Hearing Research 323 (2015) 40–50

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research paper

Efficacy and safety of N-acetylcysteine in prevention of noise induced hearing loss: A randomized clinical trial

Richard Kopke [a, *], Martin D. Slade [b], Ronald Jackson [c], Tanisha Hammill [g], Stephen Fausti [d], Brenda Lonsbury-Martin [e], Alicia Sanderson [c], Laura Dreisbach [f], Peter Rabinowitz [b], Peter Torre III [f], Ben Balough [c]

[a] Hough Ear Institute, University of Oklahoma, USA
[b] Yale University School of Medicine, New Haven, CT, USA
[c] Spatial Orientation Center, Department of Otolaryngology, Naval Medical Center, San Diego, CA, USA
[d] VA Medical Center, R & D-NCRAR, Portland, OR, USA
[e] Department of Otolaryngology, University of Colorado Health Sciences Center, Denver, CO, USA
[f] Auditory Physiology and Psychoacoustics Laboratory, San Diego State University, San Diego, CA, USA
[g] TLC Research Solutions, LLC, New Braunfels, TX, USA

ARTICLE INFO

Article history:
Received 29 April 2014
Received in revised form
31 December 2014
Accepted 7 January 2015
Available online 22 January 2015

ABSTRACT

Despite a robust hearing conservation program, military personnel continue to be at high risk for noise induced hearing loss (NIHL). For more than a decade, a number of laboratories have investigated the use of antioxidants as a safe and effective adjunct to hearing conservation programs. Of the antioxidants that have been investigated, N-acetylcysteine (NAC) has consistently reduced permanent NIHL in the laboratory, but its clinical efficacy is still controversial. This study provides a prospective, randomized, double-blinded, placebo-controlled clinical trial investigating the safety profile and the efficacy of NAC to prevent hearing loss in a military population after weapons training.

Of the 566 total study subjects, 277 received NAC while 289 were given placebo. The null hypothesis for the rate of STS was not rejected based on the measured results. While no significant differences were found for the primary outcome, rate of threshold shifts, the right ear threshold shift rate difference did approach significance ($p = 0.0562$). No significant difference was found in the second primary outcome, percentage of subjects experiencing an adverse event between placebo and NAC groups (26.7% and 27.4%, respectively, $p = 0.4465$). Results for the secondary outcome, STS rate in the trigger hand ear, did show a significant difference (34.98% for placebo-treated, 27.14% for NAC-treated, p-value = 0.0288). Additionally, post-hoc analysis showed significant differences in threshold shift rates when handedness was taken into account.

While the secondary outcomes and post-hoc analysis suggest that NAC treatment is superior to the placebo, the present study design failed to confirm this. The lack of significant differences in overall hearing loss between the treatment and placebo groups may be due to a number of factors, including suboptimal dosing, premature post-exposure audiograms, or differences in risk between ears or subjects. Based on secondary outcomes and post hoc analyses however, further studies seem warranted and are needed to clarify dose response and the factors that may have played a role in the observed results.

1. Introduction

1.1. Background

Noise-induced hearing loss is a world-wide problem both in industry and in the military. Current National Health and Nutrition Examination Survey data suggest that more than 22 million U.S.

* Corresponding author. Hough Ear Institute, 3400 NW 56th St, Oklahoma City, OK 73112-4463, USA. Tel.: +1 405 917 1270; fax: +1 619 532 5444.
E-mail address: rkopke@houghear.org (R. Kopke).

http://dx.doi.org/10.1016/j.heares.2015.01.002
0378-5955/

Acronyms, abbreviations and symbols

ABR	auditory brain stem response
ASHA	American Speech and Hearing Association
b.i.d.	bis in die (twice daily)
d	Day
dB	Decibel
DP1	distortion product
DPOAE	distortion product otoacoustic emissions
g	Gram
GSH	Glutathione
GST	glutathione-S-transferase
h	Hour
HPD	hearing protection device
i.p.	Intraperitoneal
kg	Kilogram
kHz	Kilohertz
MANOVA	multiple analysis of variance
mg	Milligram
NAC	N-acetylcysteine
NIHL	noise induced hearing loss
NF1	noise floor
PMTF	psychoacoustical modulation transfer function
PO	per os (oral)
PTS	permanent threshold shift
ROS	reactive oxygen species
SPL	sound pressure level
STS	significant threshold shift
t.i.d.	ter in die (three times per day)
TS	threshold shift
TSI	Tinnitus Severity Index
TTS	temporary threshold shift

workers are exposed to hazardous workplace noise. The Healthy People 2020 study recently found the prevalence of hearing loss among factory workers to be 42% (McCullagh et al., 2011). The military represents a high-risk population for NIHL. Despite the widespread use of HPDs within hearing conservation programs in the military, NIHL remains a major problem. Shaw and Trost (2005) reported that after a 24-year career spent predominantly aboard ships, enlisted sailors have a 46% risk of rating compensable hearing loss. American Veterans of recent conflicts in the Middle East are four times more likely to have severe hearing impairment than non-veterans (CDC, 2011). U.S. Veterans Administration claims for hearing loss and tinnitus exceeded $1.2 billion in 2009, indicative of an exponential increase in funds for such disabilities paid since 2000 (Saunders and Griest, 2009).

Although HPDs can attenuate the impact of noise exposure, they have intrinsic limitations which greatly reduce their effectiveness, including: (a) noise levels can exceed their protective capability; (b) acoustic energy can bypass HPDs and be transmitted directly through the skull; (c) the vital need for audible communication can be limited by HPDs; (d) poor compliance; (e) lack of fit; and (f) exposure to sudden, unanticipated and damaging noise when HPDs are not worn. Thus, there is a need for hearing protection above that mandated by hearing conservation programs (Humes et al., 2005). This study is the first of its kind to test the use of a nutraceutical antioxidant agent, N-acetylcysteine (NAC), as an adjunct to hearing protection devices in preventing noise-induced hearing loss in a prospective, placebo-controlled, Phase II-like trial using a large, noise-exposed military recruit population.

More than a decade ago, it was found that NIHL was not merely caused by physical damage, but that it was also metabolic in nature (Yamane et al., 1995). Acoustic over-stimulation can promote the excessive release of the neurotransmitter glutamate at the inner hair cell synapse (excitotoxicity), a rapid increase in reactive oxygen species (ROS), and release of free radicals in the cochlea, leading to apoptosis and hair cell or neuronal death (Kopke et al., 1999; Poderoso et al., 2000; Puel et al., 1998). Glutathione (GSH) is a principal cochlear antioxidant in the liver and many other organs. It reduces cochlear injury after noise exposure, possibly as a result of its role in preventing free radical damage (Ohinata et al., 2000a). However, acoustic insult can result in the depletion of endogenous reserves of glutathione resulting in cellular damage. Consequently, therapeutic interventions have focused on the administration of exogenous sources of antioxidants or the stimulation of endogenous antioxidants to prevent noise-induced ototoxicity. Many compounds have been found to be quite effective in the prevention of NIHL in the experimental laboratory setting (Lynch and Kil, 2005). Antioxidants have been reported to reduce the ototoxic effects of acoustic trauma in animal models. These preclinical studies demonstrate that antioxidant supplementation increases cellular GSH and reduces hair cell damage and permanent hearing loss from damaging noise exposures, including simulated firearms noise (impulse noise) (Kopke et al., 2005). NAC, a thiol amino acid derivative, not only acts as an antioxidant to directly scavenge hydrogen peroxide and hydrogen radicals (Aruoma et al., 1989) but also increases the synthesis of intracellular GSH (Gillissen et al., 1997). NAC is most notably recognized in clinical use as an FDA-approved treatment for acetaminophen toxicity related to GSH depletion and oxidative stress and has an extremely favorable safety profile when given in very high doses to acutely or chronically ill humans. D-methionine would be another logical choice (Rewerska et al., 2013). Ebselen represents another potential choice for a therapeutic agent (Kil et al., 2007); however, the human clinical exposure to ebselen is significantly less than for NAC.

This study was based on considerable preclinical data. NAC was chosen for this clinical study based on its demonstrated efficacy in preclinical models in several species with different types of acoustic trauma, its long clinical history of safety and tolerability, and it's relatively low cost. Chinchillas, when given NAC alone by intraperitoneal (i.p.) injection after exposure to steady state noise, had substantially reduced auditory brain stem response (ABR) threshold shifts and outer hair cell loss (Coleman et al., 2007). A similar reduction in ABR threshold shifts was obtained in chinchillas when NAC was given orally after exposure to high kurtosis noise (Bielefeld et al., 2007). In guinea pigs, when NAC was given i.p. after exposure to noise, both ABR threshold shifts and outer hair cell loss were significantly reduced (Ohinata et al., 2003). In another study, both compound action potentials and outer hair cell loss were reduced in guinea pigs with NAC treatment immediately after noise exposure (Fetoni et al., 2009). Similarly, rats administered NAC i.p. after exposure to white noise showed a reduction in ABR threshold shifts and outer hair cell loss (Wu et al., 2010). When rodents exposed to impulse noise were given NAC i.p. prior to noise exposure, hair cell loss and ABR threshold shifts were reduced by 50–80% (Kopke et al., 2005).

