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Research paper

NAC for noise: From the bench top to the clinic [☆]

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Abstract

Noise-induced hearing loss (NIHL) is an important etiology of deafness worldwide. Hearing conservation programs are in place and have reduced the prevalence of NIHL, but this disorder is still far too common. Occupational and recreational pursuits expose people to loud noise and ten million persons in the US have some degree of noise-induced hearing impairment. It is estimated that 50 million in the US and 600 million people worldwide are exposed to noise hazards occupationally. Noise deafness is still an important and frequent cause of battlefield injury in the US military. A mainstay of hearing conservation programs is personal mechanical hearing protection devices which are helpful but have inherent limitations. Research has shown that oxidative stress plays an important role in noise-induced cochlear injury resulting in the discovery that a number of antioxidant and cell death inhibiting compounds can ameliorate deafness associated with acoustic trauma. This article reviews one such compound, *N*-acetylcysteine (NAC), in terms of its efficacy in reducing hearing loss in a variety of animal models of acute acoustic trauma and hypothesizes what its therapeutic mechanisms of action might be based on the known actions of NAC. Early clinical trials with NAC are mentioned.

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Keywords: NAC; NIHL; Acute acoustic trauma; Hearing loss; Treatment

1. Introduction

The most common potentially preventable form of sensorineural hearing impairment is noise-induced hearing loss (NIHL) from acute and/or chronic acoustic overexposure.

Thirty to forty million workers in the US are at risk for NIHL due to noise exposure on the job, and NIHL contributes to the hearing loss of an estimated ten million Americans (Franks et al., 1996). It is estimated that 16% of disabling hearing loss in adults worldwide is due to occupational noise

Abbreviations: NIHL, noise-induced hearing loss; HPDs, hearing protection devices; NAC, *N*-acetylcysteine; AAT, acute acoustic trauma; FDA, U.S. Food and Drug Administration; ROS, reactive oxygen species; RNS, reactive nitrogen species; GSH, glutathione; NO, nitric oxide; 4-HNE, 4-hydroxynonenal; PCD, programmed cell death; CDPs, programmed cell death pathways; MAPK/JNK, mitogen-activated protein kinase/(C-Jun kinase); Src PTK, Src protein tyrosine kinase; ALCAR, acetyl-L-carnitine; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; L-NAME, *N*-nitro-L-arginine methyl ester; DPOAEs, distortion product otoacoustic emissions

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(Nelson et al., 2005). The military is one of the world's most noise hazardous occupations, and NIHL in the military continues to be an acute and costly problem despite hearing conservation programs emphasizing personal hearing protection devices (HPDs) (Bohner et al., 2002; Wolgemuth et al., 1995). Hearing damage from acoustic trauma accounts for up to 47% of all wounded in action evacuations from Operation Iraqi Freedom and Operation Enduring Freedom and is the fourth leading reason for medical referral for combatants returning from deployment. These injuries result in costly VA disability expenditures that are rising each year (Kopke, 2005). However, a variety of other occupations are also associated with significant acoustic overexposure including, manufacturing (Landen et al., 2004), transportation (Beckett et al., 2000; Landen et al., 2004; Landon et al., 2005), construction (Neitzel and Seixas, 2005; Seixas et al., 2005), mining (Landen et al., 2004), and others.

Hearing protection devices are an essential and partially effective part of any hearing conservation program but, due to their limitations, NIHL can still occur. These limitations include: (a) noise levels can exceed the protective capability of the device; (b) injurious acoustic energy can directly be transmitted through the skull bypassing the protective device to damage the cochlea; (c) HPD attenuation is frequency dependent; (d) their protective capability is reliant on precise fitting of the device, which under some conditions cannot always be maintained; (e) often the overriding need to communicate precludes wearing an HPD (Kopke et al., 2002). For example, long haul truck drivers are exposed to constant high level noise for many hours a week yet cannot wear HPD because of the need to hear critical environmental cues, and finally (f) damaging noise cannot always be anticipated, and short unexpected high intensity exposures can too frequently cause permanent damage (Price, 2005). 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 (Lim et al., 1971; Lim and Dunn, 1979; Slepecky, 1986). Since the discovery that noise-induced metabolic oxidative stress plays a significant role in acoustically-generated cochlear injury (Yamane et al., 1995), research has defined a variety of potential therapeutics effective in reducing the permanent hearing loss associated with acoustic overexposure in animal models (Kopke et al., 1999; Lynch and Kil, 2005). 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 pharmacologic intervention.

The ideal pharmacologic agent would specifically address known mechanisms of acoustic injury, be orally administered, be exceptionally safe, be effective and affordable. This review looks at the accumulated data

on one such agent, *N*-acetylcysteine (NAC), currently in clinical trials as an agent to prevent or treat acute acoustic trauma (AAT). NAC has been in clinical use with U.S. Food and Drug Administration (FDA) approval for several decades as an antidote to acetaminophen overdose and as a mucolytic agent (Miller and Rumack, 1983). At the present time, it is not FDA approved for any uses related to hearing loss.

