The monitoring role of otoacoustic emissions and oxidative stress markers in the protective effects of antioxidant administration in noise-exposed subjects: A pilot study

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Summary

Background:
Oxidative stress has been recently identified as the pivotal pathway of cochlear damage. The aims of this study were to evaluate whether distortion product otoacoustic emissions (DPOAEs) can discriminate normal subjects with a risk of damage induced by sound exposure, the effectiveness of OAEs in monitoring the protective effects of Coenzyme Q10 terclatrate (QTer®), and the role of blood parameters in monitoring preventive therapies.

Material/Methods:
Twenty volunteers were randomized to two groups: the first (n=10) was treated with Q-Ter® (200 mg orally once daily) for 7 days before noise exposure and the second group was treated with placebo using the same schedule. All participants were exposed to white noise of 90 dB HL for 15 minutes. DPOAEs and pure-tone audiometry (PTA) were measured before and 1 h, 16 h, and 7 and 21 days after exposure. Inflammatory and oxidative stress parameters were measured before and 2 and 24 h after exposure.

Results:
In the placebo group, DPOAE amplitudes were reduced 1 and 16 h after exposure compared with the baseline values (p<0.05). In the Q-Ter® group, DPOAEs did not show any significant difference between baseline and post-exposure (p>0.1). PTA threshold values in the Q-Ter® and placebo groups did not differ before and after exposure. No significantly different levels of the inflammatory markers were observed in the Q-Ter® and placebo groups at the different time points.

Conclusions:
This pilot study confirms that DPOAEs represent a sensitive test for monitoring the effects of noise in preclinical conditions and pharmacological treatment.

key words: Coenzyme Q-Ter® • otoacoustic emissions • hearing loss • noise • oxidative stress markers

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BACKGROUND

Noise-induced hearing loss (NIHL) is a major cause of hearing disability, accounting for about 16% of all disabling hearing losses in the adult population worldwide [1]. In the EU alone, 7% of workers report that work affects their health in the form of hearing disorders, and the cost of NIHL represents about 10% of the total compensation costs for occupational diseases. The European Agency for Safety and Health at Work estimates that the cost of untreated hearing loss to Europe ranged from €78 to 92 billion (http://osha.europa.eu). Prevention of NIHL is based on several hearing-preservation methods, such as reduction of noise sources, the use of hearing-protection devices, the development of hearing-loss screening, and basic education for the high-risk population. Such hearing-preservation programs are, however, still difficult to operate for specific groups of workers, such as individuals working on construction sites or soldiers involved in firearm use or war actions. Moreover, the continuing spread of personal music devices, such as mp3 players, among youngsters exposes these subjects to higher risks of hearing loss development. In recent years, significant contributions to identify the underlying pathways of damage have been posted, with new perspectives for clinical prevention and treatment. Death of hair cells after acoustic trauma may be due to direct mechanical trauma and/or a result of increased metabolic activity in the inner ear [2].

Several studies have shown that the generation of reactive oxygen species (ROS) and free radicals is involved in the cascade of cochlear events that induces acoustic trauma [3]. Depending on the severity, frequency, duration, and temporal characteristics of noise (impulse noise versus continuous noise), the effects on the cochlea range from a moderate disappearance of the hair cell stereocilia and moderate damage of the stria vascularis and lateral wall to a complete fracture of the organ of Corti and rupture of the Reissner’s membrane [4]. The outer hair cells (OHCs) are the most vulnerable elements in the inner ear; signs of apoptosis have been detected at this level a few minutes after noise over-exposure [5]: 24 h later a larger number of OHCs showed caspase 3 activation, while 48 h after noise exposure several nuclei were found to be positive in the TUNEL assay, indicating a massive activation of apoptosis [6].

Evoked otoacoustic emissions (EOAEs) represent an accurate, objective, fast, and noninvasive tool for assessing OHC function in experimental and clinical studies [7]. Data available in the literature suggest that EOAE measurement could be a useful method compared to conventional pure-tone audiometry (PTA) in the monitoring of both cochlear changes in a subject exposed to noise and the effects of preventive therapeutic interventions acting on OHC function. In a recent paper, DPOAEs (distortion product otoacoustic emissions) measured with a standard technique in a group of young workers exposed to brief occupational noise had good sensitivity and specificity for higher frequencies [8].