Importantly, NAC has shown promise in human tests. In a double-blind crossover study, human males received NAC (1200 mg/day) or placebo for 14 days. This study showed that NAC may prevent noise-induced TTS among occupationally noise-exposed men (Lin et al., 2010). A study involving Swedish military subjects, undergoing training in an indoor urban warfare,

setting compared NAC to placebo to measure effects on a variety of cochlear functions (Lindblad et al., 2011). The NAC treatment consisted of acetylcysteine, 200 mg, (Tika). Four tablets were taken, all of them after exposure: The first tablet, dissolved in half a glass of water, was taken directly after exposure, a second tablet 1 h later, a third at breakfast the next day, and the fourth an hour later. The data suggested a NAC treatment effect as measured by pure tone threshold shifts, psychoacoustical modulation transfer function, and transient-evoked otoacoustic emissions. Another study, carried out in a factory setting with textile workers, also suggested a positive treatment effect of NAC as measured by pure tone threshold shifts (Doosti et al., 2014). The aim of this study was to compare the protective effect of N-acetyl-cysteine (NAC) and ginseng on protection from NIHL in textile workers exposed to continuous noise in daily work settings. In this study, 48 participants were randomly allocated to three groups; Group I received NAC 1200 mg/day, Group II received ginseng 200 mg/day, and Group III (control group) received no supplement. Pure tone audiometry and high frequency audiometry were performed preshift before and after 14 days (on day 15). Linear regression analysis results showed reduced noise-induced temporary threshold shift (TTS) for NAC and ginseng groups at 4, 6 and 16 kHz ($P < 0.001$) in both ears. Furthermore, the protective effects were more prominent in NAC than ginseng.

1.2. Study aim

While use of NAC has a long standing safety profile in humans, thereby negating the need for a Phase I clinical trial, efficacy data for NIHL prevention in humans was limited. This Phase II-like study administered oral NAC to healthy Marine Corps recruit volunteers who were noise-exposed during 16 days of routine military noise during weapons training. The aim of the study was to determine whether the administration of this compound was safe and well-tolerated and could reduce the rate of noise-induced threshold shifts to a significant degree when tested as an adjunct to hearing protection devices.

2. Methods

2.1. Trial design

This was a single-center, prospective, randomized, parallel, double-blind, placebo-controlled trial that was reviewed and approved by the Institutional Review Board at the Naval Medical Center, San Diego, U.S. Navy Bureau of Medicine and Surgery and U.S. Army Medical Research and Materials Command.

2.2. Participants

Study volunteers (18–35 years of age) were recruited from trainees at the United States Marine Corps Recruit Depot, San Diego. At the time of the study, only male personnel were trained at this site. Thus, no females could be recruited for this study. The voluntary informed consent of the subjects used in this research was obtained as required by SECNAVINST 3900.39D.

2.3. Interventions

Informed consent was obtained with higher ranking personnel absent and an ombudsmen present to protect the vulnerable population of military recruits and prevent undue influence. Each volunteer underwent initial hearing screening consisting of conventional and high frequency pure tone audiometry, distortion product otoacoustic emissions testing (DPOAE), otoscopy, and immittance measures. Baseline questionnaires regarding past noise exposure, medical history, and hearing symptoms, including tinnitus, were collected. Immittance tympanometry was measured with Interacoustics impedance audiometer (Model #AT235, Minneapolis, MN, USA). Middle ear immittance was evaluated with a 0.226 kHz probe tone with a static pressure change in the ear canal equal to 200 daPa/s. Contralateral acoustic reflexes were also measured at 1000 Hz. Certified audiologists collected pure tone threshold data in four double-walled sound attenuated booths (Acoustics Systems, Austin, TX/USA). Pure tone thresholds were measured with an Interacoustics AC40 (Minneapolis, MN/USA) clinical audiometer and TDH-39P earphones using pulsed tones at 2, 3, 4, and 6 kHz, and also for ultrahigh frequencies at 8, 10, 12.5, 14, 16, 18, and 20 kHz using Koss Pro/KTX-6 earphones (Milwaukee, WI/USA). Daily calibrations were completed for earphones in all four sound booths using OSCAR electro-acoustic ear simulators (Tremetrics, Eden Prairie, MN/USA). Following behavioral audiometry, DPOAEs were measured using Starkey DP2000 (St. Louis Park, Minnesota/USA) software loaded on Dell Latitude D600 laptop computers. Tinnitus was self-reported on an analogue, eleven-point "tinnitus severity" scale where a value of 0 is associated with "very quiet" and a value of 10 is associated with "very loud." The measure was acquired prior to weapons training, on each of the 16 days of training and at ten days post weapons training. The Tinnitus Severity Index (TSI) was also administered to the subjects at the beginning and end of the study (Folmer and Griest, 2000).

Exclusion criteria included abnormal hearing in either ear (hearing thresholds greater than 25 dB HL at 2, 3, 4, or 6 kHz), asymmetry ($\geq$30 dB SPL between the ears) for high frequency thresholds ($\geq$8 kHz), abnormal pre-weapons training baseline tympanometry, any known hypersensitivity or allergy to N-acetylcysteine, or concomitant use of erythromycin.

Recording parameters for DPOAEs were: 1) $f2$ varied from 2.25 through 10 kHz (14 frequencies); 2) $f2/f1 = 1.2$; and 3) L1 = 65 dB SPL and L2 = 55 dB SPL. A complete acoustic calibration, by a trained technician of all audiometric equipment, was performed prior to, twice during, and after data collection.

Study participants were administered three 900 mg, dissolving, effervescent tablets of NAC or placebo t.i.d., for a total daily dose of 2700 mg, for each of the first 13 days of weapons training (Fig. 1). During the last three days of training in which the schedule would not allow for three meals per day, subjects consumed two doses (1800 mg), one dose (900 mg) just prior to the morning meal and a second dose (900 mg) just prior to the evening meal. Subjects consumed each dose by dissolving it into 4–8 ounces of water just prior to meals and under the close observation of study personnel. Daily drug logs were kept for each subject to record compliance.

As part of routine weapons training, all subjects were uniformly exposed to various noises including impulse, steady-state noise, as well as simulated explosions. The most common noise exposure was M-16 rifle fire, with every trainee firing 325 rounds during the training. Trainees were precluded from all recreational sources of noise exposure. While study personnel were not given access to training locations to verify if subjects wore HPDs during all noise exposures, investigators assume subjects were in compliance with repeated instructions to follow regulations which stipulate the use of HPDs.

Subjects completed questionnaires regarding adverse events prior to weapons training on each of the 16 days of dosing, and at study completion (approximately 10 days after their last dose). The principal investigator and medical monitor each received daily reports of subject adverse events and medical clinic visits for immediate review and determination of the subject's medical needs and ability to continue in the study.

Ten days after final noise exposure, subjects returned for post weapons training audiologic testing. Subjects were retested in the

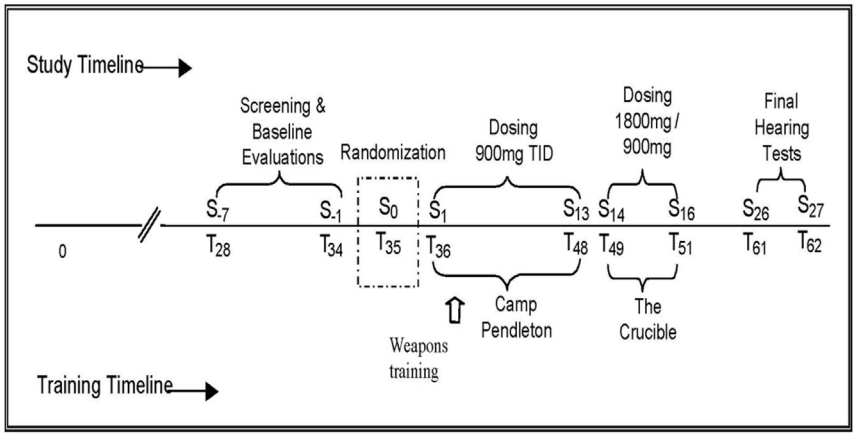

Fig. 1. Research design – study timeline. The study (S) and the training (T) events are displayed in chronological order. T28-62 refers to the days and events of training for the recruits in their regimented training program. The designations S-7 to S27 refer to the days and events of the experimental study the subjects took part in.

same booth using identical equipment as in the pre-weapons training testing. A computerized database checked the post weapons training audiogram against criteria for hearing change and automatically flagged the audiogram if these criteria were met, thereby allowing for a recheck of the hearing threshold at the frequencies in question. Subjects with a significant threshold shift (STS) underwent final tympanograms. If middle ear pathology was confirmed, these individuals were excluded from further analysis and counseled to have medical follow-up. The TSI was administered and the analogue tinnitus score obtained.

