Published in final edited form as:

Expert Opin Investig Drugs. 2015 February ; 24(2): 201–217. doi:10.1517/13543784.2015.960076.

Early investigational drugs for hearing loss

Debashree Mukherjea, PhD^{†,1,2} [Research Assistant Professor], Sumana Ghosh, MS², Puspanjali Bhatta, BS², Sandeep Sheth, PhD², Srinivasan Tupal, PhD², Vikrant Borse, BS², Thomas Brozoski, PhD³ [Professor], Kelly E Sheehan, MS⁴, Leonard P Rybak, MD, PhD^{4,5} [Professor], and Vickram Ramkumar, PhD⁶ [Professor]

¹Southern Illinois University School of Medicine, Department of Surgery, P. O. Box 19629, Springfield, IL 62794-9629, USA

²Southern Illinois University School of Medicine, Department of Pharmacology and Neuroscience, Springfield, IL, USA

³Southern Illinois University School of Medicine, Department of Surgery, Springfield, IL, USA

⁴Southern Illinois University School of Medicine, Department of Surgery, Springfield, IL, USA

⁵Southern Illinois University School of Medicine, Department of Pharmacology, P. O. Box 19649 Springfield, IL 62794-9649, USA

⁶Southern Illinois University School of Medicine, Department of Pharmacology and Neuroscience, PO Box 19629, Springfield, IL, USA

Abstract

Introduction—Sensorineural hearing loss (HL) is becoming a global phenomenon at an alarming rate. Nearly 600 million people have been estimated to have significant HL in at least one ear. There are several different causes of sensorineural HL included in this review of new investigational drugs for HL. They are noise-induced, drug-induced, sudden sensorineural HL, presbycusis and HL due to cytomegalovirus infections.

Areas covered—This review presents trends in research for new investigational drugs encompassing a variety of causes of HL. The studies presented here are the latest developments either in the research laboratories or in preclinical, Phase 0, Phase I or Phase II clinical trials for drugs targeting HL.

Expert opinion—While it is important that prophylactic measures are developed, it is extremely crucial that rescue strategies for unexpected or unavoidable cochlear insult be established. To achieve this goal for the development of drugs for HL, innovative strategies and extensive testing are required for progress from the bench to bedside. However, although a great deal of research needs to be done to achieve the ultimate goal of protecting the ear against acquired sensorineural HL, we are likely to see exciting breakthroughs in the near future.

[†]Author for correspondence: Southern Illinois University School of Medicine, Department of Surgery, Springfield, IL, USA, dmukherjea@siu.edu.

Declaration of interest: The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Keywords

aminoglycoside antibiotics; anti-inflammatory agents; antioxidants; cisplatin; cochlea; mAbs; noise-induced hearing loss; ototoxicity; presbycusis or age-induced hearing loss; reactive oxygen species; sensorineural hearing loss; short inhibitory RNA; vitamins

1. Introduction

The center for hearing and communications quotes that “approximately 12% of the U. S. population or 38 million Americans have a significant hearing loss”, among them are 3 million children with 1.3 million under the age of three [1]. Globally, it has been estimated that nearly 600 million people have hearing loss (HL) [2] and by 2050 nearly 1.2 billion people will have significant HL. The most vulnerable populations are the pediatric patient and the elderly (over 60 years of age). HL wreaks havoc on national economies and in quality of life of the individuals. There are no approved treatments available as yet other than devices such as hearing aids and cochlear implants. Therefore, it is imperative that prophylactic as well as treatment measures be developed and tested for human use.

In this review paper, we address new drugs that have potential for being used as either prophylaxis or rescue of HL. We discuss drugs that have shown promise in animal models as well as in clinical trials (Phase 0, I or II). The different types of HL addressed are: drug-induced (platinum drugs and aminoglycosides) vascular-borne, noise-induced (mechanical stress), sudden sensorineural (usually immune mediated) and HL related to aging (presbycusis). Platinum chemotherapy drugs like cisplatin and carboplatin are ototoxic, and the severity of HL depends on the cumulative dose. The HL is usually bilateral, and the most vulnerable population is pediatric patients treated for neuroblastomas and other CNS malignancies. Aminoglycosides are used in the treatment of hospital or community-acquired serious gram-negative infections, tuberculosis and tularemia. Noise-induced hearing loss (NIHL) affects ~ 30 million Americans who are exposed to hazardous levels of noise occupationally recreationally, or from environmental exposures in highly populated urban areas [3]. Sudden sensorineural hearing loss (SSHL), as the name suggests is sudden, unexplained, rapid loss of hearing and usually occurs in one ear. Age-induced HL is often manifested as the loss of ability to hear high-frequency sounds and worsens with increasing age. It is estimated that 30 – 35% of people over the age of 70 have some HL and almost 50% of the people over the age of 75 have HL related to aging [4]. Cytomegalovirus (CMV) is an important cause of congenital sensorineural HL. It is transmitted *in utero* and has devastating effects on child development.

It is generally accepted that ‘chronic’ excess accumulation of reactive oxygen species (ROS) and the accompanying inflammation in the cochlea are the basis of many forms of sensorineural hearing loss (SNHL). Thus, ROS quenchers, antioxidants and anti-inflammatory drugs that target ROS generation, inflammatory cytokines or molecules of the apoptotic pathways form major classes as potential drugs for the prophylaxis or ‘rescue’ from various kinds of SNHL. This is reflected in the type of compounds being tested in clinical trials as well as those that have shown promise in animal models. The clinical trials have been summarized from the clinical trials. gov web page (www.clinicaltrials.gov) [5],

and the preclinical and animal studies were comprehensively derived from PubMed (www.pubmed.gov) [6]. The criteria for inclusion were the studies that had to be either in the preclinical, Phase I or Phase II of clinical development. We have not included any study that is in Phase III clinical development or beyond. The different early phase interventions have been grouped according to their mode of action.

2. Antioxidants in HL

ROS generation in the inner ear has been found to play a key role in age-related [7,8], noise-related [9,10] and drug-induced HL [11,12]. This chronic ROS overload leads to the depletion of the cochlear antioxidant enzyme system (e.g., superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GSH-Px] and glutathione reductase [GSH-R] etc.), which scavenges and neutralizes the generated superoxide and hydrogen peroxide. Antioxidants are reducing agents that inhibit oxidation of other molecules, thus preventing the cascade of free radicals such as ROS. Herein, we summarize clinical trials of antioxidants and discuss their translational potential in prophylaxis and treatment of HL.

2.1 Ebselen

Ebselen contains selenium and mimics GSH-Px activity. Thus, it selectively scavenges peroxides [13-15]. Ebselen has also been shown to inhibit cyclooxygenase and lipoxygenase enzymes thereby acting as an anti-inflammatory agent.

SPI-1005 is a proprietary preparation of ebselen prepared by Sound Pharmaceuticals that allows it to be effective orally. Phase I of clinical trials to determine the safety and pharmaco-kinetics of SPI-1005 have been completed (Study to Evaluate the Safety and Pharmacokinetics of SPI-1005: NCT 01452607). During this trial, a single dose of ebselen in increasing doses (200, 400, 800 and 1600 mg) was given to the subjects who were then monitored for the safety and pharmacokinetic properties of the drug. No clinical treatment data are available yet.

Phase II clinical trials of SPI-1005: Otoprotection with SPI-1005 for Prevention of Temporary Auditory Threshold Shift (NCT01444846) to evaluate potential prevention of temporary threshold shifts in hearing that may occur after listening to music through an iPod or personal music player has been completed. The sound level was established based on prior human studies where a slight temporary auditory threshold shift was safely induced in the subjects who were randomized to either placebo or SPI-1005 and treated before and after music exposure. All subjects and investigators were blinded to the intervention. Three different dosages of SPI-1005, 200, 400 and 600 mg, were used in this study and were administered in the form of oral capsules twice daily for 4 days. The level of temporary threshold shift was determined by pure-tone audiometry. Comparisons between the treatment and placebo groups were carried out at several time points after the music exposure. Clinical data are not yet available. This clinical trial is being sponsored by Sound Pharmaceuticals, Inc. in collaboration with University of Florida. This Phase II clinical trial is still active. The details of this study have not been disclosed; therefore, the sound exposure levels or the duration of exposure are unknown. Similarly, the treatment regimen

does not specify the time of administration in relation to the sound exposure. Ebselen seems to be a strong candidate for NIHL if the clinical trial data show benefit.

Ebselen is also under clinical investigation for chemotherapy-induced ototoxicity in adult cancer patients, who can suffer both HL and tinnitus: SPI-1005 for Prevention and Treatment of Chemotherapy Induced Hearing Loss (NCT01451853). The objective of this trial is to evaluate the safety and efficacy of SPI-1005 at three dose levels when delivered orally twice daily for 3 days, surrounding each cycle of platinum chemotherapy for head and neck or non small cell lung cancer patients to prevent and treat chemotherapy-induced HL and tinnitus. This clinical trial is yet to open for recruiting.

In animal studies, ebselen was shown to improve GSH-Px levels within critical cochlear structures thereby reducing the temporary threshold shift induced by intense noise and cisplatin. Kil *et al.* reported that treatment with oral ebselen (4 mg/kg) in F-344 rat before and immediately after noise exposure reduced both outer hair cell loss and the acute swelling of stria vascularis by scavenging ROS and other free radicals and by stimulating glutathione peroxidase expression and activity [16]. Another study by Lynch *et al.* showed that oral ebselen along with allopurinol, a xanthine oxidase inhibitor, significantly protected against HL in a rat model of acute cisplatin toxicity by preventing the loss of outer hair cells. A subsequent publication showed that the combination of ebselen with allopurinol does not affect the chemotherapeutic efficacy of cisplatin in the rat model [17].

2.2 N-acetylcysteine

N-acetylcysteine (NAC) is a derivative of cysteine in which an acetyl group is attached to the nitrogen atom. It is an antioxidant with liver protecting properties. NAC is commonly sold as a dietary supplement. It is also used to treat cough. It breaks disulfide bonds in mucus and liquefies it, making it easier to expectorate. Clinically, NAC is used: as a mucolytic agent; in the management of paracetamol (acetaminophen) overdose; and in carbon monoxide poisoning. Other uses of NAC include sulfate repletion in conditions such as autism, where cysteine and related sulfur amino acids may be depleted [18]. NAC is well tolerated by humans. The first use of NAC in protection from noise-induced ototoxicity was in combination with salicylic acid [19] in the chinchilla model of HL. Since then, NAC has been shown to be somewhat protective against cisplatin ototoxicity (about ~ 20% of ears protected) in a pilot randomized study in head and neck cancer patients [20]. In this study, 2% L-NAC was administered transtympanically in one ear (the other ear served as a control) of 11 patients treated with cisplatin. Statistically significant hearing preservation was seen in two patients (18.2%), compared to the untreated control ear. However, as a group, hearing in the NAC-treated ears did not show statistically significant protection. Two per cent NAC was well-tolerated transtympanically. There is the possibility that the dose of NAC used in this study was lower than what was required to elicit a robust protective effect.