NAC functions as an antioxidant and as a substrate for glutathione synthesis and has a number of other bioeffects as well (Zafarullah et al., 2003). A number of laboratories have demonstrated in various species and models of noise damage to hearing that NAC can effectively attenuate permanent hearing loss due to AAT. Thus NAC is postulated to effectively reduce NIHL by addressing many of the recently-discovered cellular and molecular mechanisms thought to be responsible for cochlear damage due to loud noise.

2. Cellular and molecular mechanisms associated with acoustic injury to the cochlea

Over the past decade it has become clear that oxidative stress induced by acoustic overexposure in excess of the cochlea's intrinsic antioxidant stress defenses leads to cell injury, sensory cell death, and permanent hearing loss (Henderson et al., 2006). While acoustic overexposure can certainly be excessive enough to mechanically disrupt the cochlea resulting in a devastating and immediate unrecoverable injury, the clinical scenario is usually much less dramatic. Rather than macro-mechanical cochlear damage there frequently appears to be an oxidative metabolic stress resulting in cell injury and sensory cell death (Henderson et al., 1995; Lim et al., 1971; Lim and Dunn, 1979; Slepecky, 1986). Hence much acoustic injury may be partially recoverable, and permanent damage can be either prevented or treated with pharmacological agents aimed at ameliorating oxidative stress or inhibiting cell death responses.

The generators of this oxidative stress likely include acoustically-induced ischemia reperfusion, glutamate excitotoxicity, and an increase in mitochondrial free radical production due to higher metabolic demand as well as through less efficient energy production because of noise-induced injury to the mitochondria (Lamm and Arnold, 2000; Poderoso et al., 2000; Puel et al., 1998; Yamane et al., 1995).

Reactive oxygen species (ROS), reactive nitrogen species (RNS), and lipid peroxides are produced by the action of these oxidative stress generators (Ohinata et al., 2000b; Ohlemiller et al., 1999; Yamane et al., 1995; Yamashita et al., 2004). When the production of these toxins exceeds the intrinsic defense mechanisms of the cochlea, damage occurs to lipid membranes, proteins, and nuclear and mitochondrial DNA. Also, a rapid depletion of the cell's key antioxidant, reduced glutathione (GSH), occurs (Evans and Halliwell, 1999). A variety of antioxidant enzyme activities in the cochlea are modulated in response to the

stress (Jacono et al., 1998). Stress-induced kinase and other cell death pathways are activated resulting in the permanent loss of inner and outer hair cells and auditory neurons (Hu et al., 2002; Hu et al., 2000; Nicotera et al., 2003; Ogita et al., 2000; Shizuki et al., 2002; Wang et al., 2003; Ylikoski et al., 2002). Damage to supporting cells (Henderson et al., 1995) and elements of the stria vascularis also occurs (Hirose and Liberman, 2003; Wang et al., 2002).

A variety of toxic compounds may be produced in the cochlea in association with acoustic trauma. These compounds include the hydroxyl radical (Ohlemiller et al., 1999), the superoxide anion (Yamane et al., 1995), hydrogen peroxide, and related nitric oxide (NO)-based radicals, especially highly toxic peroxynitrite (Diao et al., 2003; Shi and Nuttall, 2003; Yamasoba et al., 2005). ROS and RNS interact with cellular membranes through β -peroxidation and produce lipid peroxides including malendialdehyde, 4-hydroxynonenal (4-HNE) (Yamashita et al., 2004) and isoprostanes (Ohinata et al., 2003). High levels of malendialdehyde (Zhuravskii et al., 2004) and isoprostanes have been detected in cochlear tissues after excessive noise exposure (Ohinata et al., 2000b). Isoprostanes are associated with an increase in tissue toxicity including vasoconstriction leading to ischemia (Ohinata et al., 2000b), and 4-HNE is a potent inducer of programmed cell death (PCD) (Huang et al., 2000).

The critical role of GSH as the cell's primary antioxidant defense system against AAT-induced cochlear injury has been well demonstrated (Ohinata et al., 2000a; Yamasoba et al., 1998b). Induced GSH deficiency enhances acoustic injury of the cochlea (Henderson et al., 1999) and replenishment of GSH with a glutathione prodrug such as NAC, D-methionine or an ester of GSH can reduce hearing loss from loud noise (Hight et al., 2003; Kopke et al., 2005; Kopke et al., 2002; Ohinata et al., 2000a). In response to acoustic overexposure cochlear GSH levels initially increase and then precipitously decline (Campbell et al., 2003). GSH-related enzymes, such as gamma glutamyl cysteine synthase, glutathione reductase and glutathione peroxidase, are modulated by loud noise exposure (Jacono et al., 1998; Ohlemiller et al., 2000).