2.4. Outcomes

The following primary outcomes were assessed:

1. Using pure tone audiometry, was the rate of subjects demonstrating STS, defined as an increase of $\geq$20 dB at any test frequency or an average increase of $\geq$10 dB at any two consecutive test frequencies, in either ear greater in the placebo group than the NAC group?
2. Were there significant differences when comparing NAC and placebo treated groups in frequency, intensity, or types of documented adverse events?

Secondary outcome measures assessed were:

1. Was the mean change in pure tone threshold hearing (post weapons training threshold minus pre-weapons training threshold) greater in the placebo group than the NAC group at each measured frequency for the left ear, right ear, and the average of both ears?
2. Using pure tone audiometry, was the rate of subjects demonstrating STS, defined as an increase of $\geq$20 dB at any test frequency or an average increase of $\geq$10 dB at any two consecutive test frequencies (ASHA), in the trigger hand ear greater in the placebo group than the NAC group?
3. Using pure tone audiometry, was the rate of subjects demonstrating STS, defined as an increase of $\geq$15 dB at any test frequency or an average increase of $\geq$10 dB at any two consecutive test frequencies (adaptation of the Navy definition of STS), in the trigger hand ear greater in the placebo group than the NAC group?
4. To determine if there was a significant difference in reported level of tinnitus between the NAC and the placebo groups. One outcome for this measure was an eleven point scale used to describe the loudness of tinnitus with a value of 0 associated with very quiet and a value of 10 associated with very loud. Another measure used was the Tinnitus Severity Index (TSI).

2.5. Sample size

The sample size for the trial was defined in order to be able to detect a 60% reduction in STS rate (from 11% to 4.4%) with a significance level of 95%, 80% power and a 1:1 randomization ratio. Since this was the first of its kind clinical study designed and initiated to evaluate the efficacy of NAC to reduce noise-induced STS, the estimate of reduction in STS rate was therefore of necessity based on preclinical data since there were no clinical data to evaluate to determine these parameters (Kopke et al., 2000, 2002, 2005). Additionally, the specified sample size allowed for a 20% loss to follow-up. Thus, the study recruited a total of 634 subjects, 317 in the NAC arm and 300 in the placebo arm.

2.6. Randomization

Subjects were randomized using subject coding and randomization schedules created by an unbiased biostatistician. There was a 1:1 allocation ratio for placebo and treatment. A randomized block design using blocks of eight was utilized. The study pharmacy was given the randomization and prepared NAC or placebo containers for blinded distribution to the subjects. The subject list was accessible only by the medical monitor.

2.7. Blinding

All study participants, audiologists, study coordinators, care providers and data collectors were blinded as to who received study drug or placebo. The taste, odor and appearance of both placebo and NAC formulation were exactly the same.

2.8. Statistical methods

Subject characteristics were compared between the NAC and placebo groups using pre-weapons training data. Continuous variables were evaluated with Student's t-test. For those variables with equal variances between the NAC and placebo groups, a pooled variance was utilized to determine significance. For the variables with unequal variances between the two groups, Satterthwaite's

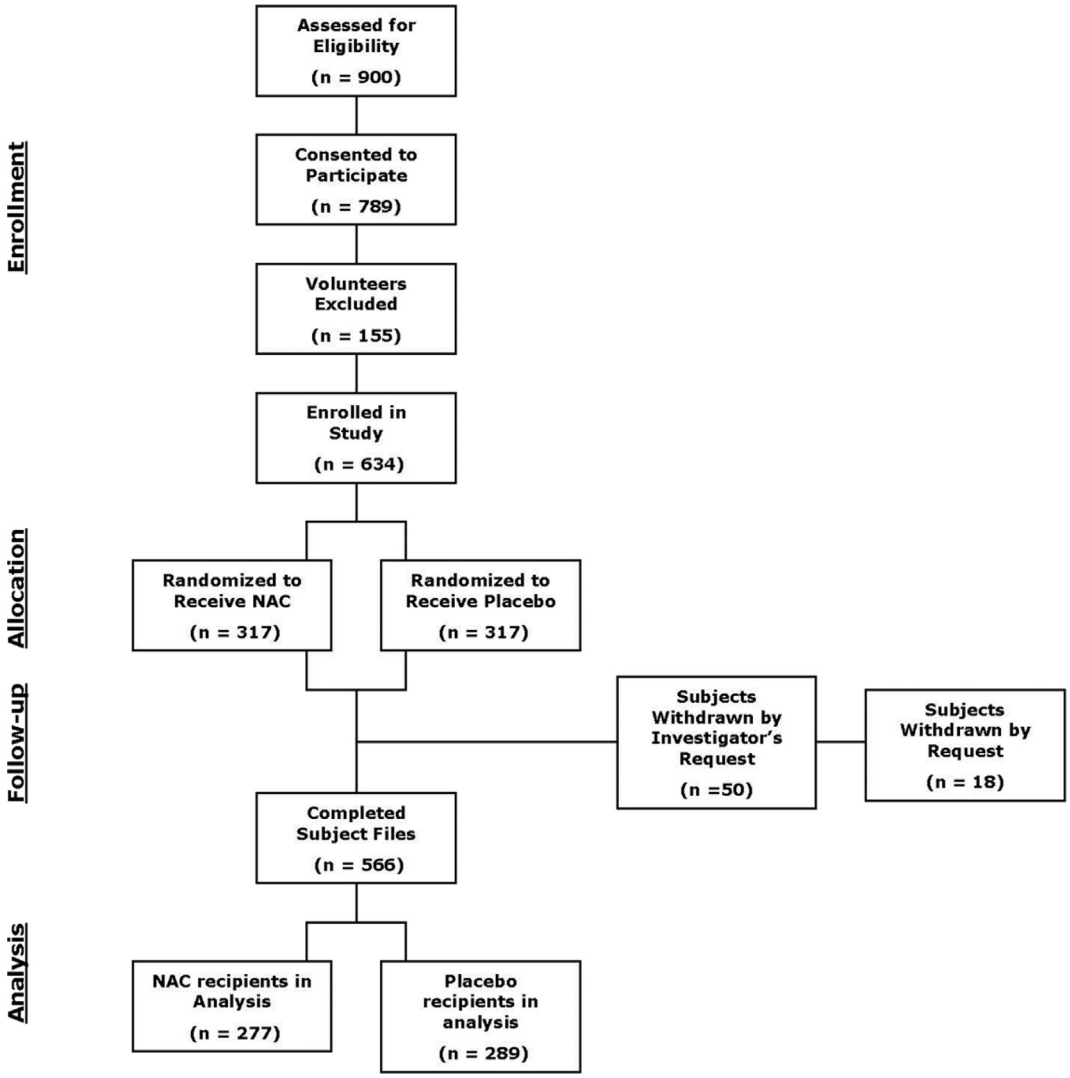

Fig. 2. Subject enrollment flow chart. Each box corresponds to a milestone in subject recruitment and enrollment, randomization, withdrawals, subjects who completed the studies and data analyzed. The number in parentheses corresponds to the number of subjects.

method was utilized. Differences in categorical variables were evaluated with Fisher's two-sided exact test.

One-sided Fisher's exact tests were utilized to determine if there was a significant decrease in STS rates for the NAC group compared to the placebo group. Both non-parametric tests (sign test) and parametric tests (Student's t-test and linear regression) were conducted to determine if there was a significant difference at each measured frequency in threshold shift changes between the NAC and the placebo groups for the left ear, the right ear, and the average of both ears. In addition to analyzing the effect of NAC at each separate audiometric frequency, multiple analysis of variance (MANOVA) regression was utilized to determine if there was an effect of NAC with regard to the change in threshold hearing for the left ear, right ear, and the average of both ears simultaneously over all tested audiometric frequencies. As a post hoc analysis, the above tests were also conducted on the trigger-hand ear, the non trigger-hand ear, the trigger-hand ear stratified by handedness, as well as the non trigger-hand ear stratified by handedness. While there were a multiplicity of analyses these were all secondary not primary outcomes of the study.

To determine if NAC had a detrimental association with adverse events, the percent of subjects in each group experiencing any adverse event was compared utilizing a one-sided Fisher's exact test. Additionally, the number of days that subjects within each group experienced adverse events was compared. Lastly, the average intensity (ranging from 1 for Mild to 4 for Severe) and frequency of adverse events experienced by subjects as well as the average severity of adverse event (frequency x intensity) were compared between the two groups. For these last four analyses (number of days, intensity, frequency, and severity of adverse events), Student's t-tests with pooled variances were utilized.