Another ongoing study that uses NAC in combination with magnesium against noise damage is reported on the clinical trials website: Prevention of Noise-induced Damage by Use of Antioxidants (NCT 01727492). The study is being conducted in Belgium, and the combination of NAC (600 mg) and magnesium (200 mg) is named Antioxidantia. This study will determine the ability of Antioxidantia to prevent NIHL and noise-induced tinnitus

(NIT), when taken orally as prior to noise exposure. Antioxidantia will be given to young adults 1 h prior to leisure noise above 100 dB for at least 3 consecutive hours. Each participant will be exposed to this noise four times, with an interval of at least 4 days between each noise exposure and will be given either the antioxidant (twice) or the placebo (twice). NIT will be scored by a visual analogue scale, and tinnitus loudness will be assessed. This will be measured prior to noise exposure (at the time of antioxidant/placebo intake) and 1 h after noise exposure. The predicted outcome is a reduction of 50% of the tinnitus loudness in the antioxidant-treated group compared with the placebo group. The study participants will also undergo audiological testing (audiometry up to 16 kHz, distortion product as well as transient-evoked otoacoustic emissions and speech-in-noise tests), and the data generated will compare the placebo results to the antioxidant trials and will be performed prior to and within 7 h after noise exposure. The latest update on this study was published by Gilles *et al.*, who reports that half of the participants has been recruited at the time of this article submission in November 2013. This is a well-designed study [21], but it has not been completed and there are no results posted as yet. We expect this study may have positive results. In the future, it will be very interesting to see whether Antioxidantia can rescue patients from NIHL and NIT when administered after noise insult.

In a different study: The Use of Anti-oxidants to Reduce Sequela of Mild Traumatic Brain Injury (mTBI) After Blast Exposure (NCT00822263), it was seen that NAC administration reduced the sequelae of mTBI in military personnel after blast exposure. The results of this study have been published [22]. Briefly, active duty military personnel diagnosed at the Al Taquaddum Level II Medical Facility (TQ), in Iraq for mTBI were administered 4-g loading dose of NAC or placebo either within 24 h or 26 – 72 h post-traumatic brain injury. The subsequent doses of 4 g daily for the next 3 days within 18 – 24 h after the first dose and then 3 g dose for the next 3 days were administered in a placebo-controlled double-blind randomized study. Eighty-one participants completed this study and were evaluated for HL and several other mTBI symptoms. The primary outcome measure for this study was the resolution of these symptoms 7 days after the blast exposure. It was reported that the outcome of ‘no day 7 symptoms’ by logistic regression analyses, indicated that NAC treatment was significantly better than placebo (OR = 3.6, $p = 0.006$). Secondary analysis also revealed that participants who received NAC within 24 h of blast had ~ 86% chance of symptom resolution versus 42% for those seen early who received placebo. No adverse side effects from the drug were reported.

This study was conducted in an active war zone and demonstrates that NAC is a well-tolerated drug and has been shown to ameliorate the severity and resolution of the mTBI sequelae. This is an acute study, and the long-term benefits of NAC in war zone personnel suffering from mTBI needs to be documented to confirm and establish that significant resolution of symptoms with NAC is actually long lasting. It would be of immense help if these participants could be contacted for a follow-up, to ensure that the benefit seen by NAC use is actually permanent, because permanent HL cannot be evaluated within 7 days of injury.

NAC is also being evaluated for its protection against cisplatin-induced ototoxicity at the Children's Hospital in Los Angeles (NAC to Prevent Cisplatin-induced Hearing Loss: NCT

02094625). Specifically, this study is to evaluate the dosage required to protect from cisplatin ototoxicity and tolerability of NAC in the pediatric population in the traditional 3 + 3 dose-escalation scheme. The patients will be evaluated for NAC and glutathione serum levels at baseline prior to the start of chemotherapy (-6 h), immediately following cisplatin (prior to NAC) (0 h), immediately following NAC (0.5 h) and 4 h post-NAC infusion (4 h). This will also help establish intra-patient and inter-patient variability in achieved serum levels. Hearing assessment and renal toxicity will be assessed up to 40 weeks. Tumor response to treatment and effect of genotype on HL and protection will be assessed up to 15 weeks post-cisplatin chemotherapy. The data collected will be compared to the patient's baseline or to normal established levels. This is a Phase I clinical trial and has not started recruiting participants at this time. However, it will be critical to closely monitor pediatric patients as NAC seems to affect the rate of metalloprotein degradation in plasma and this would be in addition to myelosuppression seen by cisplatin, Sooriyaarachchi *et al.* [23].

NIHL is another condition for which NAC is being evaluated as an otoprotectant. In this clinical trial: Antioxidation Medication for NIHL (NCT00552786). This clinical trial recruited 53 noise-exposed workers from steel industries in Taiwan. In this randomized, double-blind, crossover Phase II study, 1200 mg NAC or placebo was administered orally to the noise-exposed workers for 2 weeks. Their temporary threshold shift in hearing was examined, and blood samples were collected to determine deletion polymorphisms in the glutathione *S*-transferase (GST) T1 and GSTM1 genes. The results published for this study show that NAC had a significant protective effect against temporary threshold shifts at high frequencies; however, NAC did not affect the temporary threshold shift at low frequencies. When workers were grouped by GSTT1 and GSTM-null genotypes ($p = 0.004$), NAC's otoprotective effect seemed more prominent [24].

NAC is also being used to determine whether it can effectively prevent aminoglycoside-induced ototoxicity in peritoneal dialysis patients. In this clinical trial: Prevention of Drug Induced Ototoxicity in Peritoneal Dialysis Patients by NAC (NCT01131468). In this Phase II clinical study, NAC was evaluated for protection against HL induced by amino-glycoside and/or vancomycin in peritoneal dialysis patients. Although no improvement in hearing of the patients after 1 week was observed, there was significant hearing improvement after 4 weeks of NAC treatment ($p < 0.05$).

Earlier studies have shown that NAC can significantly reduce cochlear cell apoptosis and the generation of ROS to mitigate radiation-induced ototoxicity in patients undergoing radiotherapy [25,26]. It was also reported that NAC had a protective effect against cisplatin-mediated ototoxicity as well [25,27]. NAC can mitigate the production of ROS, significantly reducing the cascade of ROS-mediated toxic events. NAC could be an effective drug against various ototoxic agents. Further clinical studies with cisplatin, aminoglycosides and noise exposure and NAC may confirm the effectiveness of prophylactic administration of NAC against HL.

2.3 Lactated Ringer's

Lactated Ringer's is isotonic with blood and is traditionally used to combat hypovolemia due to blood loss caused by trauma, surgery or burn injuries. It is also used to counteract liver

acidosis and helps in the maintenance of stable blood pH. In ototoxicity studies, its success as a protective agent appears to be based on its ability to maintain pH balance.

Lactated Ringer's solution was serendipitously discovered to offer near complete preservation of distortion product of otoacoustic emission, when administered transtympanically, against cisplatin-induced ototoxicity by Choe *et al.* in guinea pigs [28]. These findings were confirmed and extended by Nader *et al.* and Sanli *et al.* using auditory brainstem responses in guinea pig and rats, respectively [29,30]. On the other hand, Munguia *et al.* have shown that intratympanic administration of Ringer's lactate solution through a tympanostomy tube failed to provide protection against cisplatin-induced HL in chinchillas [31].

Lactated Ringer's has been used in clinical trial: Protection from Cisplatin Ototoxicity by Lactated Ringers (NCT005 84155). This clinical trial, sponsored by University of Oklahoma, aimed to determine if the application of lactated Ringer's solution into the middle ear could prevent cisplatin-induced HL. Patients older than 18 years of age with head and neck cancer with no active external or middle ear disease were included in the study. The subjects were assigned to normal saline treated placebo comparator and lactated Ringer's treated experimental groups administered as ear drops into the external auditory canal 30 min after cisplatin chemotherapy (minimum dose of 70 mg/m²) was started and at hourly intervals for 4 h after infusion. The patients were assigned randomly as single blinded and single intervention group. Each participant received a hearing evaluation (audiogram) before each dose of cisplatin and another evaluation 2 – 4 weeks after the final treatment. This trial has been completed, but no results have been posted or published.

Another study in Phase I and Phase II clinical trial is: Trans-tympanic Ringer's Lactate for the Prevention of Cisplatin Ototoxicity (NCT01108601). In this study, a pressure-equalizing tube was inserted under local anesthesia to ensure adequate drug delivery to the middle ear, prior to chemotherapy treatment in the patients. For each patient, one ear would receive the Ringer's lactate solution (Ringer's lactate [0.03% ciprofloxacin]) and the contralateral ear will act as an untreated control. The patients were instructed to administer four drops of Ringer's lactate solution to the experimental ear twice a day during their chemotherapy treatment. Pre- and post-chemotherapy audiograms and distortion product otoacoustic emissions (DPOAEs) were to be compared to determine changes in hearing from baseline and between ears. To determine possible long-term effects hearing was to be assessed every 6 months after chemotherapy treatment for up to 4 years. This study was sponsored by McGill University Health Center, CA. The last verified information was in 2010, and the study was recruiting participants. This study may have a better chance for positive results compared to the previous study due to the insertion of ventilation tubes. Lactate is expected to provide an alternate source of bicarbonate and prevent cisplatin-induced acidosis in the cochlea. However, there are no results or updates available on this study.

2.4 Ginkgo biloba

Ginkgo biloba extract (GBE761) is a very potent superoxide and hydroxyl-free radical scavenger and can block apoptosis [32,33]. Previous animal (intraperitoneal) and clinical studies (per oral) have shown that concomitant or prior administration of GBE761 can

significantly and effectively antagonize cisplatin-induced ototoxicity and unilateral idiopathic sudden SNHL in a prospective, randomized double-blind study of 106 patients [32-37]. In the Phase II clinical trial: The Protective Effect of Ginkgo Biloba Extract on Cisplatin-induced Ototoxicity in Humans (NCT01139281), patients were randomly assigned to drug (120 mg GBE761 twice a day along with maximal cumulative cisplatin dosage of 300 mg/m²) or placebo (maximal cumulative cisplatin dosage of 300 mg/m²) group. Treatment was double blinded. Hearing was evaluated by distortion-product otoacoustic emissions assessed for 90 days. This study was sponsored by University of Brasilia and has been completed in 2010. No results or updates have been posted or published.

2. 5 Vitamins and micronutrients

Various antioxidants in the form of vitamins (β -carotene [metabolized to vitamin A *in vivo*], vitamin C, vitamin E [38-40], enzymes [manganese SOD] [41] and other dietary micronutrients [selenium and magnesium]) and NAC [37] have been successfully investigated in animals for the prevention of HL. Some of these antioxidants are currently being tested in clinical trials.

Dietary supplements and vitamins like A, C and E that scavenge ROS can act synergistically with minerals like magnesium to prevent ROS-induced damage to the inner ear. A combination of natural dietary supplements, which includes β -carotene (metabolized to vitamin A), vitamins C and E and magnesium, has shown promise for protection against NIHL in animal models [42]. The combination of these antioxidant vitamins (vitamins A, C and E) and mineral magnesium (acts in part as a vasodilator but also as an antioxidant) was evaluated for its efficacy to prevent NIHL in humans in the clinical trial: Micronutrients to Prevent Noise-induced Hearing Loss (NCT00808470). A randomized, double-blind, cross-over study was conducted in Swedish military personnel to evaluate potential protection against temporary changes in hearing induced by military weapons training, using this nutrient therapy. The study reported that the noise exposures did not result in any significant threshold shift either in the placebo or in the treatment group. No noise-induced changes were observed in any of the secondary functional test metrics [43]. However, analysis of the plasma samples after oral consumption for 2 days (short-term dosing) confirmed significant elevations of all the nutrients, except magnesium, in plasma 2 h after administration. The elevated plasma levels of the nutrients after a short-term dosing indicates that the nutrients have good bioavailability. This trial design and study protocol can be applied to other populations exposed to different noise insults.