3. How NAC may address many of the mechanisms associated with cochlear injury from AAT

As previously described, AAT appears to damage the cochlea through the generation of ROS, RNS, lipid peroxides and depletion of cochlear GSH and other cellular antioxidants. Without intrinsic antioxidant protection mitochondrial injury occurs and DNA and proteins are oxidatively damaged. A variety of programmed cell death pathways (CDPs) can be activated or necrosis can also be initiated (Hu et al., 2002; Hu et al., 2000; Yang et al., 2004). CDPs reported to be activated by acoustic trauma include pathways involving mitogen-activated protein (MAPK) kinase/(C-Jun kinase) JNK (Pirvola et al., 2000) and the intrinsic mitochondrial cell death pathway, activa-

tion of proteases known as caspases (caspases 3, 8, and 9) perhaps through an extrinsic pathway (Nicotera et al., 2003), and the Src protein tyrosine kinase (Src PTK) signaling cascade (Harris et al., 2005). As a GSH prodrug and antioxidant, NAC may ameliorate the cochlear damage through a variety of mechanisms, such as providing a substrate for cochlear GSH synthesis, free radical scavenging, and inhibition of CDP pathway activation and necrosis. GSH depletion also adds substrate for the GSH peroxidase enzyme and other GSH-related enzymes. NAC-induced GSH synthesis may also be able to ameliorate the effects of glutamate excitotoxicity as well as protect cochlear mitochondria from injury.

NAC has been recognized as a free radical scavenger for some time. NAC has been shown to be an effective scavenger of hydroxyl radical, hydrogen peroxide, and hypochlorous acid (Aruoma et al., 1989). By enhancing GSH production in cells, there may also be less production of NO, and stress-induced inducible nitric oxide synthase (iNOS) activation can be inhibited effectively reducing the production of harmful RNS (Erbas et al., 2004; Lee et al., 2004).

NAC is an effective replenisher of the key intrinsic antioxidant GSH as demonstrated in its clinical use as an effective antidote for acetaminophen overdose for many decades. Acetaminophen intoxication leads to depletion of hepatocyte GSH, unchecked oxidative stress and liver cell death. NAC given orally can rapidly replenish liver GSH and prevent hepatic necrosis. Oral NAC is metabolized to cysteine in the gut. Cysteine is oxidized to cystine which allows its transport into cells to serve as an essential precursor for GSH synthesis. Thus, the bioavailability of cysteine after oral administration of NAC is high, and the level of cysteine in plasma and tissues is more important than plasma NAC levels with regard to protective effects from oxidative stress (Cotgreave, 1997; Cotgreave et al., 1987). For example, oral NAC in humans raised plasma cysteine levels and reduced muscle fatigue and GSH oxidation (Matuszczak et al., 2005). Portal vein cysteine levels were measured to be five times higher than portal vein NAC levels 30 min after administration (Cotgreave et al., 1987). Importantly, while both NAC and cysteine were found to reduce the effect of paraquat-induced ROS, cysteine was found to be one thousand fold more active than NAC in its protective effects (Hong et al., 2005). However, NAC is preferred over cysteine as a GSH precursor or amino acid source due to differences in gut and splanchnic metabolism that make NAC much safer than the administration of oral cysteine. (Baker, 2006) Reduced toxicity of oral *N*-acetyl-cysteine (NAC) as compared to oral cysteine has been suggested to be due to slower gut absorption of NAC compared to cysteine, slow deacetylation of NAC in the gut, along with GSH synthesis from NAC in the gut all of which would result in lower cysteine concentrations in tissues. (Baker, 2006) Also, according to I. A. Cotgreave, NAC has a very different redox potential than cysteine and thus it is oxidized more slowly

than cysteine in the gut, further reducing toxicity. (personal communication, October 12, 2006).

Additionally, NAC has been shown to be a mitochondrial protectant. NAC administration was found to protect age related stress-induced mitochondrial injury in a rat model (Cocco et al., 2005; Grattagliano et al., 2004) as well as in a different stress-induced model of mitochondrial injury in mice (Quadrilatero and Hoffman-Goetz, 2004). This may be an important mechanism in the protective action of NAC in cases of AAT as it has been shown that impairing mitochondrial biogenesis enhances acoustic injury to the cochlea (Hyde and Rubel, 1995). Acoustic overexposure can also lead to the release of mitochondrial cytochrome *C* leading to activation of cell death activating caspases in cochlear hair cells (Hu et al., 2002; Hu et al., 2000). Furthermore, the important role of mitochondria injury in AAT was demonstrated by the use of another mitochondrial biogenesis molecule, acetyl-L-carnitine (ALCAR), which effectively reduced cochlear damage and hearing loss associated with both steady state and impulse noise (Kopke et al., 2005; Kopke et al., 2002).

Glutamate excitotoxicity has long been known to play a role in cochlear injury (Pujol et al., 1993; Ruel et al., 2005). The primary afferent dendrites are particularly vulnerable to injury. However, outer hair cells may also be vulnerable due to high levels of glutamate inhibiting the glutamate-cystine antiporter of the outer hair cells leading to GSH depletion (Sunami et al., 1999). Recently it has been shown that NAC can reduce glutamate toxicity in a cultured PC12 neuronal model (Penugonda et al., 2005) and in cultured hippocampal cells (Himi et al., 2003).

