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Pharmacological approaches to the prevention and treatment of cochlear injury due to noise

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Abstract
Sensorineural hearing loss (SNHL) due to the damaging effects of noise is the second leading cause of SNHL, next to presbyacusis, in the industrialized world. It is the leading cause of preventable SNHL despite the implementation of hearing conservation programs after World War II. While engineering solutions and personal hearing protection devices remain important approaches to preventing noise-induced hearing loss, they have inherent limitations. Therefore researchers from a number of laboratories worldwide have devoted much time in the last decade to elucidating the cellular and molecular mechanisms of cochlear injury due to noise. This has led to the documentation of a number of therapeutic compounds that attenuate noise damage to hearing based on known mechanisms of injury. Some of these effects are quite robust. Clinical studies are beginning to emerge.

Key words: *noise-induced hearing loss, prevention, treatment, hair cells, oxidative stress, acute acoustic trauma*

Introduction

Despite growing awareness of the need for hearing conservation programs, noise-induced hearing loss (NIHL) continues to be the most common preventable cause of sensorineural hearing loss. Although advances are being made in engineering approaches to make workplaces less noise hazardous and to develop better personal hearing protection devices, these approaches have some inherent limitations that are driving research towards an adjunctive pharmacological approach to prevent and treat noise damage to the cochlea. Over the past decade numerous studies of the cellular and molecular basis of noise-induced cochlear injury have suggested that pharmacological approaches might help to ameliorate this common hazard. In addition to the macro- and micro-mechanical cochlear damage that has consistently been documented, a number of other molecular, biochemical, and cellular processes have now been described. These include ischemia reperfusion, glutamate excitotoxicity, calcium fluxes and oxidative stress, as well as cell death processes (see article by Talaska and Schacht in this issue). Therapeutic strategies corresponding to most of these known mechanisms have been explored and proven to be successful in a variety of preclinical studies. To date, most of the data are in the realm of basic science. This article will review the pharmacological approaches aimed at the prevention and treatment of noise damage to the cochlea based on many of the known mechanisms of cochlear injury induced by noise. Mention will be made of some available and emerging clinical data.

Why might a pharmacological strategy be useful?

Currently-available hearing protection devices (HPDs) are only partially effective because 1) noise levels can exceed the protective capability of the device; 2) injurious acoustic energy can be transmitted directly through the skull, by-passing the protective device to damage the cochlea; 3) HPD attenuation is frequency dependent; 4) their protective capability relies on precise fitting of the device which cannot always be maintained; 5) often in both military and civilian applications the overriding need to communicate precludes wearing an HPD (1), and 6) damaging noise cannot always be anticipated – this frequently leads to permanent damage due to short unexpected high intensity exposures, such as explosions (2).

In the workplace 30 to 40 million Americans are at risk of NIHL (3), and worldwide estimates are that 16% of adult disabling hearing loss is due to noise

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exposure in employment. In the military, NIHL continues to be an acute and expensive problem with Veterans Affairs (VA) disability expenditures rising annually (4) even though hearing conservation programs emphasizing the use of HPDs are active and ongoing (5,6). Civilian occupations that have significant risks of acoustic overexposure include, among others, mining (7), farming and transportation (8,9), and construction (10,11).

Thus, while HPD compliance is always an issue, the aforementioned factors require additional solutions to improve hearing conservation effectiveness. More than a decade ago, it was found that NIHL was not just mechanical or physical in nature, but that cochlear injury was also metabolically induced (12–14). Since the discovery that noise-induced metabolic oxidative stress plays a significant role in acoustically-generated cochlear injury (15), research using animal models has defined a variety of potential therapeutics effective in reducing the permanent hearing loss associated with acoustic overexposure in animal models (16,17). The success of a number of compounds in preventing hearing loss suggests other strategies for hearing conservation, namely, making the cochlea more resistant to acoustic injury or treating the acutely-injured cochlea through pharmacological intervention.

Mechanism-based pharmacological strategies to prevent and treat noise damage to the cochlea

Micromechanical damage

High level noise that causes gross mechanical damage, such as the separation of the organ of Corti from the basilar membrane (18), is likely to induce injury that would not be treatable with pharmacological approaches. However, damage that may be considered micromechanical has been described, and it might be possible to pharmacologically enhance repair of these less destructive lesions. Indeed, some of these milder injuries are thought to contribute to noise-induced temporary threshold shifts (TTS), indicating that an intrinsic repair capability does exist (19–21). Micromechanical injuries described include hair cell stereocilia injury (22–24), disconnection of stereocilia tips from the tectorial membrane (19), loss of pillar cells (25), ruptured cell junctions between hair cells and supporting cells (26), and holes in the reticular lamina leading to toxic ionic perturbations (27). Treatment approaches to these mechanisms might include optimizing cellular energy states, enhancing intrinsic repair processes, or ameliorating the damaging effects of ionic or oxidative imbalance secondary to these micromechanical injuries in order to optimize the conditions for repair and recovery.

A novel therapeutic approach to mechanically-induced programmed cell death has recently been described (28). Disruption of connections between cells or cells and their extracellular matrix can induce the initiation of programmed cell death through the activation of a pathway involving Src protein tyrosine kinase (29). Chinchilla ears were pretreated topically through the round window membrane with several specific Src inhibitors in solution prior to high level noise exposure. Treated ears showed less hearing and hair cell loss (28) compared to solution-only pretreated control ears. One agent, KX1-004, was effective for both steady state and impulse noise (28).

Ischemic injury

While fairly consistent changes in cochlear anatomy and physiology due to loud sound exposure have been reported, investigators' reported observations on the effect of loud sound on cochlear blood flow are more varied. In general, however, it is thought that acoustic overexposure induces an ischemic reperfusion injury to the cochlea (see article by Talaska and Schacht in this issue). Re-oxygenation occurring with reperfusion can enhance the formation of reactive oxygen species (ROS), leading to oxidative damage. A recent study found that intense noise induces the formation of isoprostanes in the cochlea. One of the effects of these compounds is locally-induced vasoconstriction (30).

