged hypoperfusion, as is seen in myocardial infarction, in cerebrovascular accidents, and possibly in sudden sensorineural hearing loss. There is compelling evidence implicating ROS in the damage associated with cochlear ischemia, noise trauma, and ototoxicity. Specifically, localized inner ear ischemia and hypoxia (induced by selectively clamping the anteroinferior cerebellar artery) normally destroy the cochlear action potential within seconds, and the effect becomes permanent after 8 minutes of ischemia. Yet one study showed that when rats were pretreated with allopurinol or superoxide dismutase/polyethylene glycol prior to the induction of ischemia, cochlear action potential thresholds were maintained.\textsuperscript{53} This study was extended to evaluate noise-induced hearing loss, which has been shown to cause vascular perturbations. Subjects that had been pretreated with scavengers and ROS blockers experienced less of a threshold shift than did controls ($p<0.05$).\textsuperscript{53}

Aminoglycosides appear to cause their damage to the cochlea by generating ROS, which are generated after the antibiotic combines with iron to form an aminoglycoside/iron complex. Sha and Schacht reported that iron-chelating agents were useful in attenuating aminoglycoside-induced cochlear damage.\textsuperscript{2} Lautermann et al demonstrated a possible relationship between ototoxicity and dietary factors.\textsuperscript{147} Specifically, they showed that an ROS scavenger (glutathione) attenuated gentamicin-induced hearing loss in guinea pigs that had been placed on a low-protein diet (as opposed to a regular diet). Other investigators have found similar mechanisms underlying ototoxicity secondary to other compounds. For example, Clerici et al showed that direct ROS-induced cochlear injury following cisplatin administration occurred in guinea pigs, but that ROS scavengers afforded protection from cisplatin.\textsuperscript{148,149} They also demonstrated that superoxide dismutase protects against trimethyltin chloride-induced ototoxicity.\textsuperscript{149}

The lead author's institution is also investigating the effects of glutathione (L-gamma-glutamyl-L-cysteinylglycine). Glutathione is an endogenous thiol-containing amino acid that detoxifies ROS. It is also involved in the metabolism and detoxification of xenobiotics, drugs, and drug metabolites, and it offers protection from oxidizing ROS via reactions catalyzed by glutathione S-transferase, transpeptidases, transhydrogenases, peroxidasases, and reductases.\textsuperscript{150,152} Mitochondrial glutathione is critical to cell viability, and the glutathione redox cycle is a primary antioxidant defense system within the mitochondrial matrix.\textsuperscript{152}

Many studies have demonstrated that high glutathione levels have a beneficial effect on cellular function, while low levels are harmful.\textsuperscript{153-158} Specifically, it has been shown that sulphydryl compounds limit gentamicin-induced damage to outer hair cells in vitro and that in vivo gentamicin ototoxicity can be diminished with glutathione.\textsuperscript{159,160} Glutathione also offers protection from cisplatin ototoxicity.\textsuperscript{161} Conversely, systemic inhibition of glutathione synthesis potentiates the ototoxicity of the ethacrynic acid/kanamycin combination.\textsuperscript{162} and glutathione depletion potentiates cisplatin nephrotoxicity.\textsuperscript{162-164} It has also been shown that the toxicity of certain clinically used drugs occurs secondary to reduced glutathione levels and the associated increase in ROS.\textsuperscript{160,165-169} Other related studies have demonstrated an 86% age-associated reduction in glutathione levels in the auditory nerve, while other cochlear tissues maintained stable levels.\textsuperscript{170}
Methionine
Methionine, a thiol-containing essential amino acid, has metal-chelating and antioxidant properties. It has been shown that D-methionine suppresses gentamicin-induced free-radical formation in vitro and in cell cultures. Furthermore, the concurrent systemic twice-daily administration of D-methionine and gentamicin significantly attenuates auditory threshold shifts in guinea pigs. Methionine can also provide protection against the ototoxic effects of cisplatin; D-methionine suppresses cisplatin-induced damage to auditory hair cells in organotypic cultures. Other animal studies have revealed the protective effects of D-methionine in preventing the hair cell loss and acute auditory threshold shifts induced by cisplatin administration. Campbell et al reported that both D-methionine and the naturally occurring L-methionine, which is more easily metabolized, completely blocked the ototoxic effects of cisplatin for 7 days in rats. The antineoplastic activity of cisplatin, however, was significantly reduced by D- and L-methionine, as assessed by in vitro cultures of tumor cell lines and tumors implanted in vivo. Conversely, a more recent study by Li et al showed that the administration of L-methionine to the round window membrane of rats significantly attenuated the ototoxic effects of cisplatin, as measured by auditory brainstem-evoked response testing, but did not interfere with the chemotherapeutic effectiveness of cisplatin in controlling a highly metastatic form of breast cancer.