A variety of lipid peroxidation products can be detected in cochlear tissues after AAT including MDA, 4-HNE (Yamashita et al., 2004) and isoprostanes. By enhancing GSH production, NAC effectively reduced 4-HNE toxicity in a neuroblastoma cell line as well as rat brain (Neely et al., 2000). Importantly, systemically administered NAC effectively reduced cochlear levels of 8-isoprostane leading to reduced noise-induced hearing and hair cell loss in a guinea pig model of acute acoustic trauma (Ohinata et al., 2003).

The consequences of excessive noise-induced stress that cannot be adequately ameliorated with intrinsic antioxidant defense systems are programmed cell death and, to a lesser degree, necrosis of hair cells and auditory neurons. As previously mentioned in this article, the stress kinase system (MAPK/JNK) may be activated with mitochondrial release of cytochrome *C* leading to the formation of apoptosomes and the activation of caspases. After AAT, release of cytochrome *C* from mitochondria, activation of caspases 3, 8, and 9 and apoptosis of cochlear hair cells have been reported (Hu et al., 2000; Hu et al., 2002; Nicotera et al., 2004; Nicotera et al., 2003). NAC has been shown to inhibit JNK, p38 MAP kinase, and NF kappa beta activation (De Flora et al., 2001; Hashimoto et al., 2001). Additionally, NAC blocked pathways of apoptosis in organ of Corti induced by cisplatin (inducer of cochlear

oxidative stress), and also reduced BAX, poly-ARP-ribose-polymerase (PARP) cleavage, and caspase 3 and 9 activation (Dickey et al., 2005; Wu et al., 2005). NAC has also been shown to inhibit JNK activation in cultured smooth muscle and a mouse model of neurodegeneration (Kyaw et al., 2004; Park et al., 2004). Researchers (Harris et al., 2005) recently reported that AAT induced the activity of the Src protein tyrosine kinase signaling cascade and that inhibiting that cell death pathway reduced AAT-induced cochlear injury. NAC also has been reported to partially prevent the induction of Src-PTK and reduce its activity in premature cell death (Cuadrado et al., 2003). Finally, NAC has also been shown to be effective at reducing toxin and stress-induced cellular necrosis. NAC reduced carbon tetrachloride induced liver necrosis in rat (Ritter et al., 2004), and toxin induced hepatocyte necrosis (Menor et al., 2004). Interestingly and importantly, NAC given systemically (150 mg/kg) up to 6 h after ischemic injury in a rat stroke model reduced the stroke injury, brain apoptosis and inflammatory necrosis, and improved functional outcomes as it reduced penumbral damage (Khan et al., 2004).

In summary, NAC as utilized in a variety of *in vitro* and *in vivo* models, addresses most of the currently known mechanisms that may be associated with the genesis of AAT-induced cochlear injury. NAC has been documented to act as a free radical scavenger, substrate for GSH production, mitochondrial protectant, glutamate excitotoxicity inhibitor, lipid peroxidation inhibitor, and PCD and necrosis inhibitor. This information is summarized in Table 1. Current ongoing studies are more specifically further addressing these mechanisms in cochlear models of noise injury. Presently, the potency of NAC in reducing high level noise injury in preclinical AAT models would suggest that many of these ameliorative mechanisms are operant. The preclinical data for NAC as a protectant from AAT will now be reviewed.

4. NAC and acute steady state noise

Early publications suggested that cochlear antioxidant enzymes, including those involving GSH synthesis and utilization, were modulated with both conditioning and damaging steady state noise exposure (Jacono et al., 1998). Topical application to the cochlear round window membrane of an adenosine antagonist that was shown to up-regulate the activity of cochlear antioxidant enzymes also conferred protection from cochlear injury in chinchilla exposed to steady state noise (Hu et al., 1997). Systemic administration of GSH ester to rats on a low protein diet reduced hearing loss induced by high level steady state noise (Ohinata et al., 2000a). Systemically administered NAC in combination with salicylate significantly attenuated permanent threshold shifts and outer hair cell loss in chinchilla induced by 6-h, steady state 4 kHz octave band noise (Kopke et al., 2000). In a subsequent study, Ohinata and colleagues compared NAC to other systemically administered agents in effectiveness of attenuation of hear-

Table 1
Potential effects of NAC on AAT-induced cochlear pathologic mechanisms

Pathologic mechanism	NAC effect	Reference
Generation of ROS	Scavenges ROS	Aruoma, 1989
Generation of RNS	Reduces RNS production	Erbas et al. (2004) and Lee et al. (2004)
Generation of 4-HNE	Detoxifies 4-HNE	Neely et al. (2000)
Generation of isoprostanes	Reduces isoprostane formation	Ohinata et al. (2003)
Depletion of GSH	Replenishes GSH	Cotgreave et al. (1987) and Cotgreave (1997)
Mitochondrial Injury	Protects mitochondria	Grattagliano et al. (2004)
Glutamate excitotoxicity	Reduces toxicity	Penugonda et al. (2005) and Himi et al. (2003)
Caspase activation	Reduces activation	Wu et al. (2005) and Dickey et al. (2005)
MAPK/JNK activation	Reduces activation	Wu et al. (2005) and Dickey et al. (2005)
Src/PTK activation	Reduces activation	Cuadrado et al. (2003)
Necrosis/inflammation	Reduces inflammation/necrosis	Khan et al. (2004)