The overall TSI score was calculated as the sum of the scores for each of the twelve items. Generalized linear models were conducted to determine the effect of NAC on the change in the scores of each of the twelve items in the TSI as well as on the overall TSI score. Independent variables included in each of the initial models were NAC (vs Placebo) and the initial value of the parameter being analyzed. Thus, for the Overall TSI Score, the following model was created: Change in Overall TSI Score = B0 + B1*NAC + B2*Initial Overall TSI Score.

3. Results

3.1. Participant flow

A total of 789 subjects consented for the study. Of these, 634 subjects met criteria for inclusion. Eighteen subjects withdrew at

their own request and another fifty subjects were dropped from the study, primarily for attrition from recruit training for reasons not related to the study (Fig. 2). Five hundred sixty-six subjects completed the study.

3.2. Recruitment

Recruitment began in March 2004 and ended in October 2004. The study concluded when the planned sample size was reached.

3.3. Baseline data

There were no statistically significant differences between the two groups with regard to ethnicity, race, smoking history, exposure to solvents, or personal medical history (Table 1). Although the percentages of those in the placebo and NAC groups were not different in regards to prior noise exposure history, there was slightly greater average exposure to firearms in the NAC group (4.64 vs. 3.68 times during the past year, $p = 0.0454$; data not shown in Table 1). On average, subjects in the placebo group missed more training events than did their NAC counterparts (1.77 events vs. 1.00 event, $p = 0.0444$; data not shown in Table 1), possibly indicating less exposure during the study period. There were no significant differences at any frequency in the initial pre-weapons training audiograms between the two groups.

3.4. Numbers analyzed

Of the 566 total study subjects, 277 received NAC, while 289 were given placebo.

Table 1
Demographic data.

	Placebo (n = 289)	NAC (n = 277)	All (n = 566)	p-value (chi square)
Ethnicity				
Hispanic or Latino	26.2%	29.5%	27.8%	0.4072
Race				
White	84.8%	88.2%	86.5%	0.7635
American Indian or Alaskan Native	7.6%	5.0%	6.4%	
Asian	4.0%	3.8%	3.9%	
Black or African American	2.0%	1.3%	1.6%	
Native Hawaiian or Pacific Islander	1.6%	1.7%	1.6%	
Average age at pretest	19.82	19.43	19.63	0.7624
Smokers				
Ever	53.6%	55.4%	54.5%	0.6673
During past year	42.3%	45.4%	43.8%	0.4759
Packs smoked per day	27.0%	29.0%	0.28	0.7089
Noise exposure				
Power tools	91.7%	90.6%	91.2%	0.6406
Guns	54.7%	57.5%	56.0%	0.5056
Engines	83.7%	80.4%	82.1%	0.2963
Music	90.2%	92.7%	91.4%	0.2986
Other	54.7%	50.7%	52.8%	0.3428
Right “Trigger Hand”	90.8%	90.3%	90.6%	0.8476
Solvent Exposed (frequently)	14.0%	16.8%	15.4%	0.3671
Cold/ear infection in past 3 months	42.7%	43.8%	43.2%	0.7948
Aspirin/non-steroidal medication in past 24 h	13.2%	15.6%	14.3%	0.4098
Head injury in past 3 months	1.4%	1.5%	1.4%	0.9553
Missed some weapons training	4.6%	3.6%	4.1%	0.5520
Exposed to noise other recruits not exposed to	3.6%	3.7%	3.6%	0.9670

3.5. Outcomes and estimation

The effect of NAC on the percentage of threshold shifts for primary outcome measures is depicted in Table 2. There is no statistical evidence at a 95% confidence level that NAC reduced the rate of threshold shifts among this noise exposed population for the primary outcomes using the 1994 ASHA guidelines for STS. However, for measures of right ear, there was a marginally significant decrease of approximately 6% in threshold shift rate for the NAC group compared to placebo group (20.94% vs. 26.99%, $p = 0.0562$).

There was no significant difference in the percentage of subjects experiencing an adverse event between the NAC and placebo groups (27.4% and 26.7%, respectively, $p = 0.4465$). Similarly, average daily intensity, frequency and severity of adverse events showed no significant differences. A summary of the types of adverse events reported is depicted in Table 2. The most common adverse events symptoms reported were gastrointestinal. These were uniformly mild and transient. During the trial, study personnel noted that the subjects were not allowing the effervescent, formulated tablets to completely dissolve before ingestion. After instructions were reinforced, the incidence of gas and bloating declined, suggesting this was a potential source for many of the gastrointestinal adverse events.

The average changes in threshold hearing by group (NAC vs. placebo) are graphically depicted in Figs. 3 and 4 for the right and left ears, respectively. The values for these mean changes, as well as those for the average of both ears, along with their standard deviations, are included in Table 3. From this table, it can be seen that for 30 of the 33 single frequency measures for right, left and both ears that the mean change in hearing loss over the course of the study was less for the NAC subjects than for the placebo group over the measured audiometric frequencies. Based upon the Sign test, the probability of this occurring by chance for any of the measures is $p < 0.001$ suggesting NAC reduced hearing loss. Further analysis was undertaken on this data post-hoc and is described below.

The rate of subjects demonstrating STS in the trigger hand ear showed no significant difference, based on ASHA definition. The rate was 27.56% for the placebo group and 21.56% for the NAC group with a p-value of 0.0620 as shown in Table 4.

A similar analysis was applied using criteria adapted from the U.S. Navy definition for STS, 15 dB or more at a single frequency and 10 dB or more at two consecutive frequencies. When these criteria were applied, the rate of STS was significantly greater in trigger-hand ears. The rate was 34.98% for placebo-treated and 27.14% for NAC-treated subjects with a p-value of 0.0288 as shown in Table 4.

There were no statistically significant differences between the two groups with regard to self-reported tinnitus where tinnitus

Table 2
Primary outcomes.

1) Rates of threshold shift	**Group**		**p-value***
	Placebo	**NAC**	
Left Ear	19.03	21.30	0.7816
Right Ear	26.99	20.94	0.0562
Either Ear	38.41	36.82	0.3813
Both Ears	7.61	5.42	0.1877
2) Adverse events	**Placebo**	**NAC**	**Total**
Drowsiness	1	1	2
Ear pain	9	11	20
Headache	15	12	27
Increased urination	0	3	3
Indigestion	0	17	17
Stomach ache	219	222	441
Tinnitus	16	15	31
Vomiting	1	2	3
Other	8	9	17
	26.7	27.4	$p^* = 0.4465$

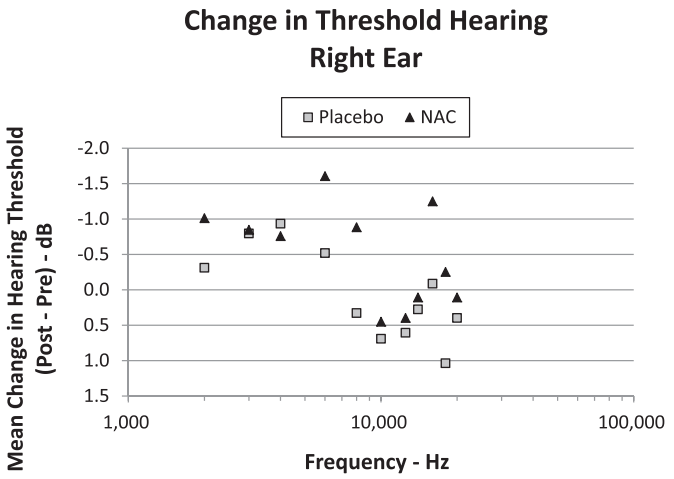

Fig. 3. Change in threshold hearing: right ear. Mean change in hearing threshold (pre-noise minus post-noise threshold in decibels) as a function of treatment (NAC vs. placebo) and by frequency for the right ear.

was measured on a scale of 0–10, with 0 being "very quiet" and 10 being "very loud". Prior to weapons training, both the placebo group and the NAC group reported average loudness values of 2.2 (sd = 2.0). Additionally, there was difference in the change in tinnitus across the study as measured by post weapons training loudness level minus pre-weapons training loudness level), with both groups recording a mean decrease in loudness of 1 point. A summary table for the results of the models utilized for the TSI data is seen in Table 5. As can be observed from the results included in the table, NAC had no effect upon the overall TSI score. Additionally, accounting for multiple comparisons, NAC had no effect upon any of the twelve items comprising the overall TSI.

