Compounds for the prevention and treatment of noise-induced hearing loss

Eric D. Lynch and Jonathan Kil

Noise-induced hearing loss (NIHL) is the leading occupational disease and a major contributor to the development of age-related hearing loss. The pharmacological prevention and treatment of NIHL has been under preclinical investigation for the past 20 years. Promising treatments have now been identified and entered into clinical development. Within the next five years, safe and effective drugs could be approved as the first generation of otoprotectants. This review covers strategies that are under investigation for NIHL. Drugs that effectively prevent and treat NIHL will have a significant impact on medical costs, disability compensation and several issues affecting the quality of life.

Noise is the greatest causative factor among the defined etiologies of hearing loss. Traditionally, prevention of noise-induced hearing loss (NIHL) has been addressed by providing wearable hearing protection and reducing noise emissions. However, for many occupations this has been insufficient, especially when noise levels exceed 130–140 decibels (dB).

According to the National Institute for Deafness and Communication Disorders (NIDCD), the American Speech, Language and Hearing Association (ASHA), and the Occupational Safety and Health Administration (OSHA) >30–40 million Americans are exposed to hazardous sound or noise levels on a regular basis. NIHL affects ~10–15 million people, of all age groups, in the USA [1,2]. NIHL is the leading occupational disease, a significant cause of disability and a major cost to society.

Many noisy occupations such as the military [3,4], construction [5,6], manufacturing, mining [7], forestry [8], farming [9], aviation [10,11], rail [12] and trucking [7] report the urgent need to develop hearing-conservation programs. Whereas annual surveillance and compliance remains an ongoing issue, the efficacy of hearing-protection devices (e.g. earplugs) and hearing-protection measures (i.e. reduced noise exposure time) could be augmented by pharmacological agents that might reduce NIHL more effectively.

In Table 1, OSHA-regulated exposures to noise are detailed according to sound pressure level, which is measured in dB as a logarithmic scale of sound intensity. Every 3 dB increase is a doubling of sound intensity. For practical purposes, OSHA states that every 5 dB increase in sound exposure level requires a 50% reduction in exposure time or duration. OSHA recommends that no one should be exposed to >140 dB of sound, even for brief periods.

Personnel in certain military and industrial occupations are at extreme risk of developing NIHL as a result of noise exposure levels often exceeding 120 dB. For example, the M16 rifle (routinely used during basic training and annual weapons qualification exercises) discharges at 156 dB. A common result is the development of a temporary threshold shift (TTS). With multiple, cumulative exposure events, significant irreversible hearing loss can occur that produces a permanent threshold shift (PTS).

Eric D. Lynch
Jonathan Kil*
4010 Stone Way N,
Suite 120,
Seattle,
WA 98103, USA
*email: jkil@soundpharmaceuticals.com

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TABLE 1

Sound pressure levels (SPL) that can irreversibly damage hearing

SPL (dB)	Duration	Sound source	Industry
140	<1 min	Firearms, jet engine	Military, aviation
130	>1 min	Drop forge, jackhammers	Manufacturing, mining, construction
120	>5 min	Amplified speaker	Musicians, recreational
110	>15 min	Engines	Rail, trucking
100	>1 h	Woodshops, chainsaws	Forestry
90	>4 h	Motorcycles, lawnmowers	Recreational
85	>8 h	Interior plane cabins	Aviation

Recent acute-noise-exposure data from a US Army Special Forces study reported 11% of 72 subjects who underwent live-fire weapons-training exercises for three days sustained permanent hearing threshold shifts [13]. A survey of US Marines exposed to live-fire exercises for 3–5 days indicated that 11% of this group had noise-induced PTS [13]. These data are consistent with the 1994 findings of Attias *et al.* [14], where 11% of 150 basic training recruits from the Israel Defense Forces experienced significant threshold shifts following 56 days of live-fire exercises. Evaluating hearing within a few hours of exposure to measure TTS (a dangerous condition in high-risk, communication-intensive environments) could double or triple this number. Furthermore, analyzing hearing loss at even one important speech frequency (e.g. 2, 3 or 4 kHz) would generate a much higher incidence level.