A retrospective clinical study by Choi et al. also highlights the importance of antioxidant vitamins (daily β -carotene and vitamins C and E) in attenuating the risk of HL in the US general population. A cross-sectional data from 2592 participants of age group 20 – 69 years showed that higher intake of these micronutrients and magnesium lowered the risk of HL as inferred from the lower (better) pure-tone averages (PTAs) at both speech (0.5, 1, 2 and 4 kHz) and high frequencies (3, 4 and 6 kHz). Moreover, a comparative study of higher intake of β -carotene or vitamin C with high magnesium versus a lower intake of both nutrients demonstrated a significantly decreased risk of HL [44]. The investigators did not clarify which causes of HL are ameliorated by antioxidant vitamins. This study included a wide

range of ages of subjects and most likely included various types of HL. Recently, in a population-based study, Kang et al. have also demonstrated a protective role of dietary vitamin intake against age-related HL. Statistical analysis from 1910 participants between age group 50 and 80 years showed that dietary supplement use of vitamin C significantly preserves hearing at all frequencies. Interestingly, though the same group reported that high serum concentration of vitamin D was correlated to HL instead of hearing protection [45]. Kang *et al.* [45] thus strongly recommend judicious intake of vitamins as a preventive measure for HL in the elderly population.

2.6 α -Lipoic acid

α -Lipoic acid, an essential cofactor for mitochondrial enzymes, has been demonstrated to exert a protective effect against cisplatin-induced HL in animal models [46,47]. A recent study by Kim *et al.* [47] reported that α -lipoic acid pre-treatment significantly reduced apoptotic cell death of the inner and outer hair cells in cisplatin-treated rat organ of Corti explants. This effect of α -lipoic acid was mediated via marked inhibition of the increase in the expression of IL-1 β and IL-6, the phosphorylation of ERK and p38, the degradation of I κ B α , the increase in intracellular levels of ROS and the activation of caspase-3 in cisplatin-treated HEI-OC1 cells. In another study, rats treated with α -lipoic acid plus cisplatin did not show significant elevation of hearing thresholds. Furthermore, α -lipoic acid acting as ROS scavenger prevented the depletion of glutathione as well as the decrease of SOD, CAT, GSH-Px and GSH-R activities in the cochlea [46]. α -Lipoic acid also showed beneficial effects against age-related HL. Administration of α -lipoic acid for 6 weeks in 24-month-old Fischer rats showed delay in the progression of age-induced HL. However, this delay was only seen to be significant at 3 kHz [48]. The authors concluded that as a mitochondrial metabolite, α -lipoic acid may reduce age-related worsening in auditory sensitivity and improve cochlear function by preventing the ROS-induced mtDNA4834bp deletion in inner ear of rats [49], thus protecting and repairing age-induced mitochondrial DNA damage.

A preliminary study conducted by Quaranta *et al.* evaluated the effect of α -lipoic acid on temporary threshold shift measured 2 min after the end of exposure induced by a 90 dB HL 3 kHz pure tone for 10 min in young normal hearing subjects (10/group). Group of subjects taking 600 mg of oral α -lipoic acid for 10 days prior to noise insult had significantly lower temporary threshold shift as compared with the control group (exposed to noise without α -lipoic acid) and the subjects who were exposed to noise 1 h after oral ingestion of α -lipoic acid. A single dose of α -lipoic acid did not induce any protection against noise-induced temporary threshold shift, whereas 10 days of treatment caused a significant lowering of the threshold, which indicates that single dosage is not sufficient to achieve the therapeutic concentration and a short course of α -lipoic acid treatment is required for necessary protection of HL [50]. Thus, multiple-dose lipoic acid appears very promising as a preventative agent against noise-induced temporary HL. Future studies are needed to determine whether this agent protects against permanent HL from noise.

3. Anti-inflammatory agents in HL

Any mode of insult or environmental stressor such as noise, ototoxic drugs like aminoglycosides, cisplatin or viral infection can activate an inflammatory cascade leading to SNHL. Auditory hair cells undergo both necrosis and apoptosis after cochlear trauma. Cochlear trauma induces oxidative stress and activate inflammatory cascade that involves caspase activity and MAPK/JNK pathways. A particular mode of insult targets a specific pathway; targeting the pathways that lead to apoptosis provides a basis for therapeutic intervention for preserving hearing: i) antioxidants decrease the level of ROS and ii) caspase inhibitors interfere with the extrinsic and intrinsic apoptotic pathways to mitigate cisplatin- or aminoglycoside-induced HL. Corticosteroids are potent anti-inflammatory agents that decrease inflammation and inhibit apoptosis via activation of pro-survival pathways. JNK inhibitors target JNK signaling pathways that are involved in labyrinthitis, acoustic trauma and aminoglycoside ototoxicity-induced HL [51]. Many researchers are focusing on the role of anti-inflammatory drugs as prophylactic agents for protection against HL.

3.1 Corticosteroids

Corticosteroids have been used in the treatment and prevention of cisplatin and aminoglycoside ototoxicity and SSHL based on their anti-inflammatory effects. Animal studies have shown that corticosteroids effectively attenuate the cisplatin- and aminoglycoside-induced HL by inhibition of the generation of ROS in the cochlea [52-54].

Recent studies by Filipo *et al.* [55] showed that intratympanic steroid therapy in moderate SSHL showed complete recovery in 76% of the patients, marked recovery (PTA improvement > 30 dB) in 8% of the patients, slight recovery (PTA improvement 10 – 30 dB) in 12% of the patients and no improvement in 4% of the patients. Control group was treated with saline intratympanic therapy and showed complete recovery in 20% of the patients. Total of 50 patients were recruited for this study. Intratympanic steroid treatment consisted of 0.3 ml of 62.5 mg/ml of prednisolone for 3 consecutive days. The control group was administered intratympanic saline for 3 consecutive days. Audiometric evaluations were performed at 7 days from the start of treatment. All patients not showing complete recovery were then administered oral prednisolone in a tapering dose for 8 days. The intratympanic steroid group did not show any further improvements with oral steroids. The control group on the other hand after oral steroid showed complete recovery in 72% of the patients and no recovery in 28% of the patients. This study underscores the importance of early treatment that was started as early as 3 – 13 days from the onset of idiopathic sudden sensorineural hearing loss (ISSHL) and that steroid therapy is effective in treatment of ISSHL. Filipo *et al.* [56] have further shown that oral steroid therapy versus short-term intratympanic steroid therapy for ISSHL showed no significant difference in PTA improvement; however, the intratympanic steroid groups showed better audiometric curves.

The use of intratympanic steroid injections as a rescue therapy in patients with SSHL who did not respond to systemic steroid therapy have been reported by several investigators [57,58]. Briefly, Ferri *et al.* [58] have reported that the patients showed full or partial recovery in hearing capability in 52.7% of the patients, 43.7% had no change in hearing and in 3.5% hearing worsened with IST. Patient profile for this study consisted of 55 patients

with refractory ISSHL who had a PTA of 30 dB or more after 10 days of intravenous steroid treatment. These patients were then treated with 0.5 ml of methylprednisolone (40 mg/ml). IST was performed every 2 or 3 days up to a total of 7 injections. In this study, the authors further delineated that the recovery associated with IST was positively correlated with time of treatment and onset of HL, severity of HL, age of the patients, status of the contralateral ear and the frequencies of HL.

In a similar study by Raghunandhan *et al.* [59] showed that high-dose intravenous steroid therapy of 30 patients with ISSNHL, complete recovery occurred in 56.66% cases and marked improvement (> 30 dB) in 16.66% patients. However, the caveat is that the treatment had to begin within 6 h from the onset of HL in order to restore normal hearing. This study also assessed the various co-morbidities influencing the outcomes as well as improvement in associated symptoms such as vertigo and tinnitus [59].

Dexamethasone and Ginaton are also being evaluated for the treatment of acute sensorineural HL in the clinical trial: Sudden Hearing Loss Multi-center Clinical Trial (NCT02026479). This study will compare two doses of dexamethasone (5 and 10 mg) as postauricular treatment and Ginaton (40 mg, thrice a day, oral). Pure-tone audiometry will be measured for the three contiguous frequencies with the worst HL at baseline and at day 30 post-treatment. This study will also evaluate tinnitus and vertigo resolution in the patients by questionnaires at baseline, 14, 30 and 90 days. This study is sponsored by Peking University People's Hospital and is not open to recruitment as yet.

Another clinical trial is testing the efficacy of fludrocortisone in the amelioration of ISSNHL: Fludrocortisone for Sudden Hearing Loss (NCT01186185). The rationale behind this study is that mineralocorticoids regulate the balance of electrolytes and water. Fludrocortisone has been shown to be effective in combination with prednisolone at low doses in a mouse model of autoimmune HL [60]. Fludrocortisone is not approved for the treatment of SSNHL, and the purpose of this study was to test whether fludrocortisone can treat sudden HL. Fludrocortisone was to be administered 0.2 mg by mouth daily for 30 days for patients with ISSNHL within the last 3 months. HL was to be determined by pure-tone and speech audiometry on completion of a 1-month course of treatment and compared with pretreatment test results. This study was sponsored by Oregon Health and Science University. There are no updates available.

Dexamethasone has also been used in the prevention of cisplatin-induced HL. Several studies have shown that intratympanic administration of dexamethasone is protective against cisplatin ototoxicity [54,61-64]. However, recent studies did not demonstrate any protection with dexamethasone when administered either intratympanically or intraperitoneally, against cisplatin-induced HL [61,62]. There is one clinical trial that is in Phase IV of clinical testing: Prevention of Cisplatin-Induced HL by Intratympanic Dexamethasone Treatment (NCT01372904).

4. Biological agents

Biological agents are being widely used in medicine as they are target-specific and readily taken up by the body. ILs are cytokines that regulate the body's immune responses. One class of biological inhibitors that seem to be otoprotective targets the IL-1 β pathway. IL-1 β is a pro-inflammatory cytokine that belongs to the IL-1 superfamily and has been implicated in tissue injury and in autoimmune diseases [65]. IL-1 β signaling can be blocked by the use of a specific antibody against IL-1 β (e.g., gevokizumab) or by another member of the IL-1 super-family, the IL-1Ra (IL-1 receptor antagonist), that binds to the IL-1R (non-productive) and effectively blocks the subsequent IL-1 β signaling [66] (e.g., anakinra).

Another biological agent that may be otoprotective is the TNF- α receptor inhibitor. TNF- α is another pro-inflammatory cytokine that belongs to the TNF superfamily and is a pro-inflammatory immune regulator that stimulates the acute phase reaction. Dysregulation in TNF- α signaling has been documented as one of the hallmarks of autoimmune diseases. TNF- α receptor antagonists are widely used in the treatment of autoimmune diseases such as rheumatoid arthritis, psoriasis and so on (e.g., etanercept).

Among the other interesting drugs being tested are the anti-angiogenic antibody (targeting angiogenic VEGF-A) bevacizumab for vestibular schwannomas; short inhibitory RNAs (siRNAs), which have shown promise in animal studies, and CMV vaccines that are being tested for use in CMV-mediated HL.