3.6. Ancillary analysis

In post hoc analysis several interesting findings were noted. More than 90% of the study recruits were right-hand dominant. As shown in Table 4, the percentage of ears demonstrating a reduced STS with treatment (using the ASHA definition) was significantly greater when an individual's handedness was factored into the analysis. For example, STS rates for right ear, right-trigger handed recruits were significantly lower for NAC-treated vs. placebo-treated subjects, 28.4% placebo vs. 21.4% NAC with a p-value of 0.0439. Although, left and right ears for left-handed trigger recruits, and left ear for right-handed individuals showed no difference between placebo compared to NAC treated subjects (p-values 0.7507, 0.9300, and 0.8376, respectively).

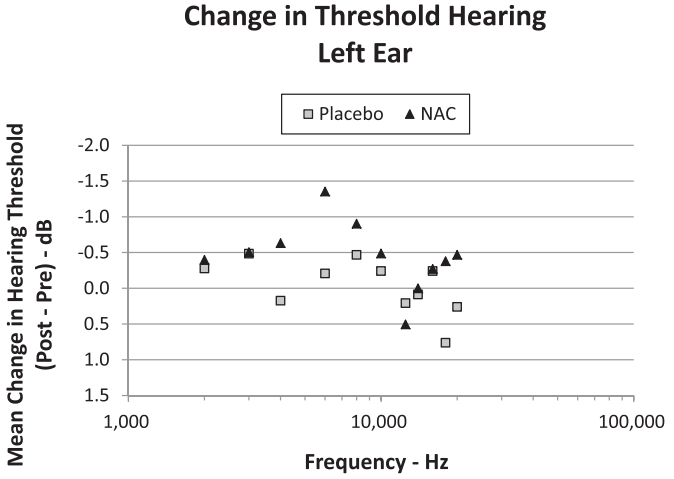

Fig. 4. Change in threshold hearing: left ear. Mean change in hearing threshold (pre-noise minus post-noise threshold in decibels) as a function of treatment (NAC vs. placebo) and by frequency for the left ear.

The percentage of ears demonstrating a reduced STS with treatment (using the adapted Navy definition) also showed significance when handedness was taken into account (Table 4). STS rates for right ear for right-trigger handed recruits were 35.80% for placebo and 27.16% for NAC with a p-value of 0.0237. Additionally, STS rates for the right ear showed treatment significance having 34.60% for placebo and 27.08% for NAC with a p-value of 0.0323. Marginal significance was shown for shifts in both ears (p-values 0.0560). The STS rate for left ear and non-trigger hand ears were not different between placebo and NAC treated groups (Table 4).

It should be noted that this is just the opposite of what has been found among those who shoot hunting rifles (Prosser et al., 1988). Discussion of this finding with a marksman revealed that there is an ear-level gas ejection port in the M-16 rifle used by these subjects to fire 325 rounds. Because of that, ear-level measurements were taken on a firing range by a certified acoustic engineer as a marksman fired a M-16 rifle. Using a Pulse System (Bruel and Krager) with 4938 pressure field ¼ microphones, it was discovered that on average there was about a 6 dB increased noise exposure level for the trigger hand ear for right-handed shooters (Table 6). This may explain why the hearing loss was generally worse in the trigger hand ear and suggests that the trigger hand ear was at greater risk for noise damage in this study. Results of multivariate analysis of variance (MANOVA) revealed that there was insufficient evidence to conclude with 95% confidence that NAC reduced the change in threshold hearing across all frequencies.

Table 7 contains the results of statistical analyses, both Student's t-test and linear regression, for the mean changes by frequency (data collected for secondary outcome one). From this table it can be seen that STS rates for only two (8000 Hz, and 18,000 Hz) of the 11 tested frequencies was significantly lower for NAC group compared to placebo group. Additionally, under Student's t-test analysis, NAC treatment also significantly reduced hearing loss rates at a third frequency, namely 6000 Hz at ($p = 0.0332$) versus placebo. However, when controlling for a subject's initial 6000 Hz hearing threshold in a linear regression model, the effect of NAC was no longer significant ($p = 0.0809$).

Analysis of DPOAE data utilizing change in DP1 as the dependent variable revealed no significant differences in left ear, right ear, and average of both ears at any measured frequency. Controlling for initial hearing, the ratio of DP1/NF1 revealed a handful of significant (albeit small in magnitude) differences between the NAC and placebo groups. These differences, however, were found at different frequencies than those found using pure tone audiometry. Likewise, there was no significant difference at any frequency between the two groups with regard to the distortion product (DP1) values for their initial otoacoustic emission testing. However, the initial DP1/NF1 ratio for the placebo group showed slightly lower response at 2250 Hz (left ear only), 3150 Hz (right ear only), and 3515 Hz (right ear only) than did the NAC group ($p = 0.0285$, 0.0491, and 0.0385, respectively).

4. Discussion

4.1. Limitations

Logistics of this study created subject bias. Notably, the subjects are all male, military and in a narrow age range. In addition, dosing could not be performed on a mg/kg basis. A larger mg/kg dose could

Table 3
Mean ± Standard Deviation (Std) change in hearing threshold (post − pre) dB.

Frequency (Hz)	Ear(s)	Mean change (post − pre) in hearing threshold (dB)			
		Placebo		NAC	
		Mean	Std Dev	Mean	Std Dev
2.0 k	Both	−0.294	4.0878	−0.704	4.0211
	Right	−0.311	5.4715	−1.011	5.2188
	Left	−0.277	5.0956	−0.397	4.7801
3.0 k	Both	−0.640	4.4611	−0.677	4.7173
	Right	−0.796	5.5954	−0.848	5.7343
	Left	−0.484	5.9606	−0.505	6.1765
4.0 k	Both	−0.554	6.2774	−0.695	5.8545
	Right	−0.934	8.1112	−0.758	6.9654
	Left	0.173	7.4048	−0.632	7.8168
6.0 k	Both	−0.363	5.9946	−1.480	6.4495
	Right	−0.519	7.5971	−1.606	8.4934
	Left	−0.208	8.1942	−1.354	8.3117
8.0 k	Both	−0.069	5.2988	−0.894	5.9238
	Right	0.329	7.1307	−0.884	7.3864
	Left	−0.467	7.0617	−0.903	7.6552
10.0 k	Both	0.225	5.6050	−0.018	5.5413
	Right	0.692	7.5373	0.451	7.2778
	Left	−0.242	6.4773	−0.487	6.7188
12.5 k	Both	0.407	5.9864	0.451	5.7163
	Right	0.606	7.0388	0.397	6.8647
	Left	0.208	7.9468	0.505	7.5851
14.0 k	Both	0.182	6.3456	0.054	6.5626
	Right	0.277	7.9882	0.108	8.2195
	Left	0.087	8.4416	0.000	8.3623
16.0 k	Both	−0.164	7.8641	−0.752	7.7107
	Right	−0.087	11.4070	−1.250	10.3740
	Left	−0.242	9.2201	−0.271	10.2420
18.0 k	Both	0.900	6.5857	−0.316	7.1167
	Right	1.038	8.4756	−0.253	9.0954
	Left	0.761	8.3401	−0.379	8.9453
20.0 k	Both	0.355	4.1174	−0.181	4.1061
	Right	0.398	4.8338	0.108	5.3320
	Left	0.260	5.4439	−0.469	5.1566

have been chosen for this study. A dose of 1g orally t.i.d has been reported to be well tolerated over a 4 week period (Tirouvanziam et al., 2006). At a dose of 1800 mg, gastrointestinal side effects may become dose-limiting. For this particular study, funding was

Table 4
Threshold shift rates (%). The shaded rows indicate measurements that showed statistical significance.

Secondary outcomes 2 and 3			
Outcome	**Placebo**	**NAC**	**p-value (1-sided exact test)**
Trigger-hand ear − ASHA definition	27.56	21.56	0.0620
Trigger-hand ear − adapted navy definition	34.98	27.14	0.0288
Post-hoc analyses			
Outcome − ASHA definition	**Placebo**	**NAC**	**p-value (1-sided exact test)**
Non trigger-hand ear	17.67	21.56	0.8962
Left ear for left trigger-hand recruits	19.23	23.08	0.7507
Right ear for left trigger-hand recruits	11.54	23.08	0.9300
Left ear for right trigger-hand recruits	18.29	21.40	0.8376
Right ear for right trigger-hand recruits	28.40	21.40	0.0439
Outcome − adaption of navy definition	**Placebo**	**NAC**	**p-value (1-sided Exact Test)**
Left ear	33.56	29.60	0.1781
Right ear	34.60	27.08	0.0323
Either ear	53.29	46.57	0.0650
Both ears	14.88	10.11	0.0560
Non-trigger hand ear	32.16	30.86	0.4064
Left ear for left trigger hand recruits	26.92	26.92	0.6223
Right ear for left trigger hand recruits	19.23	34.62	0.9418
Left ear for right trigger hand recruits	34.46	30.45	0.2660
Right Ear for Right Trigger Hand Recruits	35.80	27.16	0.0237

Table 5
Secondary outcome 4 − tinnitus severity index comparison.