Another inner-ear disorder that is highly correlated with acute and chronic NIHL is tinnitus [15,16]. In a retrospective study of 3466 claimants who sought compensation for occupational NIHL, the prevalence of those reporting tinnitus as a function of hearing loss at 4 kHz ranged from 41.7 to 56.5%, regardless of the amount of hearing loss sustained [17].

Occupational hearing loss and hearing conservation

Military hearing-conservation programs (HCPs) are limited in their ability to control exposure to high level noise, generated from weapons fire and explosives [18], partly as a result of inadequate sound attenuation by hearing-protection devices (HPDs). Personnel required to wear HPDs are often noncompliant, citing poor fit, restricted head movement, discomfort and reduced communication ability among their reasons. In combat situations, military personnel are less likely to use HPDs because of impaired communication and the need for enemy detection. Hearing loss increases with increasing noise exposure and number of years in service, especially for those in armor, artillery and infantry branches [19].

In a survey of 12,492 medical records to evaluate the Navy's HCP, Wolgemuth *et al.* [3] found that the incidence of significant threshold shift (STS) was 29% (SD = 11.1%). In the absence of criteria establishing acceptable levels of STS in HCPs the authors concluded that the incidence of STS 'may be too high', especially in certain job categories. Whereas audiogram compliance was in the 80–92.9% range, follow-up compliance was 62%, suggesting that the customary means used to evaluate HCPs were not highly correlated with STS levels. Bohnker *et al.* [4] analyzed 68,632 monitoring audiograms of enlisted personnel from the US Navy and the US Marine Corps. (1995–1999). They found that 'hearing deterioration continues to be a significant issue in force health protection' and stated a requirement for 'better programmatic processes to prevent hearing deterioration.' Looking at the industrial sector, Dobie [20] reviewed occupational HCPs and found little evidence of efficacy, which he concluded was usually because of flaws in study methodologies (e.g. treatment and control groups were not matched for age, history of hearing loss, non-occupational noise exposure or use of HPDs, among others). Irrespective of reported limitations, the epidemiology provided by HCPs reveals an urgent need to enhance current levels of hearing protection without additional restriction, discomfort or communication interference.

A drug to prevent and treat NIHL will improve military force performance and readiness, especially in combat where hearing ability can impact on soldier 'survivability'. OSHA states that on a daily basis, 40 million people in the USA are exposed to hazardous noise levels that might permanently damage hearing. Pharmacological protection will augment hearing conservation in routine training and occupational situations where most NIHL occurs, reducing the compensation costs associated with NIHL across all industries.

Pathogenesis

In response to sound waves traveling through the cochlea, auditory hair cells in the organ of Corti depolarize following the opening of mechanotransduction channels caused by the physical deflection of the stereocilia on their apical surface. The organ of Corti contains two types of auditory hair cell: inner and outer hair cells (IHC and OHC, respectively). OHCs are organized into three rows and are usually the first hair cells affected. Healthy OHCs contract in response to acoustic stimulation, resulting in an increase in sensitivity (or gain) of ~40–50 dB (active cochlear amplification) [21,22]. Mitochondria are some of the first and most affected intracellular organelles in models of NIHL. IHCs are predominantly sensory in nature and are heavily innervated by the eighth cranial (auditory) nerve.

The constitutive activity of IHCs and OHCs is dramatic given our noisy environments. The amount and type of hair cell damage depends on the frequency, intensity and duration of the noise exposure. Above a specific intensity level, OHCs show signs of metabolic exhaustion with the accumulation of reactive oxygen and reactive nitrogen species (ROS and RNS, respectively). When OHCs are permanently damaged or lost, the threshold sensitivity of the IHC increases (loss of active cochlear amplification) and it is often recorded as a threshold shift or as hearing loss.

Over the past decade, much progress has been made in our understanding of the cellular and biochemical basis of NIHL. Acute exposure to loud noise affects several structural elements in auditory hair cells, including cell membrane and intracellular biochemical pathways [23]. These changes can evoke the formation of free radicals (in particular ROS and RNS) that overwhelm resident detoxification and antioxidant mechanisms [24–27]. Others have shown a greater susceptibility to NIHL in animals and humans with dietary magnesium (Mg) deficiency [28]. Mechanistically, low Mg might contribute to a loss of membrane potential, resulting in altered or decreased sensorineural function.