ing loss, hair cell loss and lipid peroxidation in guinea pigs exposed to high level steady state noise (Ohinata et al., 2003). They found that NAC effectively attenuated lipid peroxidation in the organ of Corti, modiolar core, and lateral wall of the cochlea and provided significant protection from hair cell and hearing loss which was generally more effective when compared to the other agents: the non-specific *N*-methyl-D-aspartate (NMDA) receptor antagonist (+)-MK-801, its inactive isomer (–)-MK-801, the selective NR1/2B NMDA receptor antagonist PD 174494, and the nitric oxide synthase (NOS) inhibitor *N*-nitro-L-arginine methyl ester (*l*-NAME). Delivery of NAC by gavage was also effective in reducing permanent threshold shifts in steady state noise exposed chinchilla (Bielefeld et al., in press).

5. NAC and acute impulse and kurtotic noise

Damaging noise may often be impulse (Henderson and Hamernik, 1986), impact (Henderson et al., 1994), or complex kurtotic noise (Henderson et al., 2001). The type of noise causing the injury may be a very important consideration. For example, intense impulse noise has been shown to cause a very rapid onset of outer hair cell apoptotic changes that may differ from steady state noise (Hu et al., 2006). To assess the effectiveness of NAC for impulse noise, chinchilla were exposed to 150 pairs of simulated M-16 rifle shots rapidly over a 2 min time period (Kopke et al., 2005). Untreated noise-exposed animals experienced a 40–50 dB permanent threshold shift and an 80–100% loss of outer hair cells whereas animals treated before and after the noise with intraperitoneal-injected NAC demonstrated an approximately 30 dB reduction in permanent threshold shift and a 40–50% attenuation of outer hair cell loss (Fig. 1). A similar study of impulse noise in rats was undertaken (Duan et al., 2004). Different dosing paradigms were used for intraperitoneal injection of NAC. Using the optimal treatment paradigm there was almost complete attenuation of outer hair cell loss as well as substantial reduction of permanent threshold shift. In another study the dosing effect of NAC on the attenuation of impulse noise-induced permanent threshold shift 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 (Kopke et al., 2004) (Fig. 2).

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 (Bielefeld et al., in press). 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 (see article by Coleman et al., 2006). 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 acute acoustic trauma. These data are summarized in Table 2.

6. NAC safety

NAC, a thiol-containing amino acid derivative, is used in the United States as a nutritional supplement and also a drug which has passed the stringent safety requirements for Food and Drug Administration (FDA) approval for prescription use only. Most of the abundant safety data for oral NAC is provided by its use in acetaminophen intoxication. In 1985, oral NAC emerged as the FDA-approved gold standard for safe and efficacious treatment/prevention of hepatic damage as a result of acute acetaminophen intoxication. NAC is effective for this indication as it replenishes liver GSH and scavenges ROS induced by the acetaminophen intoxication. A NAC dose of approximately 100 g is administered orally over 72 h and has proven to dramatically reduce the extent of liver injury, and yet has been associated with few side effects (Product Information, 1998). The most common side effects associated with this oral NAC regimen are gastrointestinal and dermatological in nature.

