Distortion Product Otoacoustic Emission (DPOAE) as an Appropriate Tool in Assessment of Otoprotective Effects of Antioxidants in Noise-Induced Hearing Loss (NIHL)

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Abstract Distortion product otoacoustic emission (DPOAE) appears to be an objective sensitive test of cochlear function. The aim of this study was to investigate whether DPOAE is an appropriate tool for assessment of minute changes in cochlea due to usage of antioxidant material. 48 workers exposed to continuous noise in a textile factory were randomly assigned into three groups: (1) The Control group ($n = 16$) received no antioxidant drugs, (2) The N-acetyl-cysteine (NAC) group ($n = 16$) received oral antioxidant NAC (1200 mg/day), (3) The Ginseng group ($n = 16$) received oral antioxidant Ginseng (200 mg/day). All three groups had a follow-up period of 2 weeks. The cochlear changes were assessed using DPOAE test before starting the daily work shift on first and 15th day. The associations between groups and DPOAE amplitudes after 2 weeks were analyzed using linear regression analysis. Four separate models were fitted by side of ears and frequency. All models were adjusted for baseline amplitude. Reduced (better) amplitude at DPOAE test was found for NAC and Ginseng groups at high frequencies (4 and 6 kHz) in both ears after 2 weeks compared to control group. Moreover, NAC group showed better DPOAE amplitude than Ginseng group. In

conclusion, DPOAE seems to be an appropriate tool in assessing minute changes in the cochlea after antioxidant drugs administration.

Keywords

Distortion product otoacoustic emission (DPOAE) ·
Antioxidants · Noise-induced hearing loss (NIHL) ·
N-Acetyl-cysteine (NAC) · Ginseng

Introduction

Noise-induced hearing loss (NIHL), ranks high incidence in comparison to the other occupational diseases. It is also the second most frequent type of sensory-neural hearing loss (SNHL) after presbycusis and imposes financial damage to the society [1].

In addition to mechanical damage, metabolic disorders have important role in NIHL. Exposure to noise, causes increase of reactive oxygen species (ROS) and free radicals in cochlea, which due to over activation of mitochondria, enzymatic reactions (like xanthine oxidase), ischemic/reperfusion and excitotoxicity [2]. These free radicals are changed to hydroxyl radicals in stria vascularis with high destructive effect [3].

Reactive oxygen species cause damage to the hair cells of the cochlea [4]. The primary results will be temporary threshold shift (TTS) lasting about several days or even weeks after noise exposure [5, 6], then converts to permanent hearing loss (PTS) over the time [7].

The cochlear hair cells are divided into two types: outer hair cells (OHCs) and inner hair cells (IHCs). OHCs are the most sensitive and susceptible sensory cells of the cochlea to noise overexposure. OHCs' activities are evaluated by otoacoustic emissions (OAEs) [8]. Distortion product OAE

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(DPOAE) is specially a sensitive method for assessing fine changes in OHCs function [8–11] and monitoring cochlear conditions in specific frequency regions because it results from localized sources all along the cochlea [12].

Recently, studies on cellular basis of NIHL have led to new strategies for the use of antioxidants to prevent damage resulting from ROS in cochlea [13, 14]. N-Acetylcysteine (NAC) and Ginseng (*Panax ginseng* Meyer) both are antioxidants, NAC increases glutathione production [15] and Ginseng has an anti-ROS virtue [16, 17].

We aimed to investigate the efficacy of DPOAE in evaluation of otoprotective effects of NAC and Ginseng in male textile factory workers exposed to noise.

Methods

Study Group

The study group consists of 48 male volunteer textile factory workers aged 28–50 years. They are working 8 h a day. The participants are exposed to 88–89 decibel A (dBA) continuous noise (measured by Sound Level Meter, SLM) and don't use any hearing protection devices. This research was performed according to the guidelines of Helsinki declaration and approved by committee of ethics of the University of Social Welfare and rehabilitation sciences, Tehran.

Subjects were randomly assigned to three equal groups by random number generator software; a control group consisted of workers who didn't receive any antioxidant drugs; NAC group (received NAC 1200 mg/day); Ginseng group (received KRG 200 mg/day). All three groups had a follow-up period of 14 days (2 weeks). An explanation about the tests and safetiness of drugs were given to each subject before the study while the grouping was not clear to them. All the subjects signed informed consent.