A major intracellular antioxidant pathway that can detoxify free radicals and attenuate ROS and/or RNS involves the tripeptide glutathione (GSH) [29,30]. Loud noise can reduce GSH and increase the level of oxidized glutathione in the inner ear [31] leaving it prone to ROS- and/or RNS-mediated cell damage. GSH interacts with glutathione peroxidase (GPx), which catalyzes the ability of GSH to act as an antioxidant. Intriguingly, GPx activity also decreases following noise exposure [27].

The additive effect of increased ROS and/or RNS and depleted antioxidant capacity can lead to cell injury or death. Some of the most damaging ROS and/or RNS are those that can oxidize lipids such as hydroxynonenal (4-HNE) and peroxynitrite (ONOO–). These free radicals degrade lipids and damage membrane-bound organelles such as mitochondria and nuclei. Excess ROS and/or RNS generated by elevated hair cell metabolic activity during intense noise exposure could overwhelm the antioxidant buffering capacity of the cell, leading to permanent loss or injury of hair cells [13,32,33].

Otoprotection in preclinical development

Recent studies with antioxidants, N-methyl-D-aspartate (NMDA) antagonists, caspase or cell death inhibitors, and growth factors have some significant design limitations that restrict their direct clinical application [34]. Among these strategies, the use of antioxidants to neutralize ROS and/or RNS is an appealing early intervention step in the prevention of cellular damage in the cochlea. At present, there are no FDA-approved drug products that can reduce or prevent NIHL. However, animal work demonstrates that NIHL can be attenuated by agents that reduce the level or activity of ROS and/or RNS or of free radicals.

One of the earliest compounds tested for prevention of NIHL was allopurinol, a hypoxanthine analogue that acts as an inhibitor of xanthine oxidase and a scavenger of free radicals. Allopurinol is a prescription drug that is FDA-approved for the treatment of gout and hyperuracemia induced by cancer chemotherapy. Systemic injections of high-dose allopurinol (50 mg/kg) into live animals before, during, and after 60 h of continuous noise exposure (90 dB), reduced the level of hearing loss immediately after noise exposure had finished [35]. However, Franzé *et al.* [36] found that the same dose of allopurinol could only reduce the TTS after intense noise exposure and not the PTS measured at 15 and 30 days post-noise.

Other compounds that have demonstrated some efficacy in preventing NIHL are GSH precursors such as N-acetylcysteine (NAC) and methionine (MET). NAC is a GSH prodrug that, upon de-acetylation to L-cysteine by the liver and local tissues, enhances GSH production [30]. High-dose NAC is FDA-approved as a mucolytic agent for respiratory diseases and can reverse acute hepatoxicity following acetaminophen overdose. It is given orally or intravenously (i.v.) at 70 mg/kg for 24–48 h. In persons that have normal GSH levels, NAC is well tolerated and is taken orally at 1–3 g per day. NAC has been shown to be otoprotective when injected intraperitoneally (i.p.) at 325 mg/kg [13]. MET has also been shown to act as an otoprotectant when injected at 200 mg/kg [13,37]. MET is an essential amino acid and can be converted to cysteine, the rate-limiting substrate for GSH production. Racemic MET (D and L isoforms) is FDA-approved to acidify urine. It is well tolerated at 500–1000 mg per day when administered orally.

Other groups have focused on reducing hair cell apoptosis by disrupting mitogen-activated protein kinase (MAPK) cell death signaling through peptide inhibition of c-Jun N-terminal Kinase (JNK). D isoforms of a 20 amino acid JNKI-1 inhibiting peptide tagged with a 10 amino acid HIV–TAT peptide (to facilitate cellular uptake) have been shown to protect against NIHL in guinea pigs when delivered subcutaneously or locally (intracochlear) via catheter before and following noise exposure [38–40]. Most recently, D-JNKI-1 peptides have shown otoprotection when delivered locally to the round window membrane of the cochlea within 24 h of noise exposure, although this is less effective than intracochlear administration [40].