4.1 Gevokizumab

Gevokizumab is a mAb raised against pro-inflammatory cytokine IL-1 β [67]. It binds to IL-1 β with a strong affinity and allosterically modulates its activity. Increase in IL-1 β has been well documented in autoimmune inner ear disease (AIED) [68-70]. The clinical trial: The Effects of Gevokizumab in Corticosteroid-resistant Subjects with AIED (NCT01950312) is a Phase II open-label clinical trial. The purpose of the study is to determine whether gevokizumab can be used as an alternative therapy for the corticosteroid-resistant patients with AIED. The primary outcome will be measured after 28 – 84 days of therapeutic interventions (gevokizumab as subcutaneous injections) by determining the improvement either at PTA of ≤ 5 dB or 12% of word recognition score. This is an ongoing trial and is recruiting participants at this point. It is being sponsored by XOMA (US) LLC in collaboration with Feinstein Institute for Medical Research and National Institute on Deafness and Other Communication Disorders (NIDCD). The details regarding the dosage and treatment time points have not been provided.

Gevokizumab has wide varieties of applications for different diseases. It is in Phase III trials for different indications like erosive osteoarthritis of the hand. There have been no reports of adverse side effects.

4.2 Anakinra

Anakinra is a recombinant, nonglycosylated human IL-1 receptor antagonist (IL-1Ra). Anakinra has an extra methionine residue at the amino terminus, compared to the native IL-1R antagonist. It works by competitively binding to IL-1R type I and therefore does not

allow the IL-1 to bind to its receptor. This prevents further responses to any inflammatory and immunological stimuli. Anakinra is generated using the *Escherichia coli* expression system.

IL-1 β has been found to be crucial to the pathogenesis of autoimmune inner ear [69,71,72]. IL-1 β antagonists, such as anakinra, rilonacept and canakinumab, have been found to be effective in mitigating autoimmune HL when treatment was initiated promptly [71-75]. Pathak *et al.* showed significant increase in the release of IL-1 β from the peripheral blood mononuclear cells (PBMC) of patients with autoimmune inner ear disease (AIED) who did not respond to corticosteroid therapy [69] compared to responders. More interestingly, this study also showed that stimulation of cultured PBMCs isolated from the responders as well as non-responders for 16 h with 100 ng/ml of anakinra showed significant inhibition of IL-1 β from the monocytes of both the responders and non-responders. These data strongly suggest that anakinra may be a good choice for the treatment of AIED, especially in those patients who have not responded to corticosteroid therapy. The clinical trial: A Clinical Trial of Anakinra for Steroid-Resistant AIED (NCT01267994) aims to do just that. This is an open-label Phase I and Phase II study to determine whether the use of anakinra in corticosteroid-resistant subjects with AIED show improvement in hearing thresholds. This study will evaluate whether treatment with anakinra for 84 days will demonstrate improved hearing threshold when compared with their baseline pre-anakinra-treatment threshold in corticosteroid-resistant patients with AIED. Audiometric thresholds will be compared to those treated with a prolonged corticosteroid taper and those that elect for no further treatment. The durability of the response will be measured over a total of 180 days. This is an ongoing study, sponsored by Dr. Andrea Vambutas, North Shore Long Island Jewish Health System and in collaboration with NIDCD.

4.3 Etanercept

Etanercept is a widely used biological agent in the treatment of autoimmune diseases such as rheumatoid arthritis, psoriasis and so on by targeting TNF- α . TNF- α is a pro-inflammatory cytokine that belongs to the TNF superfamily. TNF- α is a pro-inflammatory immune regulator that stimulates the acute phase reaction. Dysregulation in TNF- α signaling has been documented as one of the hallmarks of autoimmune diseases. There is increasing evidence to support the efficacy of TNF- α receptor antagonist, etanercept, against inflammatory disease. Etanercept can mitigate the inflammation and HL in labyrinthitis induced by keyhole limpet hemocyanin (KLH) in mice [76]. In a guinea pig model of immune-mediated HL, within 3 – 5 days of KLH insult, the labyrinth developed infiltration of inflammatory cells and HL occurred. Both the systemic treatment with etanercept (2.5 mg for 30 min) and long-term infusion of etanercept (5.0 μ g/h for 7 days) into the scala tympani significantly protected the labyrinth against KLH-induced inflammation and the development of HL [76]. Etanercept prevents NIHL via improvement of cochlear blood flow *in vivo* [77]. TNF- α signaling is known to be activated upon noise exposure. Systemic administration of etanercept after loud noise exposure (106 dB SPL, 30 min) significantly attenuated NIHL. The microcirculatory analysis of cochlea showed significant increase in cochlear blood flow in strial capillary segments. Moreover, significant hearing improvement was observed in etanercept-treated groups versus controls. Hence, there appears to be a

compelling rationale for conducting further research on etanercept as a treatment strategy against NIHL.

In Wistar rats, Kaur *et al.* have shown that etanercept can significantly ameliorate cisplatin ototoxicity significantly [78,79]. Etanercept (250 µg/50 µl per ear) was administered transtympanically prior to cisplatin (11 mg/kg intraperitoneal) treatment. Auditory thresholds were measured at baseline in the naive animals and again at 72-h post-cisplatin administration. Etanercept pretreatment provided almost complete protection from cisplatin-induced HL. Outer hair cell morphology was preserved as shown by scanning electron microscopy. These data provide a strong rationale for additional studies of etanercept for prevention of cisplatin ototoxicity.

4.4 Short inhibitory RNA

siRNA is double-stranded RNA of 20 – 30 nucleotides in length and among other properties interferes with specific gene expression [80-82]. siRNA silences by preventing mRNA from translation by binding and degradation of the specific mRNA. Several studies have shown siRNA to be very efficient in the inhibition of protein expression in animals. However, siRNA has a very short life span after systemic administration, as exo- and endonucleases in blood and serum degrade it [83]. Anatomical isolation of the cochlea helps avoid this drawback; additionally, the cochlea is sparsely populated with blood vessels. Another anatomically isolated organ is the eye, which is also a target organ for gene-specific treatment options. Localized delivery of drugs and siRNA by transtympanic route by our laboratory has helped to provide treatment options and route of delivery to the inner ear. One of the earliest gene silencing studies in which siRNA was delivered by round window application required surgery, drilling a hole in the bulla of the mouse and subsequent delivery of the siRNA for the gap junction protein, β -2 (GJB2) in the mouse [84]. Round window administration of siRNA against Transient Receptor Potential Vanilloid 1 (TRPV1) gene was reported to be otoprotective against cisplatin ototoxicity. The expression of that gene was shown to be upregulated following cisplatin administration [85]. Subsequently, Mukherjea *et al.* showed that siRNA against cochlear-specific NADPH oxidase 3 gene showed a dose-dependent protection from cisplatin ototoxicity when administered by single transtympanic injections 48 h prior to cisplatin administration [86]. More animal studies from the same group also showed that siRNA against signal transducer and activator of transcription 1, a transcription factor known to be critical in cisplatin-induced inflammation of the cochlea [87], showed amelioration of cisplatin-induced HL in the rat [78]. Localized molecular targets afford these siRNA molecules almost the ideal drug status for otoprotection. Additionally, since the siRNA has a short life span, is administered transtympanically to target the cochlea alone, and required a low dose for effective treatment, it is not likely to interfere with the chemotherapeutic efficacy of cisplatin.

4.5 CMV vaccine

CMV infection during pregnancy can result in SNHL in new-borns and infants. Four independent Towne/Toledo chimera vaccines were developed by genetic recombination of unattenuated human CMV Toledo strain and immunogenic Towne strains against human CMV [88], and it induces humoral immune response. Clinical trial: Safety Study of Four

Chimera CMV Vaccines in Healthy Adult Males 30 – 50 Years of Age (NCT01195571). The purpose of this study is to assess the safety and tolerability of four new chimeric Towne/Toledo vaccines (Towne/Toledo 1, 2, 3, 4), which can be used to prevent congenital SNHL. The total number of participants will be 36 that will be allocated randomly in four different groups and will receive different types of vaccines. Vaccine will be administered at a dose of 103 p. f. u. (plaque forming unit) and for three additional individuals a dose of 102 p. f. u. will be given followed by 103 p. f. u. dose. The safety and tolerability of different doses will be followed for 12 weeks. At the end of the study, the presence of virus will be determined in different biological samples such as blood and urine of patients. However, the immune response profiles of the patients have to be carefully evaluated to determine the safety and tolerability.

5. Transport inhibitors

Proprietary compound (PF-04958242) is in clinical trials for age-related HL: A Study of The Effects of PF-04958242 in Subjects with Age-Related Hearing Loss: NCT01518920. PF-04958242 has been classified as glycine transporter (GLYT-1) inhibitor by Pfizer, Inc. in this study. This clinical trial assesses the safety and efficacy of 0.27 or 0.35 mg oral solution of PF-04958242 in subjects with age-related SNHL (presbycusis) (n = 42; 30 – 60 dB). There is no background information supporting the use of PF-04958242 in presbycusis. The patients (age = 50 – 75 years) were randomly assigned to a crossover and double-blinded trial conducted by Pfizer. Pure-tone audiometry to determine changes in baseline and 1 h post-PF-04958242 (0.27 or 0.35 mg oral solution) averaged over 2 and 4 kHz. Secondary measures of pure-tone audiometry were also collected at 5 h post-treatment. Speech discrimination score, speech-in-noise testing and tinnitus severity score were evaluated at 1 and 5 h post-treatment. This clinical trial has been completed; no results have been posted as yet.

Pfizer has also classified PF-04958242 as an AMPA receptor agonist [89] and based on AMPA receptor activation-mediated effects, this compound is currently also being developed by Pfizer for the treatment of schizophrenia (NCT01518894; NCT01511510; NCT01749098; NCT01365338). PF-04958242 is a biarylpropylsulfonamide derivative, which can inhibit glycine transporter-1 (Glyt1), thus blocking glycine reuptake. This would cause an increase in the availability of extracellular glycine in synaptic cleft. The complexity is that elevated extracellular glycine has at least two opposed effects: i) post-synaptic inhibition mediated by glycine receptors; and ii) post-synaptic excitation mediated by glycine binding to an obligatory allosteric site on NMDA glutamatergic receptors [90]. This second, excitatory effect, is quite complex. Additionally, if this drug PF-04958242 is also an AMPA agonist as per Pfizer classification, it probably works by sustaining the attention span in schizophrenia (schizophrenia is characterized [among other things] by poorly sustained cognition and weak control of attention). Ultimately, this drug seems to be working at multiple levels in the human brain and it remains to be seen how the inhibitory-excitatory balance will play out in the central auditory system with respect to HL in presbycusis. The question remains concerning the mode of action of this drug in the central auditory system in presbycusis, and whether the results will indicate that this drug is beneficial or harmful.

6. Proton pump inhibitors

Acid-base endolymph homeostasis is essential for the normal function of the inner ear. Perturbation of homeostasis in labyrinth fluids generates abnormalities of inner ear structures such as enlargement of the endolymphatic sac (ES) or vestibular aqueduct [91]. Proton pump inhibitors (PPIs) behave as an antagonist to pendrin, a protein encoded by SLC26A4 gene [92,93]. This functions as an acid-base transporter in the ES for endolymph homeostasis [94]. PPIs can modulate the function of proton pumps to maintain homeostasis in the ES. PPIs could thus be a good treatment option to prevent or treat HL caused by SLC26A4 mutations.