Inclusion criteria for the subjects in three groups were (a) age between 18 and 50 years (b) DPOAE amplitude less (better) than 35 dB sound pressure level (dB SPL) on both ears (c) no previous history of acoustic trauma (d) not taking antioxidant drugs (or supplements) and/or ototoxic drugs for the past 2 months.

All subjects were working in morning shift. Otoscopy, tympanometry and DPOAE test were performed for all subjects prior to grouping to rule out any external and middle ear pathology before taking drug. DPOAE test was conducted in a quiet examination room by an audiologist blinded to the study.

Distortion product otoacoustic emission measurements were performed using a Madsen Capella instrument at 1, 2, 4 and 6 kHz. The frequency ratio was adjusted to

$f2/f1 = 1.2$ for all participants. Also $f2$ and $f1$ level fixed at moderate level (65 and 55 dB respectively). Signal to noise ratios (SNRs) were accepted valid when exceeding 6.

We used Larson Davis Model 824, Provo, Utah SLM for measuring noise level and Madsen GN Otometrics tympanometry instrument for evaluating of external and middle ear status.

Then NAC and Ginseng was administrated to the proper groups before work shift. The subjects were asked to report any undesired effect during intervention. After 2 weeks (on 15th day) DPOAE test was repeated for all participants again.

Statistical Analysis

Results of DPOAE were entered into a computerized database making use of only numerical study identifiers. One-way analysis of variance (ANOVA) was utilized to compare DPOAE amplitudes in three groups before drug administration.

The associations between groups and DPOAE amplitudes after two weeks were analyzed using Linear Regression Analysis. Four separate models were fitted by side of ears and frequency. All models were adjusted for baseline amplitude.

Interaction between group and baseline amplitude were specifically considered and nonsignificant terms were excluded.

Statistical analysis were performed using SPSS version 16 (SPSS Inc., Chicago) software program and p value < 0.05 was considered statistically significant.

Results

Forty-eight subjects completed the study. The mean age of workers was 39.12 (± 5) years. They had worked in this textile factory for an average of 14.48 (± 6) years. There weren't any statistically significant difference in mean age and noise exposure history (employment) between three groups (Table 1). All participants had normal otoscopy and tympanometry in both ears. The baseline DPOAE amplitudes between three groups before drug administration in right and left ears respectively are shown in Tables 2 and 3. There were no significant differences between DPOAE amplitudes of three groups ($p > 0.05$).

After drug administration (for 2 weeks), DPOAE amplitude showed significant differences between three groups at 4 and 6 kHz ($p < 0.001$). Linear regression was adjusted for DPOAE amplitude before noise exposure.

Using the controls as the reference group, regression model at frequency of 4 kHz showed:

Table 1 Demographic characteristic (n = 48, male)

	Mean $\pm$ SD				<i>p</i> value*
	NAC receivers	Gin** receivers	Control group	Total	
Age (years)	38.38 $\pm$ 4.4	39.38 $\pm$ 6.2	39.62 $\pm$ 4.6	39.12 $\pm$ 5.0	0.77
Employment (years)	15.88 $\pm$ 4.9	13.19 $\pm$ 6.2	14.37 $\pm$ 7.2	14.48 $\pm$ 6.0	0.48

p value* by ANOVA analysis in three groups

Gin** demonstrates Ginseng

Table 2 Comparison of before (baseline) DPOAE amplitudes between three groups in right ear

	Mean $\pm$ SD				<i>p</i> value*
	Frequency (kHz)	NAC receivers	Gin receivers	Control group	
1		0.83 $\pm$ 3.1	1.08 $\pm$ 2.5	1.05 $\pm$ 2.7	0.96
2		0.86 $\pm$ 4.2	0.94 $\pm$ 4.4	1.01 $\pm$ 3.5	0.99
4		−2.02 $\pm$ 5.2	−1.70 $\pm$ 4.5	−1.62 $\pm$ 3.2	0.92
6		−1.10 $\pm$ 5.2	−1.34 $\pm$ 5.0	−1.20 $\pm$ 4.5	0.99

p value* by ANOVA analysis**Table 3** Comparison of before (baseline) DPOAE amplitudes between three groups in left ear