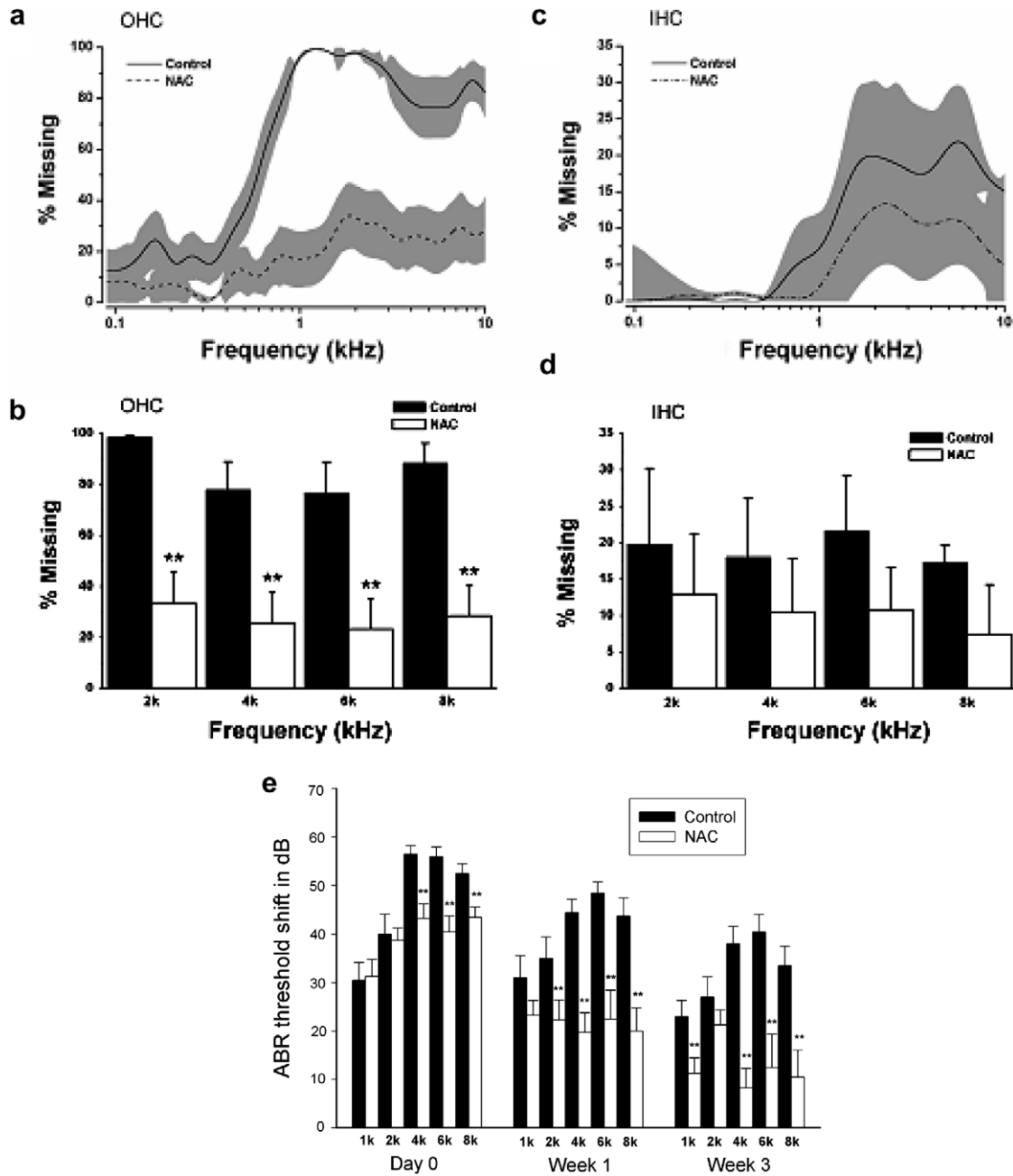


Fig. 1. (a)–(e) Hair cell counts of NAC group ($n = 12$ ears) compared with control saline group ($n = 12$ ears) after impulse noise exposure (simulated M-16 rifle noise). Figures (a) and (b) are data from OHCs, and (c) and (d) are data from IHCs. Figures (a) and (c) are cytochleograms portraying missing hair cell percentages on the Y-axis as a function of frequency on the X-axis. The gray areas represent the standard error of means. Figures (b) and (d) correspond to histogram plots of the mean percentage of hair cell missing in cochlear frequency regions ranging from 2 kHz to 8 kHz. The data is derived from (a) and (c). Asterisks represent the statistical significance level of $p < 0.01$ (**). (e) ABR threshold shifts of NAC group ($n = 12$ ears) compared with control group ($n = 12$ ears) at three time points (0, 1 and 3 weeks) after impulse noise exposure. The label “0 week” stands for the time point immediately (around 15 min) after the exposure. Error bars represent standard error of means. Asterisks represent the statistical significance level of $p < 0.01$ (**). (Reproduced by permission of *Acta Otolaryngologica*.)

NAC has been used in the clinical setting safely for over 15 years. Furthermore, the interest in this agent has also provided safety experience in numerous disease states and unique populations, and there are over 70 published placebo controlled studies on the use of NAC for a variety of indications in the literature.

Wiklund and colleagues, in a randomized, double blind, crossover study, investigated the effect of NAC or placebo

on homocysteine and lipoprotein a levels (Wiklund et al., 1996). Twelve hypercholesterolemic patients were treated with NAC doses of 2 g twice daily for two weeks and placebo (with a one week washout). Upon review of the tolerability and safety data, no difference was appreciated between the two treatment groups, and it was concluded that NAC daily doses of 4 g were well tolerated. Furthermore, no changes from baseline were observed in the clin-

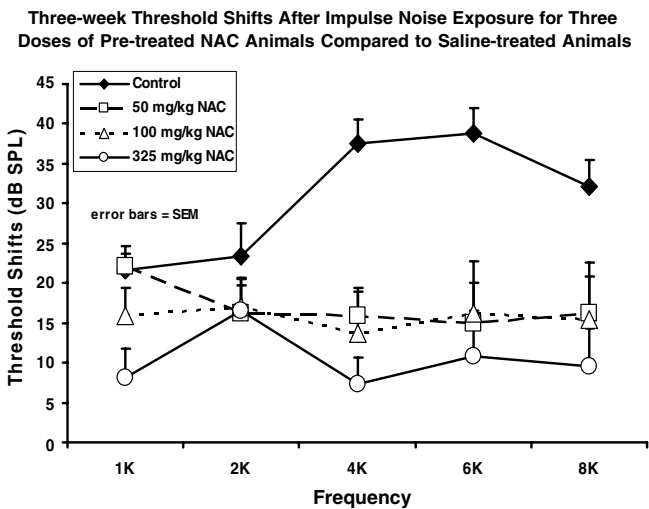


Fig. 2. Chinchilla were exposed to 150 pairs (2 per second) of simulated M-16 rifle shots. Varying the dose of NAC affected the attenuation of impulse noise-induced permanent threshold shift in chinchilla indicating that 325 mg/kg was most effective. However, significant attenuation was also noted with 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. Two-way ANOVA with repeated measures and Newman-Kuels post hoc analysis (50 mg/kg dose ($p < 0.01$), 2, 4, 6, 8 kHz, 100 mg/kg dose ($p < 0.01$), 4, 6, 8 kHz, 325 mg/kg dose ($p < 0.01$), 1, 2, 4, 6, 8 kHz). Error bars represent standard error of means. There were six animals (12 ears) in each group.

ical laboratories regardless of treatment (Wiklund et al., 1996).