A variety of compounds for protecting cochlear blood flow from noise-induced changes have been studied, including sartharan, pentoxifylline, carbogen, glucocorticoids and others. Pretreatment of animals with the angiotensin receptor antagonist sarthran during noise exposure prevented noise-induced microcirculatory ischemia and preserved auditory sensitivity at the lower frequencies (31). In another study on cochlear noise-induced threshold shifts comparing carbogen (95% O_2/5% CO_2), 5% CO_2/air and 100% oxygen given during noise exposure, carbogen resulted in reduced threshold shifts in most frequencies while the mixture of 5% CO_2/air yielded no difference in threshold shifts compared to controls and 100% oxygen resulted in a marked decrease in noise-induced threshold shifts. It was concluded that oxygen (i.e. cochlear-oxygenation) is a more important factor than CO_2 (i.e. as a vasodilator) in protection of the cochlea from noise-induced damage (32). Pentoxifyllin, a xanthine derivative that promotes capillary bed blood flow, was studied as an agent to prevent noise-induced threshold shifts. Pentoxifylline maintained cochlear

microcirculation as assessed by continuous red blood cell movement through capillaries, but did not prevent vasoconstriction or increased vascular permeability. Treatment with this drug did reduce noise-induced TTS (33).

Lamm et al. have studied a wide variety of compounds with potential effects on cochlear blood flow. For example, the effect of blood flow promoting drugs, such as hydroxyethyl starch (HES), pentoxifylline, Gingko biloba, naftidrofuryl and betahistine, and various combinations of the drugs, was studied in unexposed and steady state broad-band noise-exposed guinea pigs. Cochlear microphonics (CMs), compound action potentials (CAPs), and evoked brainstem measures (ABRs) improved significantly after HES 70, HES 200 and betahistine, resulting in partial recovery of CMs, and partial (betahistine) or even full (HES 70 and HES 200) recovery of CAPs and ABRs. Saline, pentoxifylline, Gingko biloba and naftidrofuryl had no effect on these measures in this study (34). In another very complex study, cochlear blood flow and perilymphatic partial pressure of oxygen, as well as CMs, CAPs, and ABRs were studied in noise-exposed guinea pigs during and after the following treatments: intravenous infusion of isotonic saline (placebo); blood flow promoting drugs (HES, pentoxifylline, betahistine, Gingko biloba, naftidrofuryl); anti-inflammatory agents (prednisolone, diclofenac sodium, histamine H1-receptor antagonist); isobaric oxygenation (IBO); and hyperbaric oxygenation (HBO) with and without supplements (simultaneous infusion of isotonic saline, pentoxifylline, prednisolone, or HES) (35). A sustained therapeutic effect on noise-induced cochlear ischemia was achieved only by HES, HBO+HES, and pentoxifylline. However, the best therapeutic effect on noise-induced hearing loss was achieved with a combination of HBO and prednisolone, followed by monotherapy with prednisolone or HES. All other therapies were significantly less effective or did not improve noise-induced reduction of auditory evoked potentials.

Glutamate excitotoxicity

Excessive glutamate release during acoustic overexposure may injure afferent primary auditory neurons by inducing ionic fluxes (36–38) or free radicals related to nitric oxide (NO) (39), or by interfering with the uptake of the glutathione (GSH) precursor cystine in the outer hair cells (OHCs) (40). A number of different strategies have been reported to decrease cochlear injury due to noise-induced glutamate excitotoxicity. Carbamathione is thought to interact with the redox modulatory site of the N-methyl-D-aspartate (NMDA) receptor, one of several glutamate receptors found in the cochlea and postulated to inhibit glutamate excitotoxicity by partial selective antagonism of the NMDA receptor (1). Carbamathione, when given systemically (5.6 mg/kg) shortly before and after a 6-h high level octave band noise exposure, significantly attenuated OHC and permanent hearing loss in chinchilla. The NMDA receptor antagonist MK-801 has been utilized in basic science experiments to demonstrate reduction of permanent noise-induced hearing loss (41,42). MK-801 produces complete inhibition of glutamate effects by blocking NMDA receptor-linked calcium ion channels. In one study, pretreatment of guinea pigs with MK-801 (1 mg/kg by intraperitoneal injection) prevented temporary hearing loss and swelling of spiral ganglion neuron dendrites after steady state noise exposure (42). In another study, pretreatment of rats (1 mg/kg by intraperitoneal injection) reduced compound action potential noise-induced threshold shifts but did not prevent OHC loss (41). Kynurenic acid, a broad-spectrum glutamate receptor antagonist, was applied as solution to the round window membrane of the guinea pig prior to high level steady state noise and protected against noise-induced TTS (43). Caroverine, an antagonist of two glutamate receptors (NMDA and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)), was applied onto the round window membrane of rodents, followed by steady state high level noise exposure for 1 h. Caroverine protected cochlear function against noise-induced hearing loss as measured by evoked potential hearing thresholds (44). In a comparison study, MK-801 and a more specific NMDA antagonist (selective NR1/2B NMDA receptor antagonist PD 174494) were compared to the NO synthase inhibitor L-N(omega)-Nitroarginine methyl ester (L-NAME) and the antioxidant N-acetylcysteine (NAC) in their effectiveness to reduce NIHL, cochlear HC loss and cochlear lipid peroxidation in a guinea pig model (45). MK-801 and NAC attenuated threshold shifts and hair cell loss effectively, while PD 174494 did so partially. L-NAME attenuated threshold shifts at 2 kHz but increased them at 20 kHz. Noise-induced elevation in the lipid peroxide 8-isoprostane in the cochlea was significantly attenuated by MK-801 and PD 174494 in the organ of Corti and modiolar core, by L-NAME in the lateral wall and modiolar core, and by NAC in all three regions. Overall, NAC was most effective in reducing hair cell and hearing loss in this model.

One difficulty precluding the use of a broad-spectrum glutamate antagonist to prevent NIHL may be the issue of side-effects that occur with agents such as MK-801 (46,47), and topical pretreatment of a drug to the round window

membrane would be clinically impractical. Because carbamathione is a partial NMDA receptor antagonist and is an active metabolite of the FDA-approved drug disulfiram, it might have more potential application clinically as an oral agent than other glutamate antagonists (1).

Magnesium has been studied in the context of preventing noise-induced threshold shifts, and magnesium ion supplementation is reported to ameliorate noise-induced hearing and hair cell loss associated with acoustic overexposure (48,49). Magnesium supplementation in Israeli basic trainees during basic training and weapons noise exposure was reported to reduce noise-related threshold shifts (48). Magnesium is thought to reduce glutamate excitotoxicity by countering and reducing the toxic calcium influxes induced by excessive glutamate release and may act to block the release of glutamate from presynaptic membranes (50). Additional studies suggest that magnesium supplementation may improve cochlear blood flow and oxygenation (51), prevent temporary but not permanent impulse noise-related hearing loss in rodents (52) and noise-induced TTS in humans (53).