Congenital HL is one of the most common birth defects in developed countries caused by mostly genetic factors. More than 66% of the cases of the childhood-onset HL have a genetic cause [95,96]. In search of therapies for HL, > 120 independent genes have been explored [97]. However, recessive mutations at a single locus, GJB2 or Connexin 26 (SLC26A4), account for > 50% of genetically caused HL. Additional studies have reported the presence of recessive mutations at two loci [97]. SLC26A4 mutation attributes for the enlargement of the vestibular aqueduct syndrome and the Pendred syndrome [98]. The clinical trial: Proton Pump Inhibitors in Preventing and Treating Hearing Loss in Patients with SLC26A4 Mutation (NCT00789061) reported the use of PPIs in preventing HL in patients with the SLC26A4 mutation. This study was recruiting participants until November, 2008 for Phase II and Phase III clinical trials as verified by National Taiwan University Hospital. The criteria included patients with acute HL who had homozygous or heterozygous SLC26A4 mutations. A double-blind randomized clinical trial was carried out with one dosage of PPI like taquidine or lansoprazole every day for a year. Further update is required regarding the progress of the study.

Pantoprazole is also being evaluated in the clinical trial: Pantoprazole in Preventing Nephrotoxicity and Ototoxicity from Osteosarcoma Therapy (NCT01848457). This is a 'pilot study to prevent nephrotoxicity of high-dose methotrexate (HDMTX) by prolonging the infusion duration and prevent nephrotoxicity and ototoxicity of cisplatin with pantoprazole in children, adolescents and young adults with osteosarcoma' and is currently recruiting patients for Phase II studies. The primary objectives include the evaluation of renal damage and HL. Urinary biomarkers of kidney injury will be utilized to evaluate renal damage, and audiograms will depict ototoxic effects. Radiographic and histologic studies will illustrate the effect of pantoprazole on the tumor response. The secondary objectives include the assessment of proteins that are targeted by pantoprazole. Bone-specific alkaline phosphatase will be used as a biomarker to evaluate tumor burden. A tissue microarray will be constructed to assess the proteins that are involved in the cancer drug resistance and the proteins that can be targeted by new anticancer drugs.

Many chemotherapeutic agents are metabolized and excreted by kidneys. Some of them can be retained in the kidney via specific transport systems expressed in renal proximal tubular cells [99]. By prolonging the infusion duration, the precipitation of nephrotoxic drug like HDMTX could be lowered in renal tubule to mitigate the nephrotoxicity. The cochlea is an enclosed system where the blood circulation is limited to wash out the accumulation of toxic

drugs. The organic cation transporter 2 could be blocked with pantoprazole to selectively block the uptake and accumulation of platinum drugs into renal tubular cells and cochlear hair cells.

7. Zonisamide and methylprednisolone

Zonisamide is a sulfonamide anticonvulsant drug approved by the FDA. It has been shown in animal models that zonisamide acts through several different mechanisms, making it useful in treating a broad range of seizure types [100]. These mechanisms include blocking action potentials of voltage-gated sodium channels, controlling neurotransmitter systems including GABAergic and glutamate systems, and blocking T-type calcium channels [100-103].

Mitochondrial-free radical formation is a widely studied mechanism for NIHL, but an additionally important contributor to NIHL has been shown to involve calcium homeostasis. There are many different types of calcium channels that help maintain calcium homeostasis in the cochlea, including voltage-gated calcium channels (VGCCs) [104-108]. There are two types of VGCCs: high-voltage-activated (L-Type) and low-voltage-activated (T-type) channels [109-112]. These channels are found in both the organ of Corti and the spiral ganglion neurons. NIHL was reduced by administration of anticonvulsant drugs which block the T-type VGCCs when administered either prior to or postnoise exposure [113].

Another mechanism in NIHL involves glucocorticoid signaling. Glucocorticoid drugs, such as methylprednisolone, approved by the FDA have been used clinically to treat HL in conditions including AIED, tinnitus and Meniere's disease [113-116]. The use of either glucocorticoid drugs or anticonvulsant drugs has shown partial success in the treatment and prevention of NIHL [113,117]. Bao *et al.*, demonstrated the synergistic effect of the combination of zonisamide and methylprednisolone against NIHL in C57BL/6J mice. This combination resulted in an increase in hair cell survival when mice were treated 2 h prior to noise exposure; however, the treatment did not prevent hair cell death in treatments after noise exposure. This may suggest different mechanisms involved in protection (pre) and treatment (post) of noise [118]. The clinical trial: Prevention of Noise-induced Hearing Loss (NCT02049073), intends to use this combination of zonisamide and methylprednisolone in prevention of NIHL. The purpose of this pilot study is to evaluate and determine the dose for both zonisamide (Phase I) and methylprednisolone (Phase II) as medications to prevent temporary NIHL in humans. This study is not yet open for participant recruitment and is sponsored by the Washington University School of Medicine in St. Louis, MO.

8. Conclusion

HL is rapidly taking the shape of a global challenge of considerable significance as there are no currently available approved treatments in humans. World Health Organization released the estimates of disabling HL in 2012. It was estimated that globally there are 360 million people with disabling HL, and these estimates were based on 42 population-based studies [119]. Of these, 91% are adults and 9% are children, with prevalence being highest in Southeast Asia, Asia Pacific and Sub-Saharan Africa. In the US alone, there are 38 million

people with significant HL in at least one ear, including 3 million children. The staggering societal cost for an average person with HL was \$297,000 over an individual's life span was estimated in studies conducted in 1999 and 2000. Children and young people are estimated to cost the society a staggering \$960,000 per person.

The studies discussed in this review paper point to several agents under investigation mitigating ROS generation, inflammatory mediators or the pH balance in the inner ear. Most therapies being tested are aimed at prevention of expected events of HL and target the well-established imbalances in the inner ear due to the various insults. One of the largest group of therapies target the effects of an overwhelming ROS response due to either drug/noise trauma or accumulation of ROS with ageing are ebselen, NAC, lactated Ringer's solution, ginkgo biloba, α -lipoic acid and micronutrients. The other large group of therapies targets the ROS-mediated inflammation with steroids and biological agents. The biological agents target specific downstream immune modulators associated with inflammation and HL. PPIs are another group of protective agents being tested, and their putative protection is based on the maintenance of ion homeostasis in the inner ear, which get disturbed in the event of cochlear trauma or injury. The various clinical trials listed in this review have been organized in a tabular format in Table 1.

In conclusion, it seems that prophylactic treatment with either ebselen plus allopurinol or NAC are expected to be effective for NIHL. NAC has also been shown to be protective when used as a treatment post-blast injury in active duty personnel. NAC, intratympanic corticosteroids and PPI are being tested for drug-induced HL and have a good potential for success as prophylactic agents. Corticosteroids are the most commonly used drugs for treatment of ISSNHL. Biologicals are strong candidates for the treatment of autoimmune and steroid-resistant autoimmune diseases in HL. The combination of an anticonvulsant drug that is already FDA approved and a corticosteroid to combat NIHL is an exciting concept as it constitutes a dual approach for prevention of NIHL [116]. There yet are no clear front runners for rescue of either NIHL or drug-induced HL.

The field of therapeutics seems to be directed towards the prevention of HL. However, there is a profound need to develop therapeutics for the rescue and salvage of hearing especially after unexpected exposure. There is a huge scarcity of therapeutics (experimental, preclinical or clinical) that target the above-mentioned well-established molecular pathways of cell injury and death in the inner ear postinjury. This area of 'rescue' needs immediate attention and should be tailored such that it benefits the most vulnerable pediatric population, the armed forces on the battleground and the cancer survivors treated with ototoxic drugs. The future seems to be promising for the amelioration of SNHL from a variety of causes. However, extensive clinical trials will be required to find the most effective treatments for this widespread clinical and social problem.

The future seems to be promising for the amelioration of HL from a variety of causes. However, extensive clinical trials will be required to find the most effective treatments for this widespread clinical and social problem.

9. Expert opinion

A common mechanism of acquired sensorineural HL is the production of excessive ROS and inflammation in the inner ear, resulting in damage to sensory cells in the inner ear and significant HL. The targeting of oxidative stress, inflammation and VGCCs offers promise in preventing or ameliorating acquired sensorineural HL.

Ebselen appears to be safe in a wide dosage range and seems to have good bioavailability. The efficacy of L-NAC in traumatic blast injury in military personnel is exciting.

There are numerous weaknesses revealed in our review. Many clinical trials are still ongoing, and the results are not yet available. Numerous studies require longer term follow-up, including the NAC study of traumatic blast injury. The epidemiologic studies of antioxidant vitamins and HL included a wide range of patients of different ages and did not specify what etiologies of HL were being evaluated. The lack of updated information on PPIs suggests that these drugs were not effective in patients with HL related to enlarged vestibular aqueduct syndrome. Very few clinical trials have been carried out in children receiving cisplatin. Yet, this group is the most vulnerable to cisplatin ototoxicity, with long-term effects on hearing and cognitive development. This group of patients desperately needs effective protective agents that can be administered noninvasively before or even after cisplatin therapy without compromising the therapeutic efficacy of cisplatin.

Nevertheless, this research holds great potential. The lack of interference with the chemotherapeutic efficacy of cisplatin in preclinical studies and the safety and favorable pharmacokinetics in adults suggest that ebselen will be useful for the prevention of cisplatin ototoxicity, at least in adults. Multiple doses of lipoic acid may be useful to prevent temporary NIHL, which could be critical in military operations or industrial settings. It remains to be seen whether permanent threshold shifts could be prevented or reduced. Anakinra shows promise against autoimmune-related HL by blocking inflammatory pathways. The use of anticonvulsant calcium channel blockers alone or in combination with methylprednisolone appears very likely to help prevent HL from noise. This combination may also be able to rescue hearing when administered after noise exposure.

The ultimate goal in this field is the prevention of acquired sensorineural HL in the majority of persons at risk for this significant disability. This can be achieved provided the appropriate timing, dose and method of delivery of the protective agent(s) to the inner ear are developed. The potential exists for the development and testing of drugs that could be administered after exposure to noise or ototoxic agents to rescue hearing in these individuals.

The biggest challenge is to select the agent(s) with little or no toxicity that have the proper pharmacokinetics to gain access to the inner ear and afford protection against noise or ototoxic drugs without interfering with the efficacy of the latter group. Further research on safe, novel methods of drug delivery including nanotechnology is needed. Testing of drugs, individually and in combinations, which have already been approved by the FDA, will accelerate successful clinical trials.

In the future, we predict the expansion of intratympanic drug therapy with corticosteroids and other anti-inflammatory agents, including drugs such as etanercept and antibodies targeting harmful cytokines produced in response to noise trauma or ototoxic insult. The administration of specific siRNAs to target oxidative or inflammatory pathways will be developed and refined. The systemic delivery of protective agents by nanotechnology is likely to be expanded. These would provide relatively noninvasive methods of providing the drug to the inner ear in the necessary dose and at the appropriate time to exert beneficial effects. As noise exposure is frequently not anticipated, the expanded use of rescue agents will be investigated, like the combination of anticonvulsants and methylprednisolone.

There are particular areas of research that are extremely interesting at present. The efficacy of L-NAC in traumatic blast injury in military personnel is very exciting and offers great promise for the amelioration of HL caused by this mechanism.