In a recent randomized, double blind, placebo controlled study conducted in a relatively healthy HIV patient population, daily NAC doses of 8 grams were administered for eight weeks to determine the impact on glutathione levels (De Rosa et al., 2000). Of the 81 patients randomized over half received the NAC treatment arm. When the placebo and NAC treatment arms were compared, no significant difference was observed with respect to the number of patients reporting symptoms or the average number of symptoms reported. The most common side effects

reported for both NAC and placebo arms were gastrointestinal, with seven patients from each group reported. The most common gastrointestinal complaints were diarrhea and nausea. Less frequently reported events were rash and headache. Furthermore, 75% of events reported were graded severity 1 (scale 1–4) regardless of treatment and only three patients reported events with a severity of >2 . These findings are significant as they demonstrate the mild nature of the side effects experienced at very large doses (De Rosa et al., 2000).

The most extensive safety data for oral NAC administration is provided by its use in acetaminophen intoxication. Acetaminophen overdoses greater than 150 mg/kg commonly result in a rapid and permanent hepatocellular necrosis due to oxidative stress and GSH depletion, often leading to hepatic failure and death. Oral NAC is administered for this indication at a loading dose of 140 mg/kg (≈ 11 g) followed by a maintenance dose of 70 mg/kg (≈ 5.5 g) given every 4 h for a total of 72 h. This regimen has proven to dramatically reduce the extent of liver injury when administered within 24 h of overdose. Few side effects have been associated with this regimen despite a large total NAC dose (≈ 100 g) administered over just 72-h (Product Information, 1998). The most common side effects associated with this short duration, high dose oral NAC regimen are gastrointestinal and dermatological. Specifically, nausea, vomiting, and diarrhea are reported with the oral NAC acetaminophen overdose regimen. However, it is difficult to determine the frequency of these events as they are confounded by the underlying acetaminophen overdose, which also causes these gastrointestinal symptoms. One large study investigated 1283 patients receiving the NAC regimen and concluded that no event, other than gastrointestinal, was reported with a frequency greater than 5% (Miller and Rumack, 1983). Rarely, dermatological events of rash and urticaria have also been reported by the American Society of Health-Systems Pharmacists (McEvoy, 2005). NAC should be used with caution in patients with peptic ulcer disease to avoid exacerbation, and NAC may

Table 2
Preclinical studies of NAC and AAT

Model	Dose and administration	Hair cell and hearing effect	Other effect	Reference
Steady state noise, chinchilla	325 mg/kg intraperitoneal injection	Reduced hair cell and hearing loss		Kopke et al. (2000)
Steady state noise, guinea pig	500 mg/kg intraperitoneal injection	Reduced hair cell and hearing loss	Decreased lipid peroxidation	Ohinata et al. (2003)
Impulse noise, rat	350 mg/kg intraperitoneal injection	Reduced hair cell and hearing loss		Duan et al. (2004)
Impulse noise, chinchilla	325 mg/kg intraperitoneal injection	Reduced hair cell and hearing loss		Kopke et al. (2005)
Impulse noise, chinchilla	Dose response 50, 100, 325 mg/kg intraperitoneal injection	Reduced hearing loss at each dose		Kopke et al. (2004)
Steady state noise, chinchilla	325 mg/kg by gavage	Reduced hearing loss		Bielefeld et al. (in press)
Kurtotic noise, chinchilla	325 mg/kg intraperitoneal injection	Reduced hearing loss		Bielefeld et al. (in press)

also intensify headaches in those taking nitrates for treatment of angina. There are rare reports of renal stone formation with oral intake of NAC, and therefore NAC should be avoided in patients who form cystine renal stones (Hendler and Rorvik, 2001).

7. NAC and early clinical data

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. One study examined effects of oral NAC administration in ameliorating noise-induced temporary shifts. Another study investigating the safety and efficacy of NAC in preventing permanent noise-induced threshold shifts has completed subject recruitment and data collection and is in the process of preparation for publication. An additional study is under way in the military and is examining the safety and efficacy of NAC as a treatment agent given shortly after an acute acoustic trauma.