Calcium homeostasis imbalance

Influx of calcium into the cytoplasm of OHCs is one of the effects of acoustic overstimulation in the cochlea. This calcium overload can have several consequences in the cochlea including stimulation of the mitochondria to produce more ROS, activation of cell death pathways including those involving calpains as well as the BAD (Bcl-2-associated death promoter) proapoptotic proteins in the Bcl-2 family of proteins (54–56). Experimentally, some success has been achieved by modulating the effects of noise-induced excessive calcium influx. OHC calcium influx is known to induce the expression of calcineurin, which in turn activates apoptotic pathways. The topical application of the calcineurin inhibitors FK506 and cyclosporin in guinea pigs prior to intense noise exposure was reported to significantly decrease OHC loss and reduce noise-induced threshold shifts (57). These data were confirmed in a subsequent study in guinea pigs and mice where these inhibitors were injected intraperitoneally prior to an intense steady state noise exposure (58). Yet another approach has been to attenuate hearing loss due to excessive noise through the use of calpain inhibitors, thus blocking the calpain-induced onset of programmed cell death. The infusion of the potent calpain inhibitor leupeptin into the cochlear scala tympani has been reported to decrease noise-induced hair cell loss in rodent models (59,60).

Oxidative stress

Micromechanical damage, ischemia reperfusion, and glutamate excitotoxicity all probably contribute to excessive oxidative stress in the cochlea with acute acoustic trauma (AAT). It is well documented that high level noise generates a variety of ROS, reactive nitrogen species (RNS), and lipid peroxides. Additionally, oxidant homeostasis is further deranged with the depletion of key intracellular antioxidants such as reduced GSH. Metabolic overdriving of the mitochondria by high-level noise in addition to mitochondrial injury caused by ongoing production of high levels of oxidants probably leads to increased levels of free radical species coupled with impaired energy production, leading to additional oxidative stress. Accordingly, one very successful approach in preventing and treating AAT to the cochlea has been restoring redox homeostasis. Investigations have included agents to prevent free radical production (allopurinol, deferoxamine), treatment with free radical scavengers (salicylate, mannitol, edaravone, phenyl-N-tert-butylnitrone (PBN)), up-regulating antioxidant enzyme activity (R-phenylisopropyladenosine (R-PIA)), treatment with extrinsic antioxidants (resveratrol, alpha-tocopherol, superoxide dismutase-polyethylene glycol (SOD-PEG), lipoic acid, ebselen, PBN, 4-hydroxy-PBN (4-OH-PBN)), enhancing GSH homeostasis with GSH prodrugs (NAC, GSH ester, 2-oxothiazolidine-4-carboxylate (OTC), D-methionine), and protecting and repairing mitochondria (acetyl-L-carnitine ALCAR, R-alpha-lipoic acid (LA)). While many of the above listed compounds have a primary mechanism of action, many of them have multiple mechanisms of action (e.g. free radical scavenging, GSH production, cell death inhibition, etc.).

Preventing free radical production. Allopurinol, a scavenger of some free radicals, can reduce free radical formation by inhibiting xanthine oxidase (XO) (61). Seidman et al. (62) reported that systemically injected allopurinol given before and after a prolonged steady state noise in rats partially preserved the CAP and cochlear microphonic thresholds damaged by noise. In another study using a guinea pig model with higher level steady state and impulse noise, allopurinol was found to reduce noise-induced TTS but not permanent threshold shifts (PTS) (63). Interestingly, in its role as an XO inhibitor allopurinol can actually generate damaging superoxide radicals (64). Deferoxamine mesylate (DFO) chelates iron in tissues and prevents free radical formation by inhibiting the Fenton reaction (the iron salt-dependent decomposition of dihydrogen peroxide, generating the highly reactive hydroxyl

radical, possibly via an oxoiron (IV) intermediate) (65). In one study of DFO along with other agents, pigmented female guinea pigs were exposed to noise (4 kHz octave band, 115dB SPL, 5 h). One hour before, immediately after, and 5 h after noise exposure, subjects received an injection of 5 ml saline/kg (control), or 100 mg DFO/kg (treatment) resulting in partially reduced hearing and OHC loss (66). Under some circumstances, however, DFO has been reported to display ototoxicity (67).

Free radical scavengers. Salicylate, mannitol, PBN and edaravone have all been shown to attenuate NIHL to some degree in animal models. Salicylate can act as an effective hydroxyl radical scavenger (68). Salicylate administered systemically in a guinea pig model of AAT showed limited effects on reducing PTS and hair cell loss (69). Mannitol, a hydroxyl scavenger and weak iron chelator given systemically before and after a high level steady state noise exposure in guinea pigs was shown to provide partial protection from AAT (66). Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a novel, potent, free radical scavenger currently used clinically to reduce ischemia reperfusion injury associated with stroke or myocardial infarction (70). It is given intravenously and appears to be relatively ineffective when given orally. Tanaka et al. (71) investigated the effects of edaravone against acoustic trauma in guinea pigs. Edaravone was infused into the right ear by an osmotic pump either before or up to 33 h after the 3-h exposure to 130dB noise, and less hearing loss, hair cell loss and lipid peroxidation were seen in the treated ear even up to 21 h post exposure.

PBN is a spin-trap agent that scavenges free radicals and has been reported to reduce the potentiation of NIHL caused by toxicants. In a rat model, investigators demonstrated that systemic administration of PBN reduced the potentiation of NIHL induced by co-exposure of the rats to low level carbon monoxide during the high level noise, although PBN did not significantly reduce the threshold shifts induced by noise alone (72). In a follow-on study of another potentiator of NIHL, PBN was found to reduce the additional hearing loss induced by the common industrial toxicant acrylonitrile (ACN). PBN, given to rats prior to ACN and noise, reduced noise-induced auditory threshold shifts significantly but did not reduce the hearing loss caused by the noise exposure itself (73). A related compound, 4-OH-PBN, was found to be effective in reducing AAT-induced PTS when administered 4 h after a high-level 6-h steady state noise in a dose dependent manner (Choi et al., in preparation). The compound 4-OH-PBN is the natural hepatic metabolite of PBN. It appears to reduce mitochondrial oxidative stress (74) as well as inhibit the activation of iNOS. This PBN derivative has shown promising results in reducing noise-induced permanent threshold shifts when given 4 h after noise exposure by itself or with NAC (Figure 1).