The application of other drugs already approved by the FDA to prevent NIHL is particularly fascinating. These include the calcium channel blocking anticonvulsant zonisamide. It is interesting that this agent was effective even when given after noise exposure in preclinical studies. The finding of synergistic effects of anticonvulsants with methylprednisolone against NIHL was even more intriguing. It is anticipated that the clinical trials with these combinations will prove to be safe and effective. Targeted therapy directed against cytokines in the inner ear has the potential to be effective against several causes of HL, including noise trauma and ototoxic drugs.

Although a great deal of research needs to be done to achieve the ultimate goal of protecting the ear against acquired sensorineural HL, we are likely to see exciting breakthroughs in the near future.

Acknowledgments

LP Rybak is supported by a National Institutes of Health grant RO1DC02396, while V Ramkumar is supported by NIH grant R01CA166907. D Mukherjea also has grant support from NIH with grant RO3DC011621.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Available from: <http://www.chcheating.org/about-hearing-loss/facts-about-hearing-loss>
2. Available from: <http://www.hear-it.org/2050-twice-many-hearing-loss>
3. Available from: www.asha.org
4. Available from: <https://www.nidcd.nih.gov/health/hearing/pages/presbycusis.aspx>
- 5•. Clinical Trials.gov Available from: www.clinicaltrials.gov Very useful site that lists all the clinical trials in the US.
6. PubMed Database. Available from: www.pubmed.gov
7. Riva C, Donadieu E, Magnan J, et al. Age-related hearing loss in CD/1 mice is associated to ROS formation and HIF target proteins up-regulation in the cochlea. *Exp Gerontol.* 2007; 42(4):327–36. [PubMed: 17141999]
8. Hoshino T, Tabuchi K, Nishimura B, et al. Protective role of Nrf2 in age-related hearing loss and gentamicin ototoxicity. *Biochem Biophys Res Commun.* 2011; 415(1):94–8. [PubMed: 22020098]

9. Fetoni AR, De Bartolo P, Eramo SL, et al. Noise-induced hearing loss (NIHL) as a target of oxidative stress-mediated damage: cochlear and cortical responses after an increase in antioxidant defense. *J Neurosci*. 2013; 33(9):4011–23. [PubMed: 23447610]
10. Seidman MD, Tang W, Bai VU, et al. Resveratrol decreases noise-induced cyclooxygenase-2 expression in the rat cochlea. *Otolaryngol Head Neck Surg*. 2013; 148(5):827–33. [PubMed: 23380763]
- 11••. Rybak LP, Mukherjea D, Jajoo S, et al. Cisplatin ototoxicity and protection: clinical and experimental studies. *Tohoku J Exp Med*. 2009; 219(3):177–86. Review of cisplatin-induced ototoxicity. [PubMed: 19851045]
12. Wu WJ, Sha SH, Schacht J. Recent advances in understanding aminoglycoside ototoxicity and its prevention. *Audiol Neurotol*. 2002; 7(3):171–4. [PubMed: 12053140]
13. Parnham MJ, Kindt S. A novel biologically active seleno-organic compound-III. Effects of PZ 51 (ebselen) on glutathione peroxidase and secretory activities of mouse macrophages. *Biochem Pharmacol*. 1984; 33:3247–50. [PubMed: 6593071]
14. Parnham MJ, Graf E. Seleno-organic compounds and the therapy of hydroperoxide-linked pathological conditions. *Biochem Pharmacol*. 1987; 36:3095–102. [PubMed: 3311047]
15. Masumoto H, Kissner R, Koppenol WH, et al. Kinetic study of the reaction of ebselen with peroxynitrite. *FEBS Lett*. 1996; 398:179–82. [PubMed: 8977102]
16. Kil J, Pierce C, Tran H, et al. Ebselen treatment reduces noise induced hearing loss via the mimicry and induction of glutathione peroxidase. *Hear Res*. 2007; 226:44–51. [PubMed: 17030476]
- 17•. Lynch ED, Gu R, Pierce C, et al. Reduction of acute cisplatin ototoxicity and nephrotoxicity in rats by oral administration of allopurinol and ebselen. *Hear Res*. 2005; 201:81–9. Oral antioxidants are protective in cisplatin ototoxicity and nephrotoxicity. [PubMed: 15721563]
18. Geier DA, Geier MR. A clinical and laboratory evaluation of methionine cycle-transsulfuration and androgen pathway markers in children with autistic disorders. *Horm Res*. 2006; 66(4):182–8. [PubMed: 16825783]
19. Kopke RD, Weisskopf PA, Boone JL, et al. Reduction of noise-induced hearing loss using L-NAC and salicylate in the chinchilla. *Hear Res*. 2000; 149(1-2):138–46. [PubMed: 11033253]
20. Yoo J, Hamilton SJ, Angel D, et al. Cisplatin otoprotection using transtympanic L-N-acetylcysteine: a pilot randomized study in head and neck cancer patients. *Laryngoscope*. 2014; 124(3):E87–94. [PubMed: 23946126]
21. Gilles A, Ihtijarevic B, Wouters K, et al. Using prophylactic antioxidants to prevent noise-induced hearing damage in young adults: a protocol for a double-blind, randomized controlled trial. *Trials*. 2014; 15:110. [PubMed: 24708640]
- 22••. Hoffer ME, Balaban C, Slade MD, et al. Amelioration of acute sequelae of blast induced mild traumatic brain injury by N-acetyl cysteine: a double-blind, placebo controlled study. *PLoS One*. 2013; 8(1):e54163. N-acetylcysteine protects from sequelae of blast injury. [PubMed: 23372680]
23. Sooriyaarachchi M, Narendran A, Gailer J. N-acetyl-L-cysteine modulates the metabolism of cisplatin in human plasma in vitro. *Metallomics*. 2013; (3):197–207. [PubMed: 23443244]
24. Lin CY, Wu JL, Shih TS, et al. N-Acetyl-cysteine against noise-induced temporary threshold shift in male workers. *Hear Res*. 2010; 269(1-2):42–7. [PubMed: 20638463]
25. Tokgoz B, Ucar C, Kocyigit I, et al. Protective effect of N-acetylcysteine from drug-induced ototoxicity in uraemic patients with CAPD peritonitis. *Nephrol Dial Transplant*. 2011; 26(12):4073–8. [PubMed: 21551083]
26. Low WK, Sun L, Tan MG, et al. L-N-Acetylcysteine protects against radiation-induced apoptosis in a cochlear cell line. *Acta Otolaryngol*. 2008; 128(4):440–5. [PubMed: 18368580]
27. Feghali JG, Liu W, Van De Water TR. L-n-acetyl-cysteine protection against cisplatin-induced auditory neuronal and hair cell toxicity. *Laryngoscope*. 2001; 111(7):1147–55. [PubMed: 11568534]
28. Choe WT, Chinosornvatana N, Chang KW. Prevention of cisplatin ototoxicity using transtympanic N-acetylcysteine and lactate. *Otol Neurotol*. 2004; 25(6):910–15. [PubMed: 15547419]
29. Nader ME, Théorêt Y, Saliba I. The role of intratympanic lactate injection in the prevention of cisplatin-induced ototoxicity. *Laryngoscope*. 2010; 120(6):1208–13. [PubMed: 20513041]

30. Sanli A, Aydin S, Sarisoy ZA, et al. The protective effect of dexamethasone and lactate against cisplatin-induced ototoxicity. *Turk J Med Sci*. 2011; 41(3):467–74.
31. Munguia R, Sahmkow SI, Funnell WR, et al. Transtympanic Ringer's lactate application in the prevention of cisplatin-induced ototoxicity in a chinchilla animal model. *Otolaryngol Head Neck Surg*. 2010; 143:134–14. [PubMed: 20620632]
32. Fukaya H, Kanno H. Experimental studies of the protective effect of ginkgo biloba extract (GBE) on cisplatin-induced toxicity in rats. *Nihon Jibiinkoka Gakkai Kaiho*. 1999; 102(7):907–17. [PubMed: 10459293]
33. Tian CJ, Kim YJ, Kim SW, et al. A combination of cilostazol and Ginkgo biloba extract protects against cisplatin-induced Cochleo-vestibular dysfunction by inhibiting the mitochondrial apoptotic and ERK pathways. *Cell Death Dis*. 2013; 4:e509. [PubMed: 23429295]
34. Xu O, Lu H, Li PQ, et al. Effect of combination of Ginkgo leaf extract and deferoxamine in preventing and treating ototoxicity of cisplatin. *Zhongguo ZhongXi Yi Jie He Za Zhi*. 2004; 24(10):915–18.
35. Huang X, Whitworth CA, Rybak LP. Ginkgo biloba extract (EGb 761) protects against cisplatin-induced ototoxicity in rats. *Otol Neurotol*. 2007; 28(6):828–33. [PubMed: 17450108]
36. Cakil B, Basar FS, Atmaca S, et al. The protective effect of Ginkgo biloba extract against experimental cisplatin ototoxicity: animal research using distortion product otoacoustic emissions. *J Laryngol Otol*. 2012; 126(11):1097–101. [PubMed: 22975015]
37. Burschka MA, Hassan HA, Reineke T, et al. Effect of treatment with Ginkgo biloba extract EGb 761 (oral) on unilateral idiopathic sudden hearing loss in a prospective randomized double-blind study of 106 outpatients. *Eur Arch Otorhinolaryngol*. 2001; 258(5):213–19. [PubMed: 11548897]
38. McFadden SL, Woo JM, Michalak N, Ding D. Dietary vitamin C supplementation reduces noise-induced hearing loss in guinea pigs. *Hear Res*. 2005; 202(1-2):200–8. [PubMed: 15811712]
39. Hou F, Wang S, Zhai S, et al. Effects of alpha-tocopherol on noise-induced hearing loss in guinea pigs. *Hear Res*. 2003; 179(1-2):1–8. [PubMed: 12742233]
40. Le Prell CG, Hughes LF, Miller JM. Free radical scavenger's vitamins a, c, and e plus magnesium reduce noise trauma. *Free Radic Biol Med*. 2007; 42:1454–63. [PubMed: 17395018]
41. Kinoshita M, Sakamoto T, Kashio A, et al. Age-related hearing loss in Mn-SOD heterozygous knockout mice. *Oxid Med Cell Longev*. 2013; 2013:325702. [PubMed: 23936608]
42. Le Prell CG, Yagi M, Kawamoto K, et al. Chronic excitotoxicity in the guinea pig cochlea induces temporary functional deficits without disrupting otoacoustic emissions. *J Acoust Soc Am*. 2004; 116(2):1044–56. [PubMed: 15376671]
43. Le Prell CG, Johnson AC, Lindblad AC, et al. Increased vitamin plasma levels in Swedish military personnel treated with nutrients prior to automatic weapon training. *Noise Health*. 2011; 13(55): 432–43. [PubMed: 22122960]
44. Choi YH, Miller JM, Tucker KL, et al. Antioxidant vitamins and magnesium and the risk of hearing loss in the US general population. *Am J Clin Nutr*. 2014; 99(1):148–55. [PubMed: 24196403]
45. Kang JW, Choi HS, Kim K, et al. Dietary vitamin intake correlates with hearing thresholds in the older population: the Korean National Health and Nutrition Examination Survey. *Am J Clin Nutr*. 2014; 99(6):1407–13. [PubMed: 24646817]
46. Rybak LP, Husain K, Whitworth C, et al. Dose dependent protection by lipoic acid against cisplatin-induced ototoxicity in rats: antioxidant defense system. *Toxicol Sci*. 1999; 47:195–202. [PubMed: 10220857]
47. Kim J, Cho HJ, Sagong B, et al. Alpha-lipoic acid protects against cisplatin-induced ototoxicity via the regulation of MAPKs and proinflammatory cytokines. *Biochem Biophys Res Commun*. 2014; 449(2):183–9. [PubMed: 24796665]
48. Seidman MD, Khan MJ, Shirwany N, et al. Biologic activity of mitochondrial metabolites on aging and age-related hearing loss. *Am J Otol*. 2000; 21:161–7. Antioxidants like acetyl-L-Carnitine and alpha lipoic acid prevent presbycusis. [PubMed: 10733178]
49. Peng W, Hu Y, Zhong Y, et al. Protective roles of alpha-lipoic acid in rat model of mitochondrial DNA4834bp deletion in inner ear. *J Huazhong UnivSci Technolog Med Sci*. 2010; 30(4):514–18.