Although promising, these initial discoveries have several limitations that could restrict their ability to enter human clinical trials for otoprotection. A major limitation is the route of administration and bioavailability. All of the aforementioned studies involved systemic or local injections of the compound. For human utility in an outpatient setting oral dosing is preferred to improve patient compliance, especially for more chronic therapies. A chronic therapeutic strategy is consistent with a noisy occupational or recreational environment. Another notable limitation is the level of dosing. All of the preceding studies used very high-dose levels. Although scientifically important,

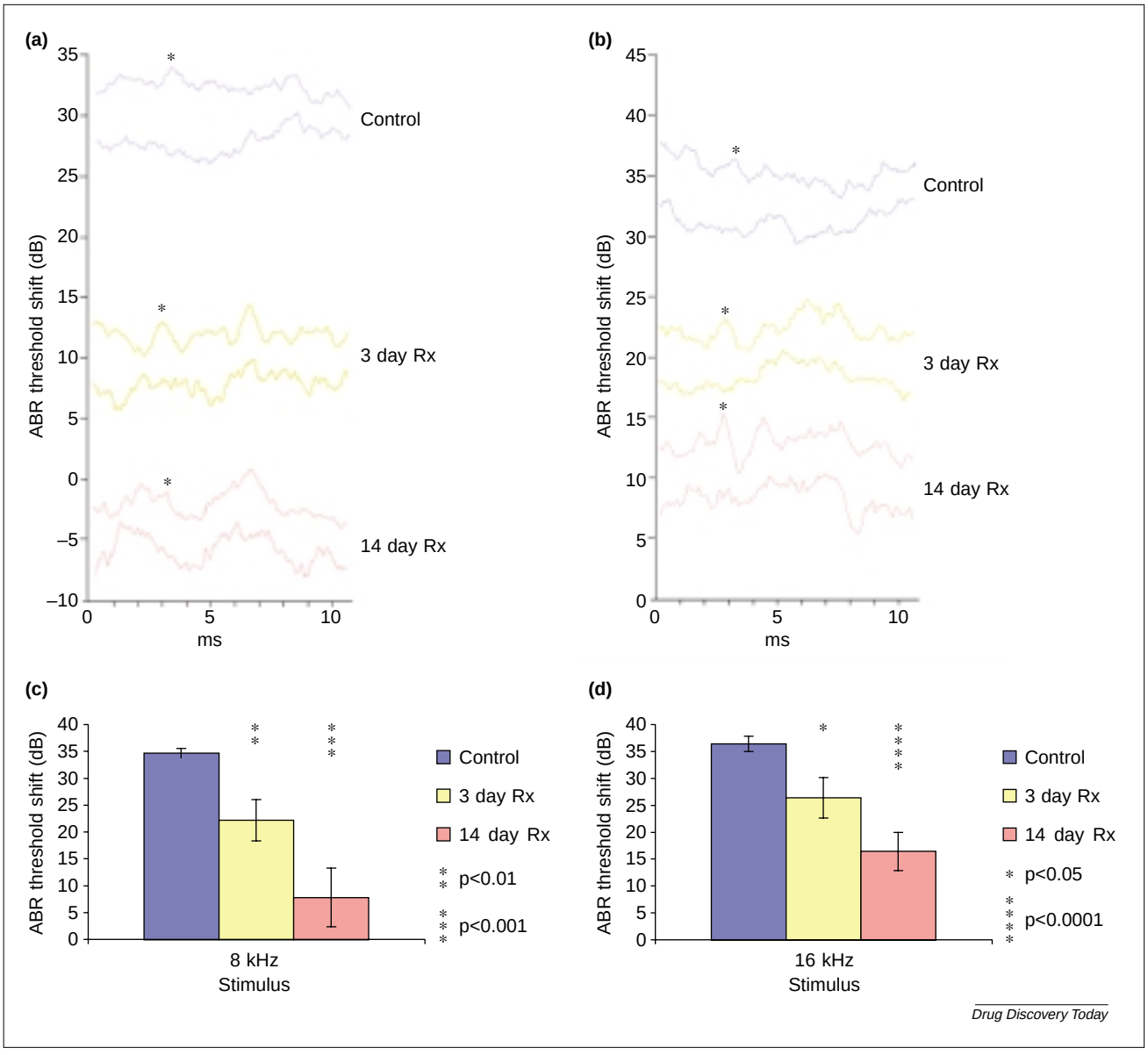

FIGURE 1

Representative auditory brainstem response (ABR) tracings from vehicle (control), 3-day, and 14-day ebselen (GPx mimic) treated F-344 rats at 15 weeks post-noise exposure. Rats were exposed for 4 h to 113 dB noise centered at 8 kHz with a 1 octave band width. Treatment was by oral gavage and began 1 day before noise exposure. The amount of ABR threshold shift or hearing loss is indicated by the upper tracing (*) for each animal, whereas the lower tracing confirms the absence of threshold or an evoked response at 5 dB below threshold at **(a)** 8 kHz and **(b)** 16kHz. X-axis in milliseconds (ms); Y axis in decibels (dB); treatment (Rx) = 4 mg/kg ebselen orally twice daily for the duration indicated. Bar charts show mean ABR threshold shifts at **(c)** 8kHz and **(d)** 16kHz for control, 3-day and 14-day ebselen treated groups (n=8 ears/group, SEM shown).

most of these compounds exhibited poor oral bioavailability, required high-dose levels or did not offer long lasting protection. In addition, the enzymes or rate-limiting catalytic proteins involved in these antioxidant pathways had not been tested. There are several limitations to this proof-of-concept work that could inhibit their direct translation into clinical testing.