Up-regulation of antioxidant enzyme activity. Exposure to low level noise has been described as a method of preconditioning the cochlea to be more resistant to subsequent potentially damaging sound exposures (75,76). At least part of the sound conditioning protective effect may be due to the up-regulation of antioxidant enzyme activity in the cochlea. Jacono et al. (77) studied the effects of conditioning noise, high level noise and a combination of conditioning and high level noise on cochlear antioxidant enzyme activity. Each of the noise exposure conditions induced changes in the activity of several antioxidant enzymes. The data suggested that changes in glutathione reductase, gamma-glutamyl cysteine synthetase and catalase played a role in attenuating hearing loss associated with sound conditioning followed by high level noise and that hair cells in the organ of Corti were protected from noise-induced damage by increasing stria vascularis activity of catalase and enzymes involved in maintaining reduced glutathione. Accordingly, another

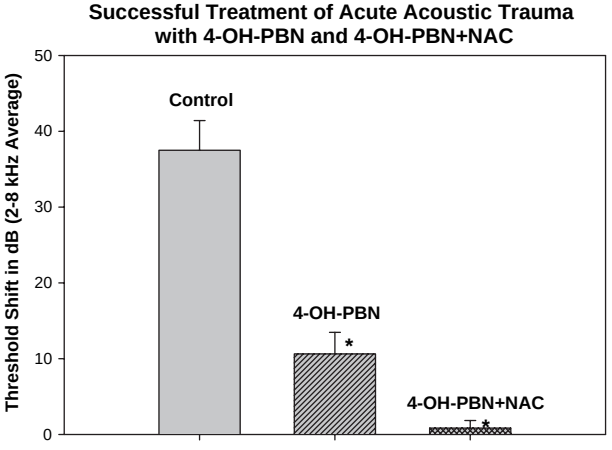

Figure 1. Effects of 4-OH-PBN (4-hydroxy–phenyl-N-tert-butylnitrone) alone and 4-OH-PBN plus NAC on noise-induced (6-h noise exposure to 4 kHz octave band noise at 105dB SPL) hearing loss. Each bar represents the average auditory brainstem response threshold shift of hearing levels 21 days post-noise compared to baseline pre-noise thresholds at 2,4,6 and 8 kHz. $n = 6$ chinchillas for each group. 4-OH-PBN, 50 mg/kg; NAC, 100 mg/kg. Drugs or carrier solution (control group) were given by intraperitoneal injection 4 h after noise exposure, and then every 12 h for an additional two days. Statistical analysis was one-way ANOVA with Scheffe post hoc analysis. Note: The two-drug combination nearly completely eliminated the permanent hearing loss ($*p < 0.05$).

experiment was designed utilizing the round window membrane application of a drug for the purpose of up-regulating cochlear antioxidant enzyme activity (78). In this study, R-PIA, a stable non-hydrolysable adenosine analogue which up-regulates antioxidant enzyme activity levels, was topically applied to the round window of the right ears of chinchillas. The animals were then exposed to a 4 kHz octave band noise at 105dB SPL for 4 h. Treated ears showed better recovery of ABR and distortion product otoacoustic emission (DPOAE) thresholds as well as less OHC loss. An additional study assessing the round window membrane pre-noise exposure application of glutathione monoethylester and R-PIA in combination demonstrated significant protection from both steady state and impulse noise-induced hearing loss (79).

Prevention with extrinsic antioxidants. Another effective strategy in preclinical studies has been delivery of a variety of extrinsic antioxidants either prior to, or shortly after, AAT with the goal of reducing PTS. Stedman's Medical Dictionary defines antioxidant as 'an agent that inhibits oxidation; any of numerous chemical substances, including certain natural body products and nutrients, that can neutralize the oxidant effect of free radicals and other substances'. Some of these antioxidants include SOD-PEG, resveratrol, alpha-tocopherol (vitamin E), ebselen, ascorbate (vitamin C), and the water-soluble vitamin E analog Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid).

Superoxide dismutase (SOD) is a constitutively-expressed antioxidant enzyme found in mammalian cochlear tissues that catalyzes the dismutation reaction, $2O_{2\cdot} - + 2H + \rightarrow H_2O_2 + O_2$ (80). Knockout of the gene for one of the SOD enzymes in mice increased their susceptibility to noise-induced PTS (81). Intraperitoneally-injected SOD was also found to be protective in reducing noise-induced TTS in guinea pigs (82). Delivery of an orally available, effective form of this compound may prove to be a therapeutic challenge.

Resveratrol (trans-3,4′,5-trihydroxystilbene), a naturally-occurring polyphenol (antioxidant compound) mainly found in grapes and red wine, has been reported to reduce noise-induced hearing loss when given to rodents. Chronic administration of this compound to rats by gavage partially attenuated noise-induced threshold shifts (83).

Hou et al. (84) reported the effects of vitamin E as prophylaxis for AAT. Pigmented guinea pigs were exposed to a noise (4 kHz octave band, 100dB SPL) 8 h/day for three consecutive days. Alpha-tocopherol (10 mg/kg or 50 mg/kg daily by intraperitoneal injection) was given three days before through three days after the noise exposure. At most frequencies and time-points tested, ABR threshold shifts of groups receiving alpha-tocopherol were significantly smaller than those of groups not receiving alpha-tocopherol.

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one), a glutathione peroxidase mimic, has also been reported to prevent AAT in several studies (85–87). Oral doses of the compound in the 4–10 mg per kg range in rats and guinea pigs have been shown to reduce both TTS and PTS in acoustic overexposure models. The mechanism of action in AAT is probably due to its antioxidant properties against ROS and RNS. In one study, guinea pigs received an oral dose of the vehicle or 10 mg/kg ebselen 1 h before exposure to 115dB SPL 4-kHz octaveband noise for 3 h. Ebselen eliminated an ABR- documented TTS and reduced swelling of the afferent dendrites beneath the inner hair cells evident immediately after noise (87). Ebselen also afforded protection from both single and repeated noise exposures (86). Ebselen is reported to be well tolerated with few side-effects and has been used in the past in stroke treatment clinical trials (88,89). Interestingly, a higher dose of ebselen did not confer additional protection from loud noise (85). One explanation for the dose response effect may be an interesting biological effect of ebselen. Ebselen has been shown to deplete cellular thiols under some conditions (90), so possibly higher doses of ebselen deplete GSH reserves and induce apoptosis, necrosis and hepatic and renal toxicities under certain conditions (90–92).

Vitamin C (ascorbate) is a water-soluble antioxidant effective against a variety of ROS, including superoxide and hydroxyl radicals. Humans and a few other mammals, such as guinea pigs, lack the terminal enzyme for vitamin C synthesis and must obtain it from dietary sources. In one study albino guinea pigs were raised for 35 days on a diet with normal, supplemented or deficient levels of ascorbate, then exposed to 4 kHz octave band noise at 114dB SPL for 6 h to induce PTS. There was less hearing loss and hair cell damage in animals on the high vitamin C diet compared to those with normal or reduced dietary vitamin C intake (93).