Different approaches to reduce or prevent the effects of noise have been employed in experimental models, ranging from restoration of the ROS balance using antioxidants or substrates for antioxidant synthesis and limitation of the amount of lipid peroxidation that occurs in the organ of Corti after noise trauma to inhibition of selected pathways that result in apoptotic cell death of the OHCs [9]. It has been also demonstrated that ROS trigger oxidative stress and the activation of mitogen-activated protein kinase (MAPK). This, in turn, increases c-Jun N-terminal kinase (JNK) signal transduction, involved in the production of proinflammatory cytokines which enhance tumor necrosis factor (TNFα) expression in the OHCs and finally trigger apoptosis [9,10]. Signs of inflammation leading to necrosis have been demonstrated in the cochlea a few minutes after noise exposure [5]. Increased antioxidant levels in the organ of Corti following oxidative stress induced by higher mitochondrial activity, glutamate excitotoxicity, and ischemia/reperfusion insult represents a rational approach against NIHL [3]; many antioxidant agents have been tested successfully. The results obtained in experimental models and supported by electrophysiological, immunohistochemical, and morphological studies encourage their clinical use; however, only a few methods can be used for testing the effectiveness of antioxidant drugs in humans, and a few clinical trials have been carried out these years.

The aims of this paper are: 1) to evaluate whether DPOAEs can discriminate among normal hearing subjects (young age, no systemic and/or ear pathologies, no occupational noise exposure) those with early cochlear damage due to loud sound exposure, 2) to assess the effectiveness of EOAEs in monitoring the protective effects of Coenzyme Q-Ter® (CoQ-Ter®), an antioxidant drug that was shown to prevent NIHL and OHC mitochondrial dysfunction in a previous experimental study [6], and 3) to evaluate whether systemic inflammatory and antioxidant parameters could be an adjunctive and effective method of monitoring the effects of drug administration to prevent NIHL in clinical practice. Preliminary data obtained comparing Q-Ter® and placebo in volunteers might represent the basis for an extensive use of this protocol in a clinical trial to evaluate the effectiveness of oral administration of CoQ-Ter® against NIHL in young workers exposed to noise.

MATERIAL AND METHODS

Subjects

Twenty young volunteer male students (40 ears) between 23 and 28 years old (mean: 26.4 years) were enrolled in this study. None of the subjects had a history of treatment with ototoxic drugs, shooting experiences, systemic disease such as diabetes, or any past or present middle ear infection. All subjects had a negative history for occupational or hobby noise exposure and were fully informed of the aim, design, and clinical applications of this study. The care of the human subjects was approved by the Ethics Committee of Turin University and the investigations were performed in accordance with the principles of the Declaration of Helsinki.

A general otolaryngological examination and standard 226-Hz probe tone tympanometry were performed in every subject to exclude middle ear pathologies. Conventional pure-tone audiometry was carried out in a soundproof room and the pure-tone thresholds of each ear were measured at frequencies of 0.125, 0.25, 0.50, 1, 2, 3, 4, 6, and 8 kHz with an Interacoustics audiometer. Subjects with pure-tone thresholds greater than 20 dB at any of the tested frequencies were excluded from the study.
DPOAE measurements

DPOAEs were recorded in a sound-attenuated chamber by an ILO-92 instrument (OtoDynamics) as previously shown in Di Girolamo et al. [11]. The acoustic stimuli were two 70-dB SPL equilevel \((L_{1e}-L_{2e})\) primary signals \(f_1\) and \(f_2\) simultaneously delivered through a catheter inserted into the external auditory canal, with an automatically determined \(f_2/f_1\) ratio of 1.22. Nine pairs of stimuli were used corresponding to \(f_2\) frequencies of 1001, 1527, 2002, 2515, 3174, 4004, 5042, and 6348 Hz. For each pair of stimuli the DPOAE level was measured (as sinusoidal acoustic emission) and the frequency was calculated using the equation \(2f_2-f_1\). Data were evaluated by assuming that \(f_1\) corresponded to the specific cochlear region considered mainly responsible for the distortion products. DPOAEs were measured and recorded as the average of four separate spectral averages of each stimulus condition. The noise level was measured at a frequency of 50 Hz above the DPOAE frequency using similar averaging techniques. For graphic display, a plot of mean DPOAE levels was constructed as a function of the stimuli (DPgram).