Parameter	Effect of NAC on change in parameter score (adjusting for initial value of meter)	
	Estimate	95% confidence interval
Make you feel irritable or nervous	0.005	(−0.101, 0.111)
Make you feel tired or stressed	0.058	(−0.044, 0.160)
Make it difficult for you to relax	−0.087	(−0.212, 0.038)
Make it uncomfortable to be in a quiet room	−0.054	(−0.162, 0.054)
Make it difficult to concentrate	−0.098	(−0.231, 0.035)
Make it harder to interact pleasantly with others	−0.015	(−0.109, 0.079)
Interfere with your required activities	0.015	(−0.052, 0.082)
Interfere with your social activities	−0.016	(−0.090, 0.058)
Interfere with your overall enjoyment of life	0.031	(−0.049, 0.111)
How much of an effort is it for you to ignore tinnitus when it is present	0.103	(−0.020, 0.226)
How much discomfort do you usually experience when your tinnitus is present	0.130	(0.010, 0.250)
Does your tinnitus interfere with sleep	0.048	(0.046, 0.142)
Overall tinnitus severity score[a]	−0.033	(−0.856, 0.790)

[a] Overall Tinnitus Severity Score calculated as the sum of the scores for the 12 items as conducted by Folmer and Griest, 2000.

not available to perform a larger dose response trial design with multiple doses. In the vast majority of articles cited in this paper, no non-monotonic dose–response effects were noted suggesting that the results in this study were not due to an excessive dose of NAC. Another challenge in this study was frequency of dose administration. Following oral administration, reduced N-acetylcysteine has a terminal half-life of 6.25 h (Holdiness, 1991). Because of the logistics of training, the drug could be administered only every eight hours and for part of the study every 12 h. This dosing schedule may have reduced effectiveness. A twice a day dosing schedule would likely have been less effective than an every eight hour schedule. The therapeutic effects of NAC are dose dependent and relatively high doses are required (Duan et al., 2004; Lorito et al., 2006, 2008). Therefore, to increase efficacy and decrease dose requirements, combining NAC with other agents may be a more effective treatment option (Choi et al., 2008; Coleman et al., 2010; Coleman J. K. et al., 2007, Ewert et al., 2012; Floyd et al., 2008; Kopke et al., 2000).

Extrapolated hearing recovery curves for noise-exposed, control, non-treated animals reveal an asymptotic curve that stabilizes at three weeks (Clifford et al., 2011). Under laboratory conditions, a final audiogram is uniformly performed at 21 days post noise (Clifford et al., 2011) to differentiate between a temporary threshold shift (TTS) which occurs immediately following noise, and permanent threshold shift (PTS) after stabilization and

Table 6
M-16 noise exposure levels. SPL measured at trigger and non trigger ear position (for a right-handed shooter).

Frequency (kHz)	SPL (dB) trigger ear	SPL (dB) non trigger ear	Difference (dB) (trigger ear − non trigger ear)
0.5	112	109	3
1.0	106	101	5
2.0	109	109	0
3.15	109	102	7
4.0	106	104	2
5.0	108	104	4
6.3	107	101	6
8.0	106	101	5
12.5	105	98	7
16.0	106	98	8

Table 7
Effect of NAC on mean change in hearing threshold (post − pre) student t-test and linear regression analysis (p-values unadjusted for multiple comparisons). The shaded rows indicate measurements that showed statistical significance.

Frequency − Hz	Ear(s)	t-test		Linear regression (controlling for initial hearing)	
		t-value	p-value	Parameter estimate	95% confidence interval
2 k	Both	1.20	0.2299	−0.3928	(−1.035, 0.250)
	Right	1.55	0.1205	−0.6174	(−1.448, 0.213)
	Left	0.29	0.7724	−0.1480	(−0.930, 0.634)
3 k	Both	0.10	0.9241	0.0937	(−0.630, 0.818)
	Right	0.11	0.9122	0.1547	(−0.712, 1.022)
	Left	0.04	0.9672	0.1312	(−0.804, 1.067)
4 k	Both	0.28	0.7821	−0.3019	(−1.249, 0.646)
	Right	−0.28	0.7815	−0.2423	(−1.384, 0.899)
	Left	0.72	0.4737	−0.4518	(−1.628, 0.724)
6 k	Both	2.13	0.0332	−0.8505	(−1.804, 0.103)
	Right	1.61	0.1086	−0.9111	(−2.065, 0.243)
	Left	1.65	0.0991	−0.7594	(−2.056, 0.537)
8 k	Both	1.75	0.0813	−0.7171	(−1.575, 0.140)
	Right	1.99	0.0473	−1.2608	(−2.366, −0.155)
	Left	0.70	0.4819	−0.0960	(−1.180, 0.988)
10 k	Both	0.52	0.6044	−0.2144	(−1.090, 0.662)
	Right	0.39	0.6994	0.0699	(−1.078, 1.218)
	Left	0.44	0.6587	−0.4376	(−1.456, 0.581)
12.5 k	Both	−0.09	0.9277	−0.0477	(−0.977, 0.881)
	Right	0.36	0.7216	−0.3022	(−1.401, 0.797)
	Left	−0.46	0.6488	0.1568	(−1.061, 1.375)
14 k	Both	0.24	0.8143	−0.2168	(−1.257, 0.823)
	Right	0.25	0.8047	−0.3356	(−1.625, 0.954)
	Left	0.12	0.9026	−0.1783	(−1.514, 1.157)
16 k	Both	0.90	0.3706	−0.7488	(−1.997, 0.499)
	Right	1.27	0.2058	−1.4303	(−3.153, 0.292)
	Left	0.03	0.9722	−0.2101	(−1.765, 1.344)
18 k	Both	2.11	0.0353	−1.2538	(−2.361, −0.147)
	Right	1.75	0.0811	−1.4275	(−2.811, −0.044)
	Left	1.57	0.1171	−1.1379	(−2.528, 0.252)
20 k	Both	1.55	0.1222	−0.4671	(−1.115, 0.180)
	Right	0.68	0.4983	−0.2795	(−1.061, 0.502)
	Left	1.63	0.1027	−0.5754	(−1.396, 0.245)

cochlear repair. Because the subjects finished their training shortly after the final audiogram and were assigned to different locations, longer term follow up was not possible. Therefore, it is possible that hearing loss recovery in this study had not stabilized and actually represented a TTS rather than PTS. Further studies should include follow-up audiograms on these Marines to ascertain whether their hearing improved and whether this was, in fact, a temporary or permanent threshold shift.

Another limitation included the lack of resources to evaluate serum NAC or cysteine levels in subjects to correlate a treatment effect with drug or metabolite level.

Other difficulties in translation from the experimental laboratory to human research include different short-term temporal patterns of noise administered in the lab versus a two-week exposure. This two-week period of rifle training has periods of quiet, and may establish a different equilibrium than that seen in the lab, allowing for establishment of a higher level of oxidative stress enzymes which maintains repair of the cochlea.

4.2. Generalisability

Since the study population was entirely male and of a specific age group, these results may not be generalisable.

4.3. Interpretation

While the primary outcome measures from this investigation suggest that taking NAC in the doses utilized for this study marginally reduced hearing loss in this noise-exposed population, some secondary outcome and post hoc testing suggested that NAC showed a small but statistically significant effect in reducing the rate of threshold shift in certain subgroup analyses. The non-parametric Sign test also showed that the NAC group had less hearing loss suggesting a treatment effect of NAC.

In this study, a 900 mg dosage of NAC administered three times a day proved to be safe and resulted in similar type and frequency of reported minor adverse events as placebo. Furthermore, taking one 1800 mg dosage of NAC in the morning and one 900 mg dosage of NAC in the evening is also safe, with no statistical difference observed in the type or frequency of reported adverse events. Thus, oral NAC is safe and well tolerated at this dose.

While a 10–11% rate of STS from noise exposure during recruit training is the norm (Marshall et al., 2009), our study population exhibited a greater than 37% rate of STS despite strict use of HPDs and a highly controlled environment free of other significant noise outside the weapons training period. The most likely reason for this high rate of threshold shift is that, in addition to the standard audiometric frequencies tested in other studies (Marshall et al., 2009), this study also measured hearing thresholds at high frequencies between 2 kHz and 20 kHz, inclusive. However, even 10–11 % is a major concern since warfighters in combat arms have many such training exercises per year and the noise injuries are likely to be cumulative.

Also, of considerable concern are the recent findings in a mouse model that noise injuries that produce only a TTS, and not a PTS, based on pure tone ABR testing, produce permanent cochlear injury that advances over time as well as permanent changes in wave I ABR amplitude (Kujawa and Liberman, 2009).