Among the many other agents available to treat the inner ear are grape-seed extract (resveratrol) from red wine and pine-bark extract, both of which are excellent antioxidants. Preliminary studies in the lead author’s laboratory are in progress to help us understand the effects of red-wine and grape-seed extracts on noise-induced and age-related hearing loss. Additionally, some nutritional supplements enhance mitochondrial function and energy output. One is a patented supplement that contains acetyl-L-carnitine, alpha lipoic acid, glutathione, and coenzyme Q-10. In animal studies, these substances have been shown to protect against age-related hearing loss. Perhaps direct perfusion of these substances to the inner ear will have an even more pronounced effect, but clearly additional studies are required.

The oxidative stress that is caused by hypoxia and ischemia produces a variety of damaging ROS, including hydrogen peroxide, the superoxide anion, and the hydroxyl radical. The accumulation of ROS, cytokines, and chemokines that is associated with hypoxia and ischemia promotes the expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, which subsequently leads to neutrophil-endothelial cell adhesion. This process leads to an increase in circulating tissue levels of various cytokines, leukotrienes, thromboxanes, platelet activating factor, complement components, elastases, and other enzymes, and it causes the formation of additional ROS. Typically, the effects of these molecules on their activation are deleterious to the cells and tissues involved. Some of these substances upregulate leukocyte adhesion receptors, increase vascular permeability, damage tissues directly, impair endothelial function, and eventually lead to edema, vascular insufficiency, and ultimately necrosis.

Anti-ICAM-1 antibody has been shown to attenuate damage in the brain, heart, kidney, and other tissues where ICAM-1 plays a critical role in ischemia-induced damage. Studies have shown that ICAM-1 also plays an important role in middle ear diseases (including otitis media and cholesteatoma), inner ear inflammation, and carcinoma of the head and neck. The lead author was involved in a recent study that assessed the possible protective effect of anti-ICAM-1 antibody against noise-induced cochlear damage by evaluating noise-induced temporary threshold shifts. Our premise was that intense noise exposure reduces cochlear blood flow and causes ischemia, which leads to the production of ROS. The accumulation of ROS then promotes the expression of ICAM-1 and initiates a cascade of events that ultimately leads to cochlear damage. Auditory brainstem-evoked response testing indicated that noise-induced temporary threshold shifts could be significantly attenuated by administering anti-ICAM-1 antibody intravenously. This protective effect suggests that there is a mechanism of inflammatory prevention whereby anti-ICAM-1 antibody prevents ICAM-1 from eliciting a deleterious response that would otherwise lead to cochlear damage.

A significant amount of data shows that ROS promote the expression of ICAM-1 on endothelial cells and subsequently on neutrophil-endothelial cell adhesion. For example, hydrogen peroxide-induced polymorphonuclear neutrophil adhesion is dependent on the rapid induction of the ICAM-1 mRNA signal and the surface expression of ICAM-1 on the endothelial cell. In addition, hydrogen peroxide-induced expression of hyper-adhesivity might amplify polymorphonuclear neutrophil attachment to the endothelium. Furthermore, Linas et al demonstrated (1) that ischemia in vivo followed by reperfusion in isolated perfused kidneys resulted in neutrophil retention, (2) that retained neutrophils adversely affected renal function, and (3) that neutrophil retention was dependent on ICAM-1 and oxygen metabolites. These findings indicate that the effect of hydrogen peroxide (i.e., ROS) in instigating neutrophil adhesion is mediated by ICAM-1. Other studies were conducted to show that ROS scavengers and blockers can attenuate ischemia/reperfusion-induced and noise-induced cochlear damage, presumably by preventing ROS from inducing the expression of ICAM-1. Findings of a study at the lead
author’s institution suggest that anti-ICAM-1 antibody has a similar effect in preventing noise-induced cochlear damage. This effect is mediated by blocking ICAM-1 without affecting cochlear blood flow. The long-term effectiveness of anti-ICAM-1 antibody is not clear and warrants further investigation.