Recently, three publications involving a mimic of GPx showed excellent otoprotection using an oral route of administration [41–43]. Efficacy in guinea pig and rat models of NIHL under TTS and PTS conditions has been reported. These data are consistent with the observation that GPx activity is decreased in the cochlea after noise exposure [27] and that deletion of the GPx1 gene confers increased susceptibility to noise damage [44]. In addition, this significant otoprotection was achieved in the low mg/kg range.

In normal cells, GPx functions at near-maximal levels. Augmentation of GPx activity with the enzyme itself is not practical because of its large size and relative instability. However, small-molecule mimics of GPx have been synthesized and had high GPx activity *in vitro* and *in vivo* [45–47]. Among the GPx mimics that have been developed, ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is the most advanced and has been shown to have excellent oral availability and low toxicity making it a suitable candidate drug for the prevention of NIHL (Figures 1 and 2).

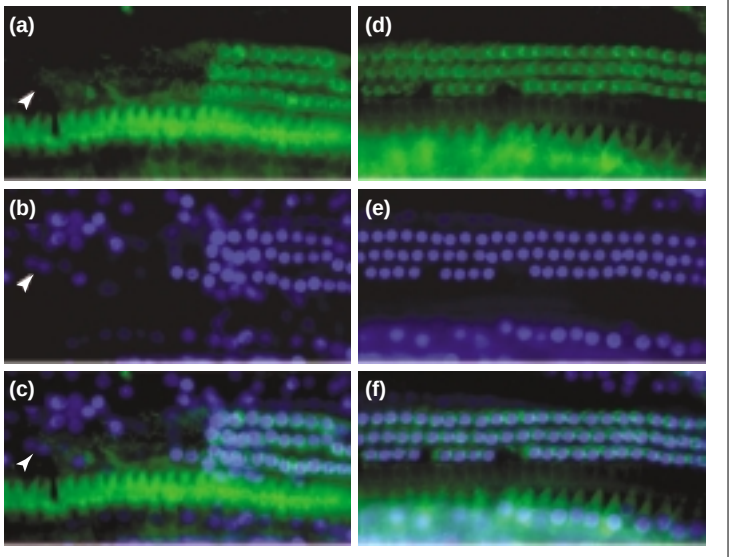

FIGURE 2

Representative fluorescent images of the organ of Corti following vehicle (control) and 3 day ebselen treatment, 1 day after a 4 h exposure to 113 dB noise centered at 8 kHz with a 1 octave band width. Mechanotransducing hair cells were identified with a fluorescent probe, AM-143 (green) that enters the stereocilia of functioning hair cells. Hair cells were co-labeled with DAPI (blue) to identify their nuclear morphology and observe evidence of pyknosis (white arrows). Single channel and merged channel views of control **(a), (b), (c)** and ebselen-treated **(d), (e), (f)** animals indicate a significant level of outer hair cell (OHC) loss in control animals and significantly less OHC loss in treated animals at a region corresponding to 8–16 kHz.