Blood tests

Blood samples were collected via venipuncture of the antecubital vein before and 2 and 24 h after Q-Ter® or placebo administration and noise exposure. Blood was collected using large-bore catheters (21 gauge) in tubes with gel and clot activator (serum samples), in heparin tubes (plasma sample), and in heparin tubes containing 8 mM N-Ethylmaleimide (NEM) (samples for nitrite determination). The blood samples were centrifuged soon after collection. For nitrite determination, the blood samples were processed using specific methodologies to limit artificial ex vivo hemolysis and levels of plasma hemoglobin. The plasma and serum samples were immediately stored at \(-80^\circ\text{C}\) until used. The analysis was performed at the University of Rome and the samples were stored in ice during transport.

Inflammatory biomarkers (HCy, hsCRP, SAA, ceruloplasmin, and RBP) were determined in the serum samples by laser nephelometry using a Dade Behring BN II Analyzer. Total antioxidant capacity (TEAC) was determined in the serum samples using a method developed by Rice-Evans and Miller [12]. The method is based on the antioxidant inhibition of the absorbance of the radical cation 2,2’-azinobis (3-ethylbenzothiazoline-6-sulphonate) (ABTS•+) formed by the interaction between ABTS and ferrylmyoglobin radical species, generated by the activation of metamyoglobin with \( \text{H}_2\text{O}_2\). Absorbance was measured with a Hewlett-Packard 8450A UV/Vis spectrophotometer (Palo Alto, CA, USA) equipped with a cuvette stirring apparatus and a constant-temperature cell holder. Nitrite concentration was determined in the plasma samples by adapting the Griess method to a chromatographic procedure. The chromatographic apparatus consisted of an HP 1100 series HPLC system (Hewlett-Packard) equipped with a diode-array detector. Coenzyme Q10 was determined with the method of Lippa et al. [13]. Briefly, 0.5 ml of plasma was extracted three times with 4 ml of aceton. The pooled extracts were evaporated to dryness. The residue was redissolved in 100 ml of absolute ethanol and 20 ml of this solution was injected into the HPLC (Beckman Gold) with the operating conditions col-
parisons were assessed with Tukey’s test (Sigmastat, USA). A p-value of <0.05 was considered significant.

**RESULTS**

**Audiological evaluation**

Before treatment and noise exposure, the DPOAE amplitudes in the subjects treated with Q-Ter® and placebo did not differ (F=1.420, P>0.254). No DPOAE amplitude modifications were observed within the test. Comparison between the Q-Ter® and placebo groups at different time points (treatment × time points) revealed statistical significance (F=17.435, P<0.001); however, no significant differences were observed comparing right and left ears (treatment × time points × side, F=0.86, P=0.48). Post hoc analysis revealed in the placebo group that DPOAE amplitudes were reduced 1 and 16 h after exposure compared with the baseline values (p<0.05). In the Q-Ter® group, however, DPOAEs did not show any significant difference between baseline and post-exposure amplitudes (p>0.1). DPOAE data collected before and 1 h, 16 h, and 7 and 21 days after exposure are shown in Figure 1. No significant differences between the two groups in DPOAE amplitude at any of the tested frequencies were present before noise trauma. One hour after exposure, the subjects in the placebo group showed a significant decrease in DPOAE amplitude for f2 values of 3174, 4004, 5042, and 6348 Hz compared with the subjects treated with Q-Ter® (p<0.05); 16 h after sound exposure a significant reduction in amplitude was observed in the placebo group for high frequencies (f2; 5042, 6348; p<0.05); and 7 and 21 days after exposure no significant differences were highlighted among the subjects treated with Q-Ter® and placebo (7 days: p=0.085, 21 days: p=0.56). The test-retest performed after a 1-minute interval under all test conditions did not show intra-subject variability.