In examining the pure tone audiologic data, several interesting findings arise from this study. First, across both the placebo and NAC treatment groups, the majority of individual mean test threshold changes were uniformly small (Figs. 3 and 4, Table 3) which may signify that hearing protectors were mostly effective and/or that other factors underlie hearing loss susceptibility in those demonstrating larger STS. This will be a challenge for any future study using this type of pharmacological intervention research protocol. Thus, an approach whereby employing pre-test procedures to identify individuals that may have inherent risk factors for NIHL such as a gene chip assay test for gene polymorphisms or a more sensitive hearing test that is predictive of NIHL susceptibility is needed to focus intervention for sensitive subpopulations.

Another challenge noted in this study is that there were often “skip lesions” where there could be a shift in a single frequency but adjacent frequencies were unaffected making the assessment of averaged data difficult. The phenomenon of “skip lesions” has been previously reported by Fausti and colleagues (Fausti et al., 1981).

Another interesting finding in this study was the effect that trigger-handedness appeared to have on the data. This prompted us to measure actual ear level noise exposures with the M-16 rifle in a detailed fashion and revealed that the trigger hand ear was exposed to significantly more acoustic trauma (Table 6). This could increase the risk to the trigger hand ear for noise exposure and threshold shift. This may explain the ear difference in threshold shift rates particularly noted in the post hoc analysis since a treatment effect may be more likely in ears with greater risk of noise-induced shift. There were likely too few left handed subjects in this study to observe a statistically significant effect.

5. Conclusions

Since the contrast between the laboratory and this clinical study is evident, further analyses of mechanisms are warranted to

determine if a true clinical benefit can be obtained. Second, although the mean change in hearing for individuals was small, there was a high rate of STS in both groups.

In laboratory experiments on animals, NAC was administered i.p., which injects agents into the tissues of the abdomen, where it will be absorbed directly into the blood stream. The oral route utilized in clinical studies only has bioavailability for NAC of approximately 9%, significantly diminishing achievable NAC plasma levels (Ewert et al., 2012). However, the greater proportion of NAC is metabolized or deacetylated in the intestine to cysteine which is transported to cells where it is utilized for synthesis of glutathione (GSH) (Olsson et al., 1988). Thus cysteine/cystine levels are likely more critical than NAC levels. Although Bielefeld et al., (Bielefeld et al., 2007) found good response after NAC at 325 mg/kg i.p., when 325 mg/kg was administered intragastrically at the same dose, hearing showed significantly less recovery towards baseline.

The main pharmacological effect of NAC is to replace depleted intracellular glutathione by supplying cystine for intracellular synthesis. Based on preclinical work the most likely mechanism that may account for the positive post hoc results in this study is that the orally administered NAC is able to replenish depleted glutathione in the cochlea occurring as a consequence of noise exposure (Fechter et al., 2003; Ohinata et al., 2000b; Yamasoba et al., 1998).

A small clinical trial investigated NAC's capability for prevention of NIHL but measured TTS only. Kramer et al. noted no difference in NAC administered versus placebo in TTS following exposure to an average of 98 dBA for 2 h in a night club (Kramer et al., 2006). However, it is difficult to draw any meaningful conclusions from this study as the number of subjects was small, and there was considerable variability in hearing threshold data between subjects. Also, in this study only a single oral dose of NAC (900 mg) was given 30 min before noise exposure.

Further studies seem warranted to explore factors related to individual hearing loss susceptibilities (genetics or stress reactions), increasing dose and dose frequency, formulation issues, other pharmacokinetics parameters, and the possibility that NAC combined with other antioxidants may be more efficacious (Ewert et al., 2012; Kopke et al., 2000).

Acknowledgments

The Chief BUMED, Navy Department, Washington DC, Clinical Investigation Program Sponsored this report-CIP# S-03-082 as required by NSHSBETHINST 6000.41B. This work was supported by U.S. Army Medical Research Acquisition Activity (USAMRAA), U.S. Special Operations Command (USSOCOM), the Bureau of Medicine and Surgery (BUMED), the Office of Naval Research (ONR), and American BioHealth Group.

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Departments of the Navy, Army, or Department of Defense or the United States Government.

This work was prepared as part of our official duties. Title 17, USC, §105 provides that 'Copyright protection under this title is not available for any work of the U.S. Government.' Title 17, USC, §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

The full clinical trial protocol is archived in the Clinical Investigations Department at the Naval Medical Center San Diego, San Diego, CA.

References

Aruoma, O.I., Halliwell, B., Hoey, B.M., Butler, J., 1989. The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. Free Radic. Biol. Med. 6 (6), 593–597.

Bielefeld, E.C., Kopke, R.D., Jackson, R.L., Coleman, J.K., Liu, J., Henderson, D., 2007. Noise protection with N-acetyl-l-cysteine (NAC) using a variety of noise exposures, NAC doses, and routes of administration. Acta Otolaryngol. 127 (9), 914–919. http://dx.doi.org/10.1080/00016480601110188.

CDC, 2011. Severe hearing impairment among military veterans–United States, 2010. MMWR. Morb. Mortal. Wkly. Rep. 60 (28), 955–958.

Choi, C.H., Chen, K., Vasquez-Weldon, A., Jackson, R.L., Floyd, R.A., Kopke, R.D., 2008. Effectiveness of 4-hydroxy phenyl N-tert-butylnitrone (4-OHPBN) alone and in combination with other antioxidant drugs in the treatment of acute acoustic trauma in chinchilla. Free Radic. Biol. Med. 44 (9), 1772–1784. http://dx.doi.org/10.1016/j.freeradbiomed.2008.02.005.

Clifford, R.E., Coleman, J.K., Balough, B.J., Liu, J., Kopke, R.D., Jackson, R.L., 2011. Low-dose D-methionine and N-acetyl-L-cysteine for protection from permanent noise-induced hearing loss in chinchillas. Otolaryngol. Head Neck Surg.: Off. J. Am. Acad. Otolaryngol. Head Neck Surg. 145 (6), 999–1006. http://dx.doi.org/10.1177/0194599811414496.

Coleman, J., Huang, X., Liu, J., Kopke, R., Jackson, R., 2010. Dosing study on the effectiveness of salicylate/N-acetylcysteine for prevention of noise-induced hearing loss. Noise Health 12 (48), 159–165. http://dx.doi.org/10.4103/1463-1741.64972.

Coleman, J.K., Kopke, R.D., Liu, J., Ge, X., Harper, E.A., Jones, G.E., Cater, T.L., Jackson, R.L., 2007. Pharmacological rescue of noise induced hearing loss using N-acetylcysteine and acetyl-L-carnitine. Hear Res. 226 (1–2), 104–113. http://dx.doi.org/10.1016/j.heares.2006.08.008.

Doosti, A., Lotfi, Y., Moossavi, A., Bakhshi, E., Talasaz, A.H., Hoorzad, A., 2014. Comparison of the effects of N-acetyl-cysteine and ginseng in prevention of noise induced hearing loss in male textile workers. Noise Health 16 (71), 223–227. http://dx.doi.org/10.4103/1463-1741.137057.

Duan, M., Qiu, J., Laurell, G., Olofsson, A., Counter, S.A., Borg, E., 2004. Dose and time-dependent protection of the antioxidant N-L-acetylcysteine against impulse noise trauma. Hear Res. 192 (1–2), 1–9. http://dx.doi.org/10.1016/j.heares.2004.02.005.

Ewert, D.L., Lu, J., Li, W., Du, X., Floyd, R., Kopke, R., 2012. Antioxidant treatment reduces blast-induced cochlear damage and hearing loss. Hear Res. 285 (1–2), 29–39. http://dx.doi.org/10.1016/j.heares.2012.01.013.

Fausti, S.A., Erickson, D.A., Frey, R.H., Rappaport, B.Z., Schechter, M.A., 1981. The effects of noise upon human hearing sensitivity from 8000 to 20,000 Hz. J. Acoust. Soc. Am. 69 (5), 1343–1347.

Fechter, L.D., Klis, S.F., Shirwany, N.A., Moore, T.G., Rao, D.B., 2003. Acrylonitrile produces transient cochlear function loss and potentiates permanent noise-induced hearing loss. Toxicol. Sci.: Off. J. Soc. Toxicol. 75 (1), 117–123. http://dx.doi.org/10.1093/toxsci/kfg169.

Fetoni, A.R., Ralli, M., Sergi, B., Parrilla, C., Troiani, D., Paludetti, G., 2009. Protective effects of N-acetylcysteine on noise-induced hearing loss in guinea pigs. Acta Otorhinolaryngol. Ital. 29 (2), 70–75.