Ebselen has strong activity against peroxynitrite (ONOO–), a super ROS and/or RNS formed by the combination of two free radicals: super oxide anion and nitric oxide [48–50]. It reduces cytochrome *c* release from mitochondria and nuclear damage [51] during lipid peroxidation. Because ebselen acts as a catalyst and is not consumed during detoxification reactions [52], low doses might prove to be effective at reducing NIHL. Preclinical studies in rats and guinea pigs indicate that noise induced TTS and PTS can be reduced by ebselen when it is administered orally in the 4–10 mg/kg range [41,42]. This corresponds to a 280–700 mg dose in humans, a dose previously shown to be well-tolerated [53,54].

In addition to its function as a GPx mimic, ebselen has been described as having the properties of: thioredoxin reductase [55], dehydroascorbate reductase and thioltransferase [56], anti-inflammatory compounds [45,57] and anti-apoptotic compounds [51,58]. In general, ebselen is capable of reducing oxidative stress levels in various cell types through a variety of mechanisms. Intense noise exposure can lead to increased oxidative stress causing OHC loss via activation of apoptotic and necrotic pathways. Noise exposure causes the release of cytochrome *c* from mitochondria in apoptotic and necrotic cells. The release of cytochrome *c* in a subpopulation of OHCs takes place early in the cell death process, before any outward signs of necrosis or apoptosis [59]. Ebselen might exert its protective effect in the cochlea through the inhibition of cytochrome *c* release from mitochondria in OHCs, as has been demonstrated in other systems [51,60]. Membrane-lipid peroxidation in the cochlea of animals exposed to high levels of noise has been demonstrated to be a predisposing factor in the permanent loss of OHC [32]. The precise biochemical mechanism(s) of ebselen-mediated protection in the cochlea of animals is unknown but is probably associated with the attenuation of ROS- and/or RNS-mediated damage.

Otoprotection in clinical development

Mg

In a double-blind placebo-controlled study involving 300 young, healthy military recruits, those supplemented daily with 4 g of oral Mg granulate verum (6.7 mmol Mg aspartate) showed significantly less PTS than those in the placebo control group (11.2% versus 21.5%) one week post noise [14]. Analysis of Mg levels in serum, erythrocytes and mononuclear cells showed a strong negative correlation between mononuclear Mg levels and the development of PTS that was independent of treatment group. However, a weak correlation between serum Mg levels and PTS was reported in a study of 68 male soldiers that had been exposed to high-level weapons noise over an 8–18 year period [61]. Analysis of Mg supplementation in soldiers exposed to low-level noise also shows reduced TTS levels, although no significant changes in serum or mononuclear cell Mg levels were identified between treated and placebo groups [62].

NAC

In a double-blind placebo-controlled study involving 600 young, healthy US Marine recruits, 900 mg NAC (effervescent tablet) was dosed orally, three times daily for two continuous weeks to reduce PTS during weapons training. At present the data from this trial are being analyzed. In a recent study, normal-hearing adults were dosed orally with placebo or 900 mg NAC 30 min before entering a nightclub where they were exposed to two hours of loud music. Personal dosimeters recorded a mean noise level of 98.1 dB (A-weighted). An average of 14 dB TTS at 4kHz was reported in subjects immediately after exposure (within 15 minutes). No significant differences between groups were identified [63]. This observation might be related to the requirement of high dose NAC to effectively prevent NIHL in animal models [13,37] or the limited ability of NAC to prevent TTS.

Ebselen

In an upcoming double-blind placebo-controlled Phase II study of oral ebselen, 60 young, healthy US Army recruits will receive doses of ebselen twice daily for two continuous weeks during weapons training. They will be assessed within six hours to determine TTS and subsequently at two and four weeks post-noise to determine PTS.