	Mean $\pm$ SD				<i>p</i> value*
	Frequency (kHz)	NAC receivers	Gin receivers	Control group	
1		0.95 $\pm$ 3.4	1.04 $\pm$ 3.4	1.03 $\pm$ 2.9	0.99
2		0.79 $\pm$ 3.5	0.86 $\pm$ 3.9	0.82 $\pm$ 3.8	0.98
4		−1.07 $\pm$ 4.6	−1.80 $\pm$ 5.3	−1.63 $\pm$ 3.4	0.94
6		−2.20 $\pm$ 5.7	−1.93 $\pm$ 5.2	−1.34 $\pm$ 5.1	0.89

p value* by ANOVA analysis*In Right Ear*

$$\text{After} = -0.40 + 0.93(\text{NAC}) + 0.75(\text{Ginseng}) + 1.0(\text{before})$$

In Left Ear

$$\text{After} = -0.36 + 0.84(\text{NAC}) + 0.70(\text{Ginseng}) + 0.99(\text{before})$$

Using the controls as the reference group, regression model at frequency of 6 kHz show:

In Right Ear

$$\text{After} = -0.34 + 0.77(\text{NAC}) + 0.72(\text{Ginseng}) + 1.0(\text{before})$$

In Left Ear

$$\text{After} = -0.31 + 0.76(\text{NAC}) + 0.69(\text{Ginseng}) + 1.0(\text{before})$$

The calculated amplitude after NAC usage increased in comparison with control group, by 0.93 and 0.84 dB SPL at 4 kHz in right and left ears, also 0.77 and 0.76 dB SPL at 6 kHz in right and left ears respectively.

Besides, the calculated amplitude after Ginseng usage increased in comparison with control group by 0.75 and

0.70 dB SPL at 4 kHz in right and left ears, also 0.72 and 0.69 dB SPL at 6 kHz in right and left ears respective.

Discussion

Using the objective methods in assessing hearing status is uncommon in industry, despite having high value in uncooperative subjects. Evoked potential tests and steady state responses are some kinds of these methods, but need of high technology regarding recording parameters and time consuming are their problems. In contrast, OAE (DPOAE) is known as a fast test of evaluating early cochlear damage due to noise exposure [18, 19]. Exposure to noise causes damage of OHCs. As DPOAE directly measures OHCs function, it's amplitude will be influenced and depressed after noise exposure [20].

Previous study has shown that noise decreases the level of GSH in the inner ear [21]. NAC can reduce NIHL by increasing the intracellular levels of GSH, and scavenging ROS [22]. Our study showed that DPOAE amplitude at high frequencies (4 and 6 kHz) reduced in subjects receiving antioxidant NAC. It has been reported that early damage of noise typically appears at high frequencies first

[23] and the most susceptible region to noise exposure is 3–6 kHz [24]. Our result is consistent with those of Lin et al. [25] human clinical trial, used pure tone audiometry (PTA) test in his study which showed oral administration of NAC at 1,200 mg/day for 2 weeks induce reduction in noise-induced TTS at high frequencies (4.6 and 8 kHz). Another study by Kramer et al. [26], used DPOAE test demonstrated no statistically significant difference in TTS found between the NAC receivers (900 mg 30 min before live music exposure) and placebo receivers. The different result of this study with ours may be related to the rate of administration (given only once) and low dosage (900 mg) of NAC.

We found small but significant decrease in DPOAE amplitude at high frequencies (4 and 6 kHz) in participants receiving Ginseng. In an animal model, Kang et al. (2010) studied the effects of Korean Red Ginseng (KRG) in noise-induced TTS and observed fast recovery of hearing in mice fed with KRG (200 mg/kg) one hour and one day after noise exposure for 3 days and concluded that KRG is an inhibitor of TTS after noise exposure. They suggested the following reasons; (1) an anti-ROS effect of KRG and (2) lack of expression of 8-oxoguanine in the stria vascularis of inner ears of animals fed with KRG [27].

As some studies have pointed that DPOAE evaluation in moderate level induces the fewest errors in results [28, 29], we feel our results enjoy high validity since DPOAE measurement was performed in the moderate level (65 and 55 dB).

In conclusion, the DPOAE is an opportunity to assess cochlear minute changes specifically for OHCs function. Its advantage is objectivity, accuracy, noninvasiveness and efficacy, fastness and not requires the acoustical room. Our findings suggest the employment of DPOAE as part of hearing evaluation test battery in routine screening and diagnostic tests for noise exposure evaluation of effects of antioxidant drugs on cochlea. Generalization of our results concerning the protective effects of these two drugs needs trials with different dosage of them in larger population.

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