A study by (Kramer et al., 2006) was a randomized, double blind, placebo controlled design involving voluntary discothèque attendees. This study was confounded by the small number of subjects, variability in noise exposure levels for each group, and measures which evaluated only temporary shifts in thresholds. In the study 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. Noise dosimetry was performed on study groups of four subjects who stayed together in the club during the musical performances. There were no reported side effects from the NAC ingestion. There were no significant differences in temporary induced pure tone threshold shifts (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 (Duan et al., 2004; Kopke et al., 2004, 2005, 2000; Ohinata et al., 2003). These data are consistent with the hypothesis that the cochlear mechanisms of noise-induced TTS and PTS differ considerably. The most consistent correlates of noise-induced PTS have been postulated to be inner and outer hair cell loss and nerve fiber degeneration occurring through cell death (Nordman et al., 2000; Liberman and Beil, 1979; Bohne and Clark, 1982) Different processes have been reported to be correlates of noise-induced TTS, including pillar buckling and uncoupling of the outer hair cell stereocilia from the tectorial membrane (Nordman et al., 2000), temporary afferent

nerve injury (Robertson, 1983; Puel et al., 1998) and reversible stereociliary damage (Gao et al., 1992; Canlon, 1988). Whereas NAC may prevent the GSH depletion and oxidative stress leading to hair cell and neuronal loss, it has not been shown to prevent the mechanical, neurite, and stereocilia injuries thought to cause TTS.

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 informed consenting, received baseline pure tone audiometry, DPOAEs and filled out tinnitus and side effect questionnaires. Subjects then 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. The study has now been completed and suggests safety and a favorable biologic response for NAC such that a dose ranging study is now being planned (Kopke et al., in preparation).

An additional study, termed REACTOR, is currently recruiting subjects at several US military centers to determine in a prospective, randomized, double blinded placebo controlled study if oral NAC given shortly after acute acoustic trauma induced hearing loss can reduce the amount of permanent threshold shift. Completion of the study is anticipated in the next 12–18 months.

8. Summary

Acute acoustic trauma and NIHL are still very prevalent threats to hearing health despite continued deployment of hearing conservation programs. New and accumulating data regarding the role of oxidative stress and cochlear cell death in the pathogenesis of cochlear injury are providing a rational mechanism-based approach for preventing and treating noise-induced hearing loss with pharmacologic agents.

Acoustic overexposure leads to ischemia reperfusion, excessive glutamate release, overproduction of ROS, mitochondrial injury, lipid peroxidation, GSH depletion and loss of hair cells and neurons through PCD and inflammatory pathways. Not surprisingly, a wide variety of interventions have now been found to reduce NIHL in animal models. Successful approaches have included a wide variety of antioxidants, including GSH prodrugs such as NAC (Kopke et al., 2000), D-methionine (Kopke et al., 2002), 2-oxothiazolidine-4-carboxylate (OTC) (Yamasoba et al., 1998a) and GSH esters (Hight et al., 2003); other antioxidants such as allopurinol (Seidman et al., 1993), edavarone (Takemoto et al., 2004), lipoic acid (Seidman et al., 2000), resveratrol (Seidman et al., 2003), α tocopherol (Hou et al., 2003), ebselen (Yamasoba et al., 2005), and SOD-PEG (Seidman et al., 1993); acetyl-L-carnitine (ALCAR) (Kopke et al.), antioxidant enzyme inducers such as R-PIA (Hight

et al., 2003); glutamate antagonists such as magnesium (Attias et al., 2004), carbamathione (Kopke et al., 2002), caroverine (Chen et al., 2001), MK-801 (Ohinata et al., 2003), and riluzole (Ruel et al., 2005); NOS inhibitors such as *l*-NAME (Ohinata et al., 2003); and a variety of other compounds such as mannitol (Yamasoba et al., 1999), iron chelators (Yamasoba et al., 1999), cell death inhibitors (Pirvola et al., 2000; Harris et al., 2005), glucocorticoids (Takemura et al., 2004), growth factors (Shoji et al., 2000; Sugahara et al., 2001), and cyclosporine A (Minami et al., 2004). For a succinct review see (Lynch and Kil, 2005).

Each of these approaches has advantages and disadvantages. The ideal pharmacologic agent would specifically address known mechanisms of acoustic injury, and it would be orally administered, exceptionally safe, effective, and affordable.

While many of these technologies and compounds may prove beneficial in human clinical trials, (in fact, magnesium supplementation was reported to reduce noise-induced hearing loss in Israeli soldiers (Attias et al., 1994)) NAC is emerging as a compound that meets many of the criteria for an ideal agent. It addresses many of the known mechanisms of cochlear injury due to acoustic trauma, functioning as an antioxidant, glutathione replenisher, mitochondrial protectant, and necrosis and cell death inhibitor. Its effectiveness in animal models has been documented for oral and parenteral administration in several species for steady state, impulse, and kurtotic noise exposures, in several different laboratories. It is orally administered, exceptionally safe, relatively inexpensive, and initial clinical reports are encouraging in terms of its safety and a suggestion of positive biological activity in reducing noise-induced threshold shifts.

Further clinical and basic science study is underway. With time and further clinical research a pharmacological approach which is best suited for prevention and treatment of AAT will emerge. This advancement will be adjunctive to other hearing conservation programs already deployed and significantly further reduce the incidence of noise-induced hearing loss worldwide.

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