Another very interesting study reported that relatively high doses of salicylate and Trolox given either before or after AAT could attenuate hearing loss in a guinea pig model (94). In this study, the combination of compounds was given either before damaging noise exposure or up to several days afterwards. By extending the length of treatment to cover a delayed oxidative stress burst occurring 7 to 10 days after the acute noise exposure, investigators

were able to demonstrate some reduced PTS even when the compounds were given beginning three days after noise exposure.

Enhancing GSH homeostasis. One very successful approach toward preventing and treating hearing loss due to AAT in rodent models has been through the enhancement of GSH production by administration of pharmaceuticals that cells use in the synthesis of GSH. With AAT rapid depletion of a cell's key antioxidant, reduced glutathione (GSH), occurs (95). The critical role of GSH as the cell's primary antioxidant defense system against AAT-induced cochlear injury has been well demonstrated (96,97). In response to acoustic overexposure, cochlear GSH levels initially increase and then precipitously decline (98). GSH-related enzymes, such as gamma glutamyl cysteine synthase, glutathione reductase and glutathione peroxidase, are modulated by loud noise exposure (77,99). Induced GSH deficiency enhances acoustic injury of the cochlea (100) and replenishment of GSH with a glutathione prodrug such as NAC, methionine or an ester of GSH can reduce hearing loss from loud noise (1,79,96,101).

NAC and other precursors of GSH have been widely studied for the prevention and treatment of AAT in rodent models. Systemic administration of GSH ester to rats on a low protein diet reduced hearing loss induced by high level steady state noise (96). Chinchilla systemically administered a NAC-salicylate combination showed significantly attenuated PTS and OHC loss induced by 6-h, steady state 4 kHz octave band noise (102). In a subsequent study, Ohinata et al. compared NAC to other systemically administered agents in effectiveness of attenuation of hearing loss, hair cell loss and lipid peroxidation in guinea pigs exposed to high level steady state noise (45). They found that NAC effectively attenuated lipid peroxidation in the organ of Corti, modiolar core, and lateral wall of the cochlea, and reduced hair cell and hearing loss. Delivery of NAC by gavage was also effective in reducing PTS in steady state noise-exposed chinchilla (103).

Impulse (104), impact (105), or complex kurtotic noise (106) may damage the ear. The type of noise causing the injury may be a very important consideration. For example, intense impulse noise has been shown to cause very rapid onset of OHC apoptosis compared to steady state noise (107). Chinchilla were exposed to 150 pairs of simulated M-16 rifle shots rapidly over a 2-min time period (101). Untreated noise-exposed animals experienced a 40–50dB PTS and 80–100% loss of OHC, whereas animals treated before and after the noise with intraperitoneally-injected NAC demonstrated an approximately 30dB reduction in PTS and a 40–50% attenuation of OHC loss (Figure 2). A similar study of impulse noise in rats was undertaken (108). Different dosing paradigms were used for intraperitoneal injection of NAC. Using the optimal treatment paradigm there was almost complete attenuation of OHC loss as well as substantial reduction of PTS. In another study the dosing effect of NAC on the attenuation of impulse noise-induced PTS in chinchilla indicated that a dose of 325 mg/kg was most effective. However, significant attenuation was also noted with 50, and 100 mg/kg doses. NAC was administered by intraperitoneal injection twice daily for two days prior to noise, 1 h before noise, 1 h after noise, and twice daily for the next 48 h (109). The effectiveness of intraperitoneally-injected NAC to reduce threshold shifts induced by kurtotic noise in chinchilla was studied. Three weeks after noise exposure a significant reduction in PTS was noted (103).

In summary, intraperitoneal and orally administered NAC at a variety of doses produced significant reductions in hair cell and hearing loss when administered 48 h before and after acute damaging noise exposures. NAC has also been shown to decrease permanent AAT-induced threshold shifts when given shortly after an acute noise exposure (110). The effectiveness of NAC was tested for three different types of noise, in several species, and in a number of different laboratories, suggesting on the basis of preclinical data that NAC might be effective in human clinical trials to prevent AAT. Other GSH prodrugs have also been reported to decrease hearing loss secondary to AAT. D-methionine was found to be effective in reducing permanent hearing and hair cell loss in chinchilla when administered intraperitoneally before and after a 6-h noise exposure (1). Another GSH prodrug, OTC, was shown to partially prevent permanent hearing loss in guinea pigs when administered systemically by daily injection during a five day steady state noise exposure (111).

Enhancing mitochondrial protection and repair

Another effective experimental approach has been to administer therapeutic molecules that protect or enhance the repair of mitochondria. It has been shown that inhibiting mitochondrial repair can enhance the damage associated with acoustic overexposure (112). Damage to mitochondria or mitochondrial DNA mutations has been associated with a variety of hearing loss disorders including acoustic trauma-caused hearing loss (1,110,113). Acetyl-L-carnitine (ALCAR) is one agent that has been tested in rodent models of AAT. ALCAR improves mitochondrial energy production and can