Neurotrophic factors
Among the many causes of sensorineural hearing loss are noise trauma, aging, ototoxicity, and genetic, vascular, and viral influences. Ultimately, the damage caused by these processes leads to a uniform degeneration of hair cells, auditory neurons, or the VIIIth cranial nerve. Endogenous factors—including epidermal growth factor, transforming growth factor alpha, insulin, insulin-like growth factor-1, insulin-like growth factor-2, and glial cell-line-derived neurotrophic factor (GDNF)—have been shown to play roles in the renewal and repair of damaged populations of hair cells in the mammalian labyrinth. In mammals, this process seems to be limited to the vestibule, where supporting cells transdifferentiate into hair cells and/or damaged hair cells are repaired. By enhancing these endogenous protective mechanisms, potential therapeutic options for hearing loss might be revealed.

Gao reported a study in which exogenous neurotrophic factors were applied to postnatal rat cochlear explant cultures that had been exposed to different classes of ototoxins: neurotrophin-4/5, brain-derived neurotrophic factor (BDNF), and neurotrophin-3 were found to protect spiral ganglion neurons from ototoxicity induced by gentamicin, sodium salicylate, and cisplatin. Moreover, Gao found that concanavalin A, a lectin molecule, also significantly protected hair cells from gentamicin ototoxicity. In another study, Altschuler et al found that BDNF and GDNF enhanced spiral ganglion cell survival, while a combination of BDNF and fibroblast growth factor induced peripheral process regrowth. Others have shown that chronic infusion of both neurotrophin-3 and GDNF into the inner ear provided significant protection from acoustic trauma in guinea pigs.

Staecker et al reported (1) that direct infusion of either neurotrophin-3 or BDNF into the scala tympani of a guinea pig cochlea after a loss of auditory hair cells compensates for the loss of trophic support (i.e., the inner hair cells), (2) that the infused neurotrophin protects the auditory neurons of the spiral ganglion from cell death induced by neurotrophin molecule withdrawal, and (3) that either BDNF or neurotrophin-3 stimulated neuritic overgrowth of the VIIIth cranial nerve peripheral processes. In an in vivo gene therapy study, Staecker et al introduced the gene for BDNF into the cochlea of mice that had neomycin-induced cochlear damage via the replication-defective HSVdNflac viral vector. This BDNF gene therapy in the cochlea resulted in the prevention of the cochlear neuronal degeneration that was observed in the controls.

Many other gene-altering protective opportunities exist to repair defective or malfunctioning cells, including the use of other gene vectors and antisense oligonucleotides. Antisense oligonucleotides are small sections of synthesized cDNA (20 to 25 base pairs in length) that are complementary to a sequence of base pairs that are present in the mRNA of the gene that is targeted for disruption. The antisense oligonucleotide binds to the mRNA and causes enzymes in the cell to degrade it, thereby effectively preventing translation of the targeted gene. Antisense oligonucleotides were shown to be effective in vitro for targeting molecules in the cell-death pathway (e.g., c-Jun) in dissociated cell cultures of the rat spiral ganglion that had been exposed to oxidative stress with cisplatin. It is feasible that the infusion of antisense oligonucleotides targeted to certain genes in a cell-death signal pathway might also be effective in vivo because infused antisense oligonucleotides targeted to the GluR2 AMPA receptor have been shown to modify synaptic excitatory transmission in the cochlea of guinea pigs.

Conclusion
Our management of inner ear diseases is limited only by our current lack of a full understanding of their pathophysiology. Once a mechanism has been elucidated, novel compounds or existing medications might prove to play a critical role in the management of an array of otologic symptoms and disorders.

It is important to remember that the types of treatments discussed in this article presume end-organ dysfunction. Although many otopathologies are end-organ in etiology, it is possible that many forms of tinnitus, for example, are centrally mediated and might not respond to local therapy at the inner ear. If central tinnitus represents overexcitation or overstimulation because of a loss of inhibition, it might be possible to precisely determine its site of origin by functional magnetic resonance imaging, single-photon emission computed tomography, positron-emission tomography, or other imaging modalities. It might then be possible to temporarily inhibit the precise area in the brain and alleviate the tinnitus through electrical stimulation or deafferentation with lidocaine. Provided that no adverse sequelae occur (i.e., an expressive or receptive aphasia or the loss of an important auditory memory), this defined microarea of the brain could be ablated by either excision or cryotherapy, which might even result in a complete cure for some forms of tinnitus. From a more global perspective, the utility of pharmacologic manipulation of the membranous labyrinth might play a considerable role in the treatment of a variety of otologic disorders.
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