50. Quaranta N, Dicorato A, Matera V, et al. The effect of alpha-lipoic acid on temporary threshold shift in humans: a preliminary study. *Acta Otorhinolaryngol Ital.* 2012; 32:380–5. [PubMed: 23349557]
51. Abi-Hachem RN, Zine A, Van De Water TR. The injured cochlea as a target for inflammatory processes, initiation of cell death pathways and application of related otoprotectives strategies. *Recent Pat CNS Drug Discov.* 2010; 5(2):147–63. [PubMed: 20167005]
52. Himeno C, Komeda M, Izumikawa M, et al. Intra-cochlear administration of dexamethasone attenuates aminoglycoside ototoxicity in the guinea pig. *Hear Res.* 2002; 67:61–70.
53. Park SK, Choi D, Russell P, et al. Protective effect of corticosteroid against the cytotoxicity of aminoglycoside otic drops on isolated cochlear outer hair cells. *Laryngoscope.* 2004; 114(4):768–71. [PubMed: 15064639]
54. Paksoy M, Aydurhan E, Sanli A, et al. The protective effects of intratympanic dexamethasone and vitamin E on cisplatin-induced ototoxicity are demonstrated in rats. *Med Oncol.* 2011; 28(2):615–21. [PubMed: 20300971]
55. Filipo R, Attanasio G, Russo FY, et al. Intratympanic steroid therapy in moderate sudden hearing loss: a randomized, triple-blind, placebo-controlled trial. *Laryngoscope.* 2013; 123(3):774–8. Intratympanic steroid therapy in sudden sensorineural hearing loss. [PubMed: 23378346]
56. Filipo R, Attanasio G, Russo FY, et al. Oral versus short-term intratympanic prednisolone therapy for idiopathic sudden hearing loss. *Audiol Neurotol.* 2014; 19(4):225–33. [PubMed: 24992949]
57. Wu HP, Chou YF, Yu SH, et al. Intratympanic steroid injections as a salvage treatment for sudden sensorineural hearing loss: a randomized, double-blind, placebo-controlled study. *Otol Neurotol.* 2011; 32(5):774–9. [PubMed: 21646929]
58. Ferri E, Frisina A, Fasson AC, et al. Intratympanic steroid treatment for idiopathic sudden sensorineural hearing loss after failure of intravenous therapy. *ISRN Otolaryngol.* 2012; 2012:647271. [PubMed: 23724270]
59. Raghunandhan S, Agarwal AK, Natarajan K, et al. Effect of intravenous administration of steroids in the management of sudden sensori-neural hearing loss: our experience. *Indian J Otolaryngol Head Neck Surg.* 2013; 65(3):229–33. [PubMed: 24427572]
60. Trune DR, Kempton JB. Low dose combination steroids control autoimmune mouse hearing loss. *J Neuroimmunol.* 2010; 229(1-2):140–5. [PubMed: 20800906]
61. Shafik AG, Elkabarthy RH, Thabet MT, et al. Effect of intratympanic dexamethasone administration on cisplatin-induced ototoxicity in adult guinea pigs. *Auris Nasus Larynx.* 2013; 40(1):51–60. [PubMed: 22884636]
62. Murphy D, Daniel SJ. Intratympanic dexamethasone to prevent cisplatin ototoxicity: a guinea pig model. *Otolaryngol Head Neck Surg.* 2011; 145(3):452–7. [PubMed: 21521888]
63. Hill GW, Morest DK, Parham K. Cisplatin-induced ototoxicity: effect of intratympanic dexamethasone injections. *Otol Neurotol.* 2008; 29(7):1005–11. [PubMed: 18716567]
64. Daldal A, Odabasi O, Serbetcioglu B. The protective effect of intratympanic dexamethasone on cisplatin-induced ototoxicity in guinea pigs. *Otolaryngol Head Neck Surg.* 2007; 137(5):747–52. [PubMed: 17967639]
65. Weber A, Wasiliew P, Kracht M. Interleukin-1 (IL-1) pathway. *Sci Signal.* 2010; 3(105):cm1. [PubMed: 20086235]
66. Perrier S, Darakhshan F, Hajduch E. IL-1 receptor antagonist in metabolic diseases: Dr Jekyll or Mr Hyde? *FEBS Lett.* 2006; 580(27):6289–94. [PubMed: 17097645]
67. Owyang AM, Issafras H, Corbin J, et al. XOMA 052, a potent, high-affinity monoclonal antibody for the treatment of IL-1beta-mediated diseases. *MAbs.* 2011; 3(1):49–60. [PubMed: 21048425]
68. Zhao R, Zhou H, Su SB. A critical role for interleukin-1beta in the progression of autoimmune diseases. *Int Immunopharmacol.* 2013; 17(3):658–69. [PubMed: 24012439]
69. Pathak S, Goldofsky E, Vivas EX, et al. IL-1beta is overexpressed and aberrantly regulated in corticosteroid nonresponders with autoimmune inner ear disease. *J Immunol.* 2011; 186(3):1870–9. [PubMed: 21199898]
70. Vambutas A, DeVoti J, Goldofsky E, et al. Alternate splicing of interleukin-1 receptor type II (IL1R2) in vitro correlates with clinical glucocorticoid responsiveness in patients with AIED. *PLoS One.* 2009; 4(4):e5293. [PubMed: 19401759]

71. Goldbach-Mansky R, Dailey NJ, Canna SW, et al. Neonatal-onset multisystem inflammatory disease responsive to interleukin-1beta inhibition. *N Engl J Med*. 2006; 355(6):581–92. [PubMed: 16899778]
72. Dinarello CA, Simon A, van der Meer JW. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nat Rev Drug Discov*. 2012; 11(8):633–52. [PubMed: 22850787]
73. Kuemmerle-Deschner JB, Lohse P, Koetter I, et al. NLRP3 E311K mutation in a large family with Muckle-Wells syndrome—description of a heterogeneous phenotype and response to treatment. *Arthritis Res Ther*. 2011; 13(6):R196. [PubMed: 22146561]
74. Kuemmerle-Deschner JB, Tyrrell PN, Koetter I, et al. Efficacy and safety of anakinra therapy in pediatric and adult patients with the autoinflammatory Muckle-Wells syndrome. *Arthritis Rheum*. 2011; 63(3):840–9. [PubMed: 21360513]
75. Sibley CH, Plass N, Snow J, et al. Sustained response and prevention of damage progression in patients with neonatal-onset multisystem inflammatory disease treated with anakinra: a cohort study to determine three- and five-year outcomes. *Arthritis Rheum*. 2012; 64(7):2375–86. [PubMed: 22294344]
76. Wang J, Van De Water TR, Bonny C, et al. A peptide inhibitor of c-Jun N-terminal kinase protects against both aminoglycoside and acoustic trauma-induced auditory hair cell death and hearing loss. *J Neurosci*. 2003; 23:8596–60. [PubMed: 13679429]
77. Arpornchayanon W, Canis M, Ihler F, et al. TNF-alpha inhibition using etanercept prevents noise-induced hearing loss by improvement of cochlear blood flow in vivo. *Int J Audiol*. 2013; 52(8):545–52. [PubMed: 23786392]
78. Kaur T, Mukherjea D, Sheehan K, et al. Short interfering RNA against STAT1 attenuates cisplatin-induced ototoxicity in the rat by suppressing inflammation. *Cell Death Dis*. 2011; 2:e180. [PubMed: 21776018]
79. Rybak LP, Mukherjea D, Jajoo S, et al. siRNA-mediated knock-down of NOX3: therapy for hearing loss? *Cell Mol Life Sci*. 2012; 69(14):2429–34. [PubMed: 22562580]
80. Fire A, Xu S, Montgomery MK, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*. 1998; 391:806–11. [PubMed: 9486653]
81. Tabara H, Yasuda J. Physiological and technological aspects of RNAi. *Tanpakushitsu Kakusan Koso*. 2003; 48(4 Suppl):469–79. [PubMed: 12696156]
82. Mello CC. A conversation with Craig C Mello on the discovery of RNAi. *Cell Death Differ*. 2007; 14(12):1981–4. [PubMed: 18007669]
83. Hoerter JHA, Walter NG. Chemical modification resolves the asymmetry of siRNA strand degradation in human blood serum. *RNA*. 2007; 13(11):1887–93. [PubMed: 17804643]
84. Maeda Y, Fukushima K, Nishizaki K, et al. In vitro and in vivo suppression of GJB2 expression by RNA interference. *Hum Mol Genet*. 2005; 14:1641–50. [PubMed: 15857852]
85. Mukherjea D, Jajoo S, Whitworth C, et al. Short interfering RNA against transient receptor potential vanilloid 1 attenuates cisplatin-induced hearing loss in the rat. *J Neurosci*. 2008; 28(49):13056–65. [PubMed: 19052196]
86. Mukherjea D, Jajoo S, Sheehan K, et al. NOX3 NADPH oxidase couples transient receptor potential vanilloid 1 to signal transducer and activator of transcription 1-mediated inflammation and hearing loss. *Antioxid Redox Signal*. 2011; 14(6):999–1010. [PubMed: 20712533]
87. Schmitt NC, Rubel EW, Nathanson NM. Cisplatin-induced hair cell death requires STAT1 and is attenuated by epigallocatechin gallate. *J Neurosci*. 2009; 29(12):3843–51. [PubMed: 19321781]
88. Heineman TC, Schleiss M, Bernstein DI, et al. A phase 1 study of 4 live, recombinant human cytomegalovirus Towne/Toledo chimeric vaccines. *J Infect Dis*. 2006; 193(10):1350–60. [PubMed: 16619181]
89. Available from: http://www.pharmamedtechbi.com/~media/Supporting%20Documents/The%20Pink%20Sheet/75/1/Pfizer_research.pdf
90. Harvey RJ, Yee BK. Glycine transporters as novel therapeutic targets in schizophrenia, alcohol dependence and pain. *Nat Rev Drug Discov*. 2013; 12(11):866–85. Interesting paper. [PubMed: 24172334]