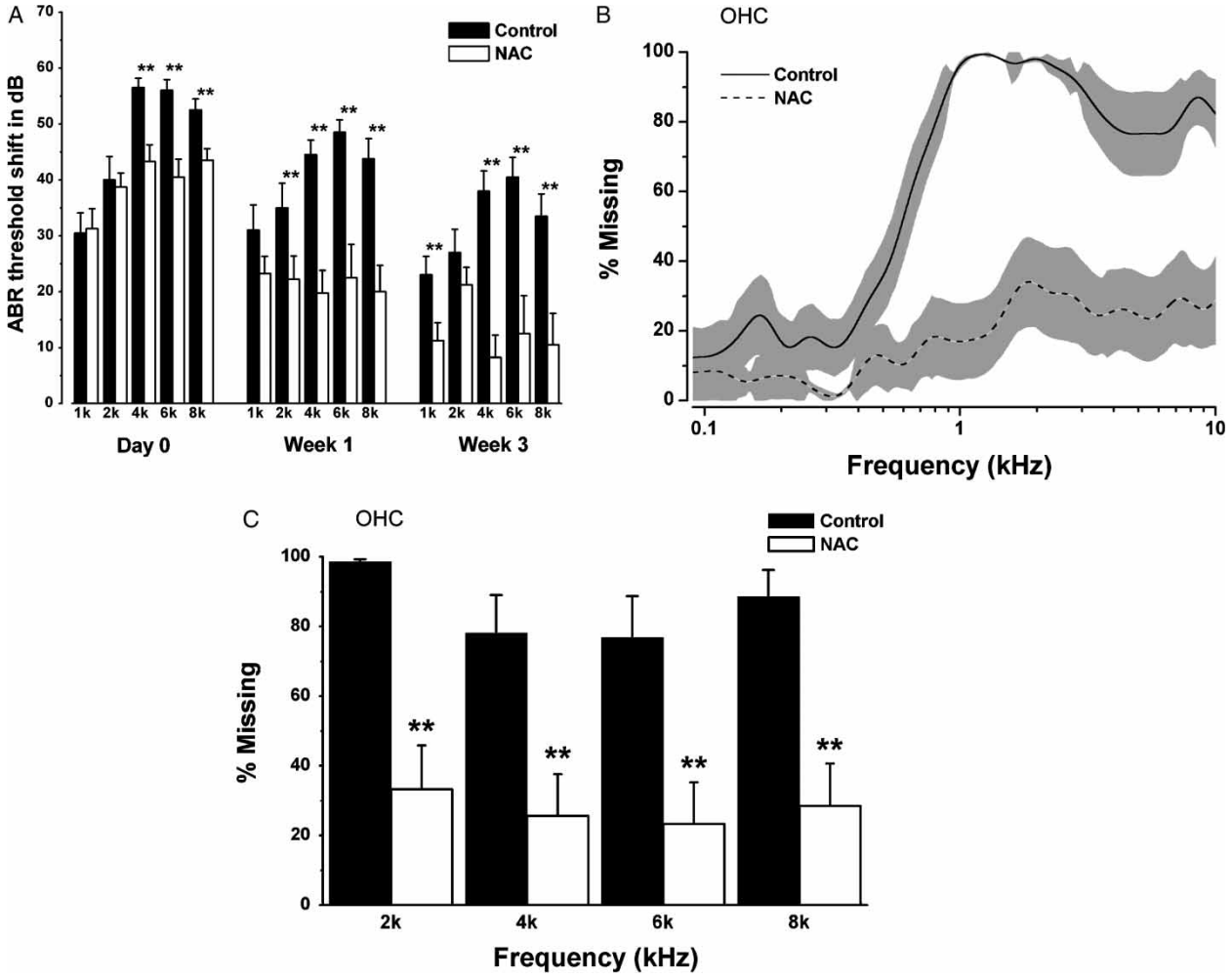

Figure 2. Effects of NAC (N-acetylcysteine) on impulse noise-induced (155dB peak SPL for 150 repetitions) hearing loss and cochlear hair cell survival. A: Comparisons of auditory brainstem response (ABR) threshold shift between the NAC-treated and the control groups ($n = 12$ ears for both groups), at three time-points: immediately after impulse noise exposure (day 0), 1 week, and 3 weeks. Error bars represent the SE of the means. $^{**}p < 0.01$ for LSD post hoc comparisons made between control and treated ears at each frequency and time point. NAC (325 mg/kg) or carrier (control) given by intraperitoneal injection twice a day (b.i.d.) for 48 h and 1 h prior to and 1 h after noise exposure and then b.i.d. for the following two days. Significant differences were found between the control and treated groups at one and three weeks for most frequencies tested. There were progressive threshold shift decreases in the treated group. B: Comparison of cochlear outer hair cell counts between the NAC-treated and the control groups ($n = 12$ ears for both groups). Lines represent the mean missing hair cell percentage as a function of frequency and the shaded areas represent the SE of the mean. C: The mean percentages of hair cells missing in cochlear regions corresponding to frequencies ranging from 2 to 8 kHz. The data are derived from panel B. $^{*}p < 0.05$, $^{**}p < 0.01$ for LSD post hoc comparisons made between control and NAC-treated animals at each frequency. NAC (325 mg/kg) or carrier (control) given by intraperitoneal injection twice a day (b.i.d.) for 48 h and 1 h prior to and 1 h after noise exposure and then b.i.d. for the following two days. (Figure courtesy of Acta Otolaryngologica.).

restore cardiolipin and carnitine levels that are critically depleted in oxidatively-stressed tissues (114). Furthermore, ALCAR can enhance the activity of some mitochondrial respiratory enzymes, enhance mitochondrial DNA transcription, restore mitochondrial metabolite transport, and protect mitochondrial membrane integrity (115). Systemic administration of ALCAR has been shown to dramatically reduce the permanent hearing loss induced both by acute steady state (1) and impulse (101) noise in chinchilla when given before and after or even shortly after noise exposure (110) (Figure 3). Another mitochondrial metabolite, alpha lipoic acid, was also reported to attenuate noise-induced PTS in rodents as well as to reduce evidence of oxidative stress in cochlear tissues (116). Lipoic acid administration has been shown to slow age-related neuronal mitochondrial decay and improve neurologic function in rats (117).

Cell death inhibition

Evidence is accumulating that the oxidative stress associated with AAT often leads to programmed cell

death. Accordingly, a variety of cell death inhibitors have been administered systemically or topically to the round window membrane and reported to attenuate AAT-induced deafness. Although GSH and a variety of antioxidants may decrease cell death through antioxidant effects, specific cell death inhibitors have been applied in models of AAT. There is growing evidence implicating the activation of cell death pathways involving mitogen-activated protein kinases (MAPKs) and the c-Jun N-terminal kinases (JNKs). For example, the systemic administration of a small molecule kinase inhibitor derived from an indolocarbazole compound known as CEP-1347 blocks JNK cell death pathway activation and partially attenuated noise-induced hearing loss in rodents (118). Another strategy has been the topical application to the round window membrane of the cell death inhibitor D-JNK-1 (119). Local delivery of D-JNKI-1 prevented acoustic trauma-induced permanent hearing loss in a dose-dependent manner in rodents (119). Caspases, a family of cysteine proteases, are present in an inactive pro-caspase form. When caspases are activated either through an extrinsic injury-mediated cell death receptor or an intrinsic mitochondrial pathway, programmed cell death is initiated (120). Activations of caspases have been reported in the cochlea after AAT (121,122). Theoretically, specific broad spectrum