Floyd, R.A., Kopke, R.D., Choi, C.H., Foster, S.B., Doblas, S., Towner, R.A., 2008. Nitrones as therapeutics. Free Radic. Biol. Med. 45 (10), 1361–1374. http://dx.doi.org/10.1016/j.freeradbiomed.2008.08.017.

Folmer, R.L., Griest, S.E., 2000. Tinnitus and insomnia. Am. J. Otolaryngol. 21 (5), 287–293. http://dx.doi.org/10.1053/ajot.2000.9871.

Gillissen, A., Jaworska, M., Orth, M., Coffiner, M., Maes, P., App, E.M., Cantin, A.M., Schultze-Werninghaus, G., 1997. Nacystelyn, a novel lysine salt of N-acetylcysteine, to augment cellular antioxidant defence in vitro. Respir. Med. 91 (3), 159–168.

Holdiness, M.R., 1991. Clinical pharmacokinetics of N-acetylcysteine. Clin. Pharmacokinet. 20 (2), 123–134. http://dx.doi.org/10.2165/00003088-199120020-00004.

Humes, L.E., Joellenbeck, L.M., Durch, J.S. (Eds.), 2005. Noise and Military Service: Implications for Hearing Loss and Tinnitus. The National Academies Press, Washington, DC.

Kil, J., Pierce, C., Tran, H., Gu, R., Lynch, E.D., 2007. Ebselen treatment reduces noise induced hearing loss via the mimicry and induction of glutathione peroxidase. Hear. Res. 226 (1–2), 44–51. http://dx.doi.org/10.1016/j.heares.2006.08.006.

Kopke, R.D., Allen, K.A., Henderson, D., Hoffer, M., Frenz, D., Van de Water, T., 1999. A radical demise. Toxins and trauma share common pathways in hair cell death. Ann. N. Y. Acad. Sci. 884, 171–191.

Kopke, R.D., Bielefeld, E., Liu, J., Zheng, J., Jackson, R., Henderson, D., Coleman, J.K., 2005. Prevention of impulse noise-induced hearing loss with antioxidants. Acta Otolaryngol. 125 (3), 235–243.

Kopke, R.D., Coleman, J.K., Liu, J., Campbell, K.C., Riffenburgh, R.H., 2002. Candidate's thesis: enhancing intrinsic cochlear stress defenses to reduce noise-induced hearing loss. Laryngoscope 112 (9), 1515–1532. http://dx.doi.org/10.1097/00005537-200209000-00001.

Kopke, R.D., Weisskopf, P.A., Boone, J.L., Jackson, R.L., Wester, D.C., Hoffer, M.E., Lambert, D.C., Charon, C.C., Ding, D.L., McBride, D., 2000. Reduction of noise-induced hearing loss using L-NAC and salicylate in the chinchilla. Hear Res. 149 (1–2), 138–146.

Kramer, S., Dreisbach, L., Lockwood, J., Baldwin, K., Kopke, R., Scranton, S., O'Leary, M., 2006. Efficacy of the antioxidant N-acetylcysteine (NAC) in protecting ears exposed to loud music. J. Am. Acad. Audiol. 17 (4), 265–278.

Kujawa, S.G., Liberman, M.C., 2009. Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J. Neurosci. 29 (45), 14077–14085. http://dx.doi.org/10.1523/JNEUROSCI.2845-09.2009.

Lin, C.Y., Wu, J.L., Shih, T.S., Tsai, P.J., Sun, Y.M., Ma, M.C., Guo, Y.L., 2010. N-Acetylcysteine against noise-induced temporary threshold shift in male workers. Hear Res. 269 (1–2), 42–47. http://dx.doi.org/10.1016/j.heares.2010.07.005.

Lindblad, A.C., Rosenhall, U., Olofsson, A., Hagerman, B., 2011. The efficacy of N-acetylcysteine to protect the human cochlea from subclinical hearing loss caused by impulse noise: a controlled trial. Noise Health 13 (55), 392–401. http://dx.doi.org/10.4103/1463-1741.90293.

Lorito, G., Giordano, P., Prosser, S., Martini, A., Hatzopoulos, S., 2006. Noise-induced hearing loss: a study on the pharmacological protection in the Sprague Dawley rat with N-acetyl-cysteine. Acta Otorhinolaryngol. Ital. 26 (3), 133–139.

Lorito, G., Giordano, P., Petruccelli, J., Martini, A., Hatzopoulos, S., 2008. Different strategies in treating noise induced hearing loss with N-acetylcysteine. Med. Sci. Monit. 14 (8), BR159–164.

Lynch, E.D., Kil, J., 2005. Compounds for the prevention and treatment of noise-induced hearing loss. Drug Discov. Today 10 (19), 1291–1298. http://dx.doi.org/10.1016/S1359-6446(05)03561-0.

Marshall, L., Lapsley Miller, J.A., Heller, L.M., Wolgemuth, K.S., Hughes, L.M., Smith, S.D., Kopke, R.D., 2009. Detecting incipient inner-ear damage from impulse noise with otoacoustic emissions. J. Acoust. Soc. Am. 125 (2), 995–1013. http://dx.doi.org/10.1121/1.3050304.

McCullagh, M.C., Raymond, D., Kerr, M.J., Lusk, S.L., 2011. Prevalence of hearing loss and accuracy of self-report among factory workers. Noise Health 13 (54), 340–347. http://dx.doi.org/10.4103/1463-1741.85504.

Ohinata, Y., Miller, J.M., Schacht, J., 2003. Protection from noise-induced lipid peroxidation and hair cell loss in the cochlea. Brain Res. 966 (2), 265–273.

Ohinata, Y., Miller, J.M., Altschuler, R.A., Schacht, J., 2000a. Intense noise induces formation of vasoactive lipid peroxidation products in the cochlea. Brain Res. 878 (1–2), 163–173.

Ohinata, Y., Yamasoba, T., Schacht, J., Miller, J.M., 2000b. Glutathione limits noise-induced hearing loss. Hear. Res. 146 (1–2), 28–34.

Olsson, B., Johansson, M., Gabrielsson, J., Bolme, P., 1988. Pharmacokinetics and bioavailability of reduced and oxidized N-acetylcysteine. Eur. J. Clin. Pharmacol. 34 (1), 77–82.

Poderoso, J.J., Boveris, A., Cadenas, E., 2000. Mitochondrial oxidative stress: a self-propagating process with implications for signaling cascades. Biofactors 11 (1–2), 43–45.

Prosser, S., Tartari, M.C., Arslan, E., 1988. Hearing loss in sports hunters exposed to occupational noise. Br. J. Audiol. 22 (2), 85–91.

Puel, J.L., Ruel, J., Gervais d'Aldin, C., Pujol, R., 1998. Excitotoxicity and repair of cochlear synapses after noise-trauma induced hearing loss. Neuroreport 9 (9), 2109–2114.

Rewerska, A., Pawelczyk, M., Rajkowska, E., Politanski, P., Sliwinska-Kowalska, M., 2013. Evaluating D-methionine dose to attenuate oxidative stress-mediated hearing loss following overexposure to noise. Eur. Arch. Oto-Rhino-Laryngol.: Off. J. Eur. Fed. Oto-Rhino-Laryngol. Soc. 270 (4), 1513–1520. http://dx.doi.org/10.1007/s00405-012-2265-3.

Saunders, G.H., Griest, S.E., 2009. Hearing loss in veterans and the need for hearing loss prevention programs. Noise Health 11 (42), 14–21.

Shaw, G., Trost, R., 2005. Statistical Analysis of Hearing Loss Among Navy Personnel. Center for Naval Analysis. CRM D0011228.A2/Final.

Tirouvanziam, R., Conrad, C.K., Bottiglieri, T., Herzenberg, L.A., Moss, R.B., 2006. High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis. Proc. Natl. Acad. Sci. U. S. A. 103 (12), 4628–4633. http://dx.doi.org/10.1073/pnas.0511304103.

Wu, H.P., Hsu, C.J., Cheng, T.J., Guo, Y.L., 2010. N-acetylcysteine attenuates noise-induced permanent hearing loss in diabetic rats. Hear Res. 267 (1–2), 71–77. http://dx.doi.org/10.1016/j.heares.2010.03.082.

Yamane, H., Nakai, Y., Takayama, M., Konishi, K., Iguchi, H., Nakagawa, T., Shibata, S., Kato, A., Sunami, K., Kawakatsu, C., 1995. The emergence of free radicals after acoustic trauma and strial blood flow. Acta Oto-laryngol. Suppl. 519, 87–92.

Yamasoba, T., Harris, C., Shoji, F., Lee, R.J., Nuttall, A.L., Miller, J.M., 1998. Influence of intense sound exposure on glutathione synthesis in the cochlea. Brain Res. 804 (1), 72–78.