TABLE 2

Summary of compounds tested to prevent NIHL

Class	Compound	Effective dose	ROA	Comments	Refs
Antioxidants					
GSH prodrug	Allopurinol	50–100 mg/kg	i.p.	TTS reduction	[35,36,64]
	ALCAR	100 mg/kg	i.p.	PTS reduction; limited study	[65]
	Edavarone	17 mM	local	PTS reduction	[66]
	lipoic acid	50–200 mg/kg	i.p., p.o.	TTS and PTS reduction	[67]
	Resveratrol	430 μg/kg	p.o.	TTS and PTS reduction; limited study; extensive pretreatment	[68]
	R-PIA	50 mM	local	PTS reduction; limited study	[69]
	α-tocopherol	10–50 mg/kg	i.p.	TTS and PTS reduction	[70]
	Methionine	200 mg/kg	i.p.	PTS reduction	[13,71]
	Monoethylester	50–150 mM	local	TTS and PTS reduction	[69]
	NAC	325 mg/kg	i.p.	TTS and PTS reduction; Ph-III NIHL completed	[65]
	OTC	735 mg/kg	i.p.	Limited PTS protection	[72]
Antioxidant enzymes					
GPx	Ebselen/SPI-1005	4–30 mg/kg	p.o.	TTS and PTS reduction; acute stroke studies halted; NIHL Ph I/II upcoming	[41–43,54]
SOD	SOD–PEG	2000 μg	i.m.	Limited study; TTS reduction; potential for SOD paradox	[35,64]
Calcineurin inhibitors	Cyclosporin A	10 μg/ml	local	TTS and PTS reduction; limited study	[73]
	FK506	1–10 μg/ml	local	TTS and PTS reduction; limited study	[73]
Diuretics	Mannitol	15 mg/kg	i.p.	Limited study; PTS reduction	[74]
Glucocorticoids	Dexamethasone	100 ng/ml	local	Limited study; PTS reduction is U- shaped	[75]
Growth factors	aFGF	1000 ng/ml	local	PTS reduction; limited study	[76]
	GDNF	100 ng/ml	local	PTS reduction; higher dose was ototoxic	[77,74]
Iron chelators	Deferoxamine	100 mg/kg	s.c.	Limited study; PTS reduction; clinically observed ototoxicity	[74]
JNK Inhibitors	CEP-1347	1 mg/kg	s.c.	PTS reduction, limited study; Parkinson's Disease studies halted	[39]
	D-JNKI-1	1–100 μM	local	TTS and PTS reduction	[40]
Magnesium	Mg	4 g in humans	p.o.	TTS and PTS reduction; efficacy correlates with Mg deficiency versus treatment	[14,61,62]
NMDA antagonists	Carbamathione	5.6 mg/kg	i.p.	PTS reduction; limited study	[65]
	Caroverine	1.6–12.8 mg/ml	local	PTS reduction; transient block of sound transduction	[78]
	MK-801	1 mg/kg	i.p.	PTS reduction; limited study	[32,79]
	PD 174494	10 mg/kg	i.p.	Limited PTS protection	[32]
NOS inhibitors	L-NAME	1 mg/kg	i.p.	Limited study; some ototoxicity seen at higher frequencies	[32]

Abbreviations: ROA, route of administration; i.p., intraperitoneal; p.o., oral; i.m., intramuscular; s.c., subcutaneous.

Although limited, these initial clinical studies of NIHL indicate that the incidence of TTS, PTS and tinnitus can be determined quickly. This allows for short clinical trial periods when compared with the clinical trial periods of other debilitating neurosensory diseases. This observation is a clear advantage in developing novel drugs for NIHL and tinnitus.

Summary

Several pathways are worth considering for the development of otoprotective compounds (summarized in Table 2). In general, the population utilizing these drugs will be healthy individuals who are at risk of developing permanent hearing loss as a result of occupational exposure to noise. Any significant, adverse events will not be tolerated. It is also unlikely that healthy individuals would undergo surgical procedures, receive repeated systemic injections or ingest beverages to prevent NIHL.

Finally, the characteristics of the drug itself, with regards to manufacturing costs and stability, will need to be considered. If these criteria can be met, market acceptance and support will be driven largely by the clear medical need for a drug and the savings associated with reduced medical and disability costs and training of personnel.

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