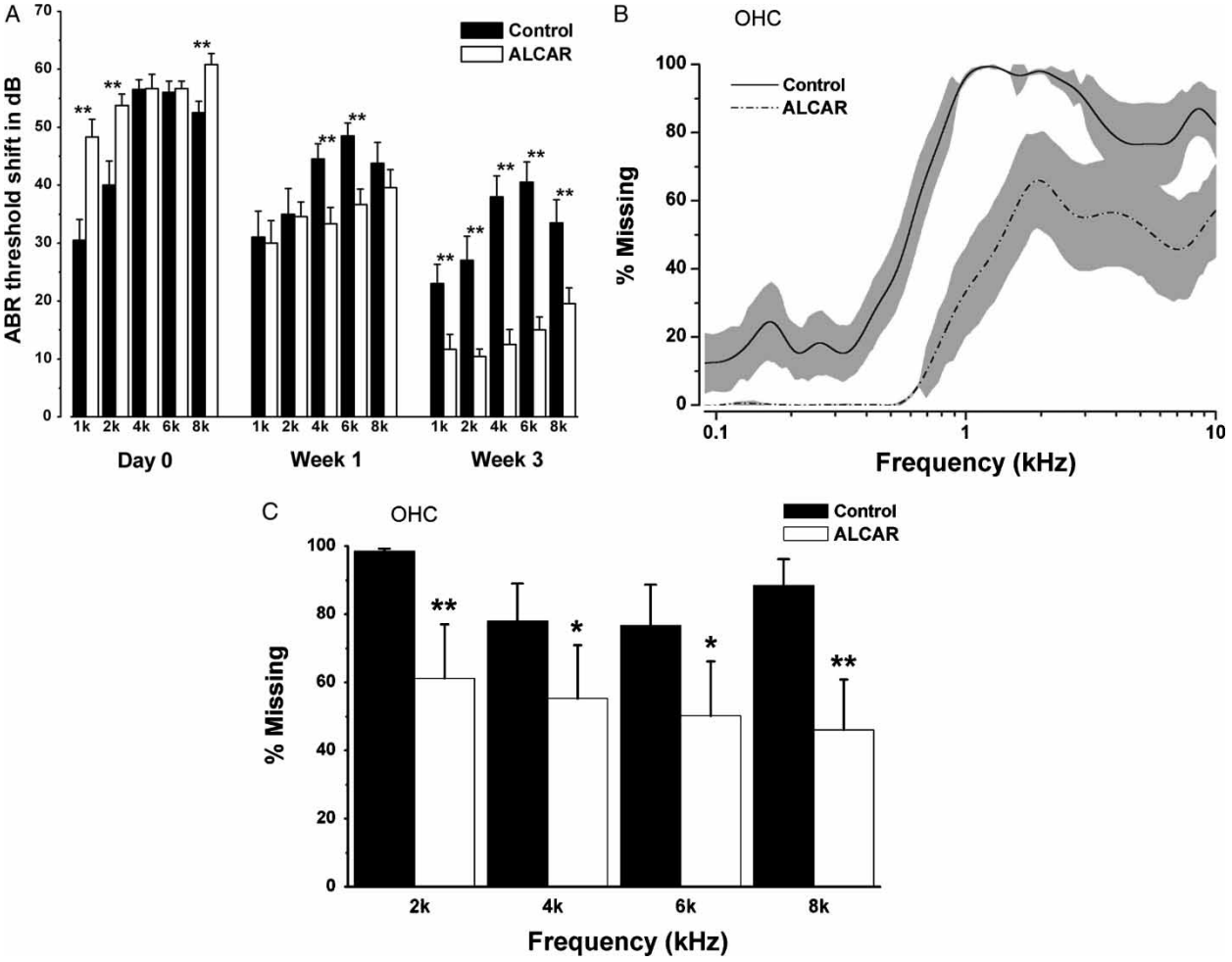

Figure 3. Effects of ALCAR (acetyl-L-carnitine) on impulse noise-induced (155dB peak SPL for 150 repetitions) hearing loss and cochlear hair cell survival. A: Comparisons of ABR threshold shift between the ALCAR-treated and the control groups ($n = 12$ ears for both groups), at three time points: immediately after impulse noise exposure (day 0), 1 week, and 3 weeks. Error bars represent the SE of the means. $^{**}p < 0.01$ for LSD post hoc comparisons made between control and treated ears at each frequency and time-point. Significant differences were found between the control and treated groups at three weeks for all frequencies tested. There were progressive threshold shift decreases in the treated group. B: Comparison of cochlear outer hair cell counts between the ALCAR-treated and the control groups ($n = 12$ ears for both groups). Lines represent the mean missing hair cell percentage as a function of frequency and the shaded areas represent the SE of the mean. C: The mean percentages of hair cells missing in cochlear regions corresponding to frequencies ranging from 2 to 8 kHz. The data are derived from panel B. $^{*}p < 0.05$, $^{**}p < 0.01$ for LSD post hoc comparisons made between control and ALCAR-treated animals at each frequency. ALCAR (100 mg/kg) or carrier (control) given by intraperitoneal injection twice a day (b.i.d.) for 48 h and 1 h prior to and 1 h after noise exposure and then b.i.d. for the following two days. (Figure courtesy of Acta Otolaryngologica)

caspase inhibitors might also prevent noise-induced cochlear programmed cell death in sensory cells (120). Finally, Hu et al. (107) reported attenuation of AAT-induced cochlear injury with an inhibitor of Src protein tyrosine kinase cell death cascade. Their studies revealed that Src inhibition can reduce hair cell loss and hearing loss resulting from exposure to both continuous and impulse noise. Importantly, both local and systemic applications of the drug were effective. Riluzole (2-amino-6-trifluoromethoxy benzothiazole), a neuroprotective agent that prevents apoptosis- and necrosis-induced cell death, when perfused into the cochlea via an osmotic mini-pump prevented hair cell degeneration and provided a dose dependent reduction of permanent hearing loss (123).

Growth factors

Several neurotrophic factors have been tested for their ability to reduce noise-induced PTS when infused locally into the cochlea. These include glial-derived neurotrophic factor (GDNF), neurotrophic factor-3 (NT-3), and brain-derived neurotrophic factor (BDNF). These growth factors may reduce apoptosis of injured hair cells and neurons and may also enhance intrinsic cochlear antioxidant defenses. Local application of GDNF to one ear reduced hearing and hair cell loss in that ear before and 2 h after acoustic trauma, but not 4 or 6 h after, indicating that topical GDNF treatment prevents cochlear sensory cell damage and hearing loss if present during or shortly after acoustic trauma (124). In another study, intracochlear perfusion of BDNF was compared to NT-3 as a preventative strategy against AAT cochlear injury. NT-3 was effective but BDNF was not in this rodent model (125).