91. Maciaszczyk K, Lewi ski A. Phenotypes of SLC26A4 gene mutations: pendred syndrome and hypoacusis with enlarged vestibular aqueduct. *Neuro Endocrinol Lett.* 2008; 29(1):29–36. [PubMed: 18283249]
92. Yang T, Vidarsson H, Rodrigo-Blomqvist S, et al. Transcriptional control of SLC26A4 is involved in Pendred syndrome and nonsyndromic enlargement of vestibular aqueduct (DFNB4). *Am J Hum Genet.* 2007; 80(6):1055–63. [PubMed: 17503324]
93. Li X, Sanneman JD, Harbidge DG, et al. SLC26A4 targeted to the endolymphatic sac rescues hearing and balance in Slc26a4 mutant mice. *PLoS Genet.* 2013; 9(7):e1003641. [PubMed: 23874234]
94. Dou H, Xu J, Wang Z, et al. Co-expression of pendrin, vacuolar H⁺-ATPase alpha4-subunit and carbonic anhydrase II in epithelial cells of the murine endolymphatic sac. *J Histochem Cytochem.* 2004; 52(10):1377–84. [PubMed: 15385584]
95. Phillips L, Bitner-Glindzicz M, Lench N, et al. The future role of genetic screening to detect newborns at risk of childhood-onset hearing loss. *Int J Audiol.* 2013; 52(2):124–33. [PubMed: 23131088]
96. Hilgert N, Smith RJ, Van Camp G. Forty-six genes causing nonsyndromic hearing impairment: which ones should be analyzed in DNA diagnostics? *Mutat Res.* 2009; 681(2-3):189–96. [PubMed: 18804553]
97. Nance WE. The genetics of deafness. *Ment Retard Dev Disabil Res Rev.* 2003; 9(2):109–19. [PubMed: 12784229]
98. Miyagawa M, Nishio SY, Usam S. Consortium DGS. Mutation spectrum and genotype-phenotype correlation of hearing loss patients caused by SLC26A4 mutations in the Japanese: a large cohort study. *J Hum Genet.* 2014; 59(5):262–8. [PubMed: 24599119]
99. Nagai J, Takano M. Molecular-targeted approaches to reduce renal accumulation of nephrotoxic drugs. *Expert Opin Drug Metab Toxicol.* 2010; 6(9):1125–38. [PubMed: 20536274]
100. Baulac M. Introduction to zonisamide. *Epilepsy Res.* 2006; 68(Suppl 2):S3–9. [PubMed: 16413170]
101. Rock DM, Macdonald RL, Taylor CP. Blockade of sustained repetitive action potentials in cultured spinal cord neurons by zonisamide (AD 810, CI 912), a novel anticonvulsant. *Epilepsy Res.* 1989; 3(2):138–43. [PubMed: 2565229]
102. Ueda Y, Doi T, Tokumaru J, et al. Effect of zonisamide on molecular regulation of glutamate and GABA transporter proteins during epileptogenesis in rats with hippocampal seizures. *Brain Res Mol Brain Res.* 2003; 116(1–2):1–6. [PubMed: 12941455]
103. Kito M, Maehara M, Watanabe K. Mechanisms of T-type calcium channel blockade by zonisamide. *Seizure.* 1996; 5(2):115–19. [PubMed: 8795126]
104. Adamson CL, Reid MA, Davis RL. Opposite actions of brain-derived neurotrophic factor and neurotrophin-3 on firing features and ion channel composition of murine spiral ganglion neurons. *J Neurosci.* 2002; 22:1385–96. [PubMed: 11850465]
105. Errington AC, Stohr T, Lees G. Voltage gated ion channels: targets for anticonvulsant drugs. *Curr Top Med Chem.* 2005; 5:15–30. [PubMed: 15638775]
106. Fuchs P. The synaptic physiology of cochlear hair cells. *Audiol Neurotol.* 2002; 7:40–4. [PubMed: 11914525]
107. Rodriguez-Contreras A, Yamoah EN. Direct measurement of single-channel Ca²⁺ currents in bullfrog hair cells reveals two distinct channel subtypes. *J Physiol.* 2001; 534:669–89. [PubMed: 11483699]
108. Schnee ME, Ricci AJ. Biophysical and pharmacological characterization of voltage-gated calcium currents in turtle auditory hair cells. *J Physiol.* 2003; 549:697–717. [PubMed: 12740421]
109. Lacinova L, Klugbauer N, Hofmann F. Low voltage activated calcium channels: from genes to function. *Gen Physiol Biophys.* 2000; 19:121–36. [PubMed: 11156438]
110. Perez-Reyes E. Molecular characterization of a novel family of low voltage-activated, T-type, calcium channels. *J Bioenerg Biomembr.* 1998; 30:313–18. [PubMed: 9758328]
111. Triggle DJ. L-type calcium channels. *Curr Pharm Des.* 2006; 12:443e457. [PubMed: 16472138]
112. Yunker AM, McEnery MW. Low-voltage-activated (“T-Type”) calcium channels in review. *J Bioenerg Biomembr.* 2003; 35:533–75. [PubMed: 15000520]

113. Shen H, Zhang B, Shin JH, et al. Prophylactic and therapeutic functions of T-type calcium blockers against noise-induced hearing loss. *Hear Res.* 2007; 226:52–60. [PubMed: 17291698]
114. Dodson KM, Woodson E, Sismanis A. Intratympanic steroid perfusion for the treatment of Meniere's disease: a retrospective study. *Ear Nose Throat J.* 2004; 83:394–8. [PubMed: 15266874]
115. MacArthur CJ, Kempton JB, DeGagne J, et al. Control of chronic otitis media and sensorineural hearing loss in C3H/HeJ mice: glucocorticoids vs. mineralocorticoids. *Otolaryngol HeadNeck Surg.* 2008; 139:646–53.
116. Trune DR, Canlon B. Corticosteroid therapy for hearing and balance disorders. *Anat Rec.* 2012; 295:1928–43.
117. Canlon B, Meltser I, Johansson P, Tahera Y. Glucocorticoid receptors modulate auditory sensitivity to acoustic trauma. *Hear Res.* 2007; 226:61–9. [PubMed: 16843624]
- 118•. Bao J, Hungerford M, Luxmore R, et al. Prophylactic and therapeutic functions of drug combinations against noise-induced hearing loss. *Hear Res.* 2013; 304:33–40. Shows the combination of zonisamide and corticosteroid effectiveness in prevention of noise-induced hearing loss. [PubMed: 23792074]
119. Available from: http://www.who.int/pbd/deafness/WHO_GE_HL.pdf

Article highlights

- “Chronic” accumulation of reactive oxygen species (ROS) and the resulting inflammation are the basis of many forms of hearing loss (HL). Thus, ROS quenchers, antioxidants, anti-inflammatory drugs and biological agents that target ROS generation, inflammatory mediators of the apoptotic pathways form major classes as potential drugs for the prophylactic treatment of various kinds of HL.
- Current clinical and preclinical studies show an overwhelming majority of developmental drugs targeting either the ROS generated or the inflammation associated with HL. There are few drugs targeting ion homeostasis and voltage gated calcium channels.
- The most promising drug candidates seem to be ebselen and NAC, though long term evaluations are needed.
- NAC has been shown to be effective in mild traumatic blast injury, however, long term follow up is lacking.
- Intra tympanic steroid administration seems to be the best treatment for sudden sensorineural hearing loss and for salvage treatment in refractory cases.

This box summarizes key points contained in the article.

Table 1
Early trials for HL

Name	NCT	Status	Agents (drugs) to prevent hearing loss	Condition
Study to evaluate the safety and pharmacokinetics of SPI-1005	NCT01452607	Phase I completed	SPI-1005 (proprietary preparation of ebselen that contains the mineral selenium)	To evaluate the safety and pharmacokinetics of SPI-1005
Otoprotection with SPI-1005 for prevention of temporary auditory threshold shift	NCT01444846	Phase II ongoing	SPI-1005	NIHL
SPI-1005 for prevention and treatment of chemotherapy induced hearing loss	NCT01451853	Phase II not yet open for recruiting	SPI-1005	Cisplatin-induced Hearing loss
Prevention of noise-induced damage by use of antioxidants	NCT 01727492	Phase II currently recruiting participants	Antioxidantia (600 mg NAC and 200 mg magnesium)	Noise-induced tinnitus NIHL
The use of anti-oxidants to reduce sequela of mild traumatic brain injury (mTBI) after blast exposure	NCT00822263	Completed	N-acetyl-cysteine (NAC)	Mild traumatic brain injury
NAC to prevent cisplatin-induced hearing loss	NCT 02094625	Not yet open for recruiting	NAC	Neuroectodermal tumors, primitive Liver neoplasms Neoplasms, germ cell and embryonal Osteosarcoma Other childhood cancers using cisplatin-based regimens
Antioxidation medication for NIHL	NCT00552786	Phase II completed	NAC Glucose	Hearing loss
Prevention of drug induced ototoxicity in peritoneal dialysis patients by NAC	NCT01131468	Phase II completed	NAC	Drug-induced ototoxicity in peritoneal dialysis patients
Protection from cisplatin ototoxicity by lactated ringers	NCT00584155	Phase I completed	Normal saline and 0.3% ofloxacin lactated Ringer's (0.03% ofloxacin)	Hearing loss
Transtympanic ringer's lactate for the prevention of cisplatin ototoxicity	NCT01108601	Phase I Phase II recruitment status is unknown	Ringer's lactate (0.03% ciprofloxacin)	Hearing loss
The protective effect of ginkgo biloba extract on cisplatin-induced ototoxicity in humans	NCT01139281	Phase II completed	GBE761 Ginkgo biloba extract	Ototoxicity Hearing loss
Micronutrients to prevent NIHL	NCT00808470	Phase II completed	Dietary supplement consisting of β -carotene, vitamins C and E, magnesium	NIHL
Sudden hearing loss multi-center clinical trial	NCT02026479	Not yet open for recruitment	Dexamethasone phosphate Ginaton	Full-frequency sudden hearing loss

Name	NCT	Status	Agents (drugs) to prevent hearing loss	Condition
Fludrocortisone for sudden hearing loss	NCT01186185	Recruitment status is unknown	Fludrocortisone	Hearing loss, sensorineural
A study of the effects of PF-04958242 in subjects with age-related hearing loss	NCT01518920	Phase I completed	PF-04958242	Hearing loss, sensorineural
Proton pump inhibitors in preventing and treating hearing loss in patients with SLC26A4 Mutation	NCT00789061	Phase II	Proton pump inhibitor	Hearing loss
Pantoprazole in preventing nephrotoxicity and ototoxicity from osteosarcoma therapy	NCT01848457	Phase III Not yet open for recruitment	Pantoprazole	Osteosarcoma Nephrotoxicity Ototoxicity
The effects of gevokizumab in corticosteroid-resistant subjects with AIED	NCT01950312	Phase II Currently recruiting participants	Gevokizumab	AIED
A clinical trial of anakinra for steroid-resistant AIED	NCT01267994	Phase II Currently recruiting participants	Anakinra	Sensorineural hearing loss AIED
Safety study of four chimera cytomegalovirus (cmv) vaccines in healthy adult males 30 – 50 years of age	NCT01195571	Phase II Not currently recruiting participants	Biological: CMV vaccine (4 Towne-Toledo chimera vaccines)	CMV
Prevention of NIHL	NCT02049073	Phase I Currently Recruiting Participants	Zonisamide Methyl-prednisone	NIHL

AIED: Autoimmune inner ear disease; NIHL: Noise-induced hearing loss.