Treatment

Pharmacological strategies for AAT could be applied in two different paradigms. Medicines could be given before, during, and shortly after the anticipated noise exposure (prevention) or given shortly after an acute noise exposure when a threshold shift has occurred (treatment). Data suggest that there may be a therapeutic window after an acute noise exposure when injured cochlear tissues could be treated to prevent apoptosis (1). After an acute damaging noise exposure, it may take a number of days before maximal hair cell loss has occurred. Additionally, a second or delayed burst of oxidative stress has been reported in rodent models 7–10 days after the acute noise exposure (94). Not surprisingly, some success has been reported when pharmacological treatment is given shortly after an acute noise injury. NAC, ALCAR, 4-OH- PBN and combinations of those antioxidants have been shown to attenuate noise-induced PTS in rodent models (110) (Figure 1). Treatment with a combination of Trolox and salicylate was reported to reduce PTS even when administered five days after the noise exposure if the drugs were given continuously through the delayed secondary burst of oxidative stress (94). As previously mentioned, edaravone infused into cochlear perilymph of rodents reduced cochlear damage and hearing loss even if given up to 21 h post exposure (71). It may be that with a moderate to severe acoustic injury the topical application of a treatment agent to the round window membrane might be a reasonable strategy if it were shown to be more effective than an oral agent given at the same time. Intraperitoneal injection of riluzole rescued the cochlea within a therapeutic window of 24 h after acoustic trauma but less efficiently than intracochlear perfusion (123).

Clinical considerations

The ideal pharmacological agent would specifically address known mechanisms of acoustic injury, be administered orally, be exceptionally safe, be effective and be affordable. In certain situations a prophylactic approach might be preferable where pharmacological protection is used in conjunction with hearing protection when noise exposures are particularly loud or prolonged. With acoustic injuries due to accidents, post-treatment of the injury would also be oral but topical application might be a useful route under some circumstances. Several agents have now been tested in clinical trials. These include magnesium supplementation, the calcium channel blocker diltiazem, dextran/pentoxifylline, and N-acetylcysteine (NAC).

Magnesium supplementation has been reported to be effective and safe as an agent to prevent NIHL in clinical trials (48,49). Attias et al. (48) examined the efficacy of supplemental magnesium in reducing noise-induced PTS in a military setting. Subjects wore hearing protection as they were undergoing weapons training and also received either 167 mg of magnesium aspartate or placebo in a liquid drink in a double-blind fashion for eight weeks to augment the protection against rifle fire noise afforded by earplugs. The authors reported that hearing threshold shifts were significantly more frequent and more severe in the placebo group compared to the magnesium group. However, PTS was defined as a threshold greater than 25 rather than a change from baseline, and this may have affected the data. The supplement was well

tolerated. Other investigators reported no correlation between serum magnesium levels and several parameters of measured NIHL in humans (126).

The perioperative efficacy of a calcium channel blocker (diltiazem) was tested in a prospective, randomized, double-blinded study involving 100 subjects to assess its ability to prevent acoustic trauma from otologic drill noise during otologic surgery. Neither the experimental nor control groups showed much postoperative hearing loss. There was a tendency for better results in the therapy group, but this was not statistically significant (127).

In another study, dextran-40 and/or pentoxifylline were studied in a randomized, double-blind, placebo-controlled study for AAT as well as sudden hearing loss. It was concluded in this particular study that there were, in fact, no clinically relevant differences in hearing gains for sudden hearing loss or acute acoustic trauma when treatment groups were compared to placebo treatments (128).

To date, several preliminary clinical trials have either been completed or initiated looking at the safety and efficacy of NAC in reducing noise-induced auditory changes. The first study by Kramer et al. (129) was a randomized, double-blind, placebo-controlled design involving voluntary discothèque attendees. This preliminary study was confounded by the small number of subjects, variability in noise exposure levels for each group, and measures that evaluated only temporary shifts in thresholds. Properly consented subjects ($n = 31$) were assessed for baseline pure tone audiometry and distortion product otoacoustic emissions (DPOAEs) and were randomized to receive either oral NAC (900 mg single dose as an effervescent tablet dissolved in tap water) or placebo (an effervescent tablet of identical taste and odor to the NAC agent) 1 h prior to attending a local discothèque for 2 h. Upon exiting the discothèque, audiometric measures were again performed in a sound-treated van. Side-effect questionnaires and tinnitus questionnaires were also administered. There were no reported side-effects from the NAC ingestion. There were no significant differences in TTS or temporary noise-induced DPOAE amplitude shifts or delays or tinnitus measures. Because the majority of reported animal data do not document a large beneficial effect of NAC in reducing temporary pure tone threshold shifts, the outcome of the study was not completely unexpected (45,101,102,108,109). These data are consistent with the hypothesis that the cochlear mechanisms of noise-induced TTS and PTS differ considerably. Another study evaluated oral NAC versus placebo in a prospective, randomized, double-blinded, placebo-controlled study in terms of safety and efficacy in reducing auditory threshold shifts, changes in DPOAEs, and tinnitus in 566 military subjects undergoing routine weapons training. Subjects underwent two weeks of required routine weapons training with M-16 rifles in which all subjects were issued and wore in-the-ear insert hearing protection devices. Subjects were randomized to receive either 900 mg of NAC or placebo three times a day with each meal. Data from the completed study are still being analyzed, but preliminary results show that the side-effect profile for NAC was no different than placebo. In addition, there appeared in the initial analysis of data to be a significant reduction in pure tone threshold shift rates in the ear exposed to the most intense rifle noise for the NAC group compared to the placebo group. While the findings of this study must be considered preliminary, these results are encouraging and a dose ranging study is being initiated (Kopke et al., in preparation).

Clinical studies of additional agents are expected. Much is yet to be learned regarding safety, as well as dose, route, and timing of administration, in addition to pharmacokinetics and efficacy. Studies such as these for acute acoustic trauma are challenging since a population at risk must be identified among whom hearing protection is already being used, numerous variables must be controlled for, and for accidental acoustic injuries quick identification of subjects followed by timely informed consent and treatment can be quite difficult.

Summary

Sensorineural hearing loss due to AAT and other types of noise exposure occupationally or recreationally continues to be an important worldwide problem. Hearing conservation programs using engineering solutions for noise reduction and individual HPDs are becoming increasingly emphasized. Nevertheless, these approaches have inherent limitations so that researchers are exploring potential pharmacological approaches toward making the cochlea more resistant to, or enhancing the recovery from, noise damage. A great deal of research effort over the past decade has uncovered many cellular and molecular mechanisms associated with cochlear injury and hearing loss due to noise. Many compounds delivered to the cochlea by a variety of routes have shown effects in reducing cochlear hair cell loss and hearing loss in experimental studies. Some of these compounds have shown robust effects in rodents and are associated with few side-effects in humans. Other new chemical entities need further study regarding animal and human safety. A few clinical studies have begun to emerge. These studies are quite difficult to perform but must be performed

to establish the safety and efficacy of this pharmacological approach. It is very possible that, in the future, pharmacological approaches will have a place as an adjunct in hearing conservation programs.

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