PTA threshold values in the Q-Ter® and placebo groups did not differ from the pre-treatment and pre-exposure values (PTA ≤10 dB), as shown in Figure 2. No differences were found between Q-Ter® and placebo treatments before and 1 h, and 7 days after loud sound exposure (p>0.05).

**Blood antioxidant and inflammatory markers**

The blood markers were analyzed in each study subject before and 2 and 24 h after noise exposure (Figure 3). Among the inflammatory markers, no significantly different levels were observed in the treated and placebo groups in the dosage measured at different time points. The homocysteine levels were higher in the Q-Ter® group than in the placebo group, with no change over time (F=37.098, P<0.001). No difference could be found between the two groups before and after exposure (F=0.0781, P=0.925). Other inflammatory markers, such as serum amyloid A lipoprotein (a), ceruloplasmin, and C-reactive protein, revealed no significant alterations between the two groups or before and after sound exposure (p>0.1). Oxidative stress markers were also analyzed before and after noise exposure, revealing a significantly lower concentration of nitrates in the patients treated with Q-Ter® than in the subjects in the placebo group (F=35.23, P<0.001); nevertheless, no significant difference between the groups before and after sound exposure was observed (F=0.654, P=0.551).

In addition, the total antioxidant capacity (TEAC assay) of blood plasma did not differ among the subjects in the two groups nor before and after noise exposure (p>0.1).

Participants in the Q-Ter® group showed a lower blood concentration of CoQ10 before and 2 h after loud sound exposure compared with the placebo group; in contrast, an increase in CoQ10 was observed 24 h after noise exposure in the subjects treated with Q-Ter® to a value higher than the subjects in the placebo group. The changes were not significant (F=1.567, P=0.218). Vitamin E was also analyzed in these groups; no significant alterations in its blood levels were observed between the two groups or before and after sound exposure (p>0.1).

**DISCUSSION**

In this study, loud sound exposure in volunteer subjects caused a depression of OHC function that resulted in a significant reduction of DPOAEs during the first 16 hours after noise exposure. The DPOAE amplitude values were significantly increased by the intake of Q-Ter® for 7 days before exposure. However, no significant PTA variations were observed by pure-tone audiometry. One of the aims of this study was to investigate the effectiveness of OAE-based tests for the detection of very low levels of hearing loss induced by loud sound as a model of moderate noise exposure in workers. In several other studies, DPOAEs have been found to be more sensitive than pure-tone audiometry, although these data remain controversial. For several authors, although OAEs are a fast, objective, and easy-to-perform test to detect early cochlear damage in NIHL, intra-individual variability and high false-positive rates limit their use for hearing preservation programs [15,16]. Lapsley-Miller et al. [17] demonstrated OAE sensitivity to aircraft noise exposure with poor correlation of OAE amplitudes and audiometric threshold shifts. Cross-section statistical studies showed that both transient evoked otoacoustic emission (TEOAE) signal-to-noise ratio (SNR) and DPOAE levels were able to discriminate between normal-hearing and hearing-impaired ears [18,19]. The sensitivity of TEOAEs was good for frequencies below 2 kHz, while DPOAEs were more sensitive for higher frequencies [8,20]. Also, the correlation with pure-tone audiometry was significantly better for DPOAEs than TEOAEs. Finally, the effects of inter-individual variability seem to interfere with DPOAEs less than TEOAEs at low levels of NIHL [8]. In our study, DPOAEs were repeated twice and only those without significant changes in the DPOAE waveform were accepted. However, the increased knowledge of the cochlear mechanisms involved in OAE generation minimizes the effects of this variability introducing appropriate data acquisition and analysis procedures.

Vinck et al. [21] analyzed the hearing function in humans before and after visiting a discotheque and found that pure-tone audiometry completely recovered after the temporary threshold shift (TTS), whereas TEOAEs and DPOAEs did not recover completely to the pre-exposure reference levels, indicating a higher sensitivity to cochlear damage. Kramer et al. [22] studied subjects exposed to loud music and found no statistically significant differences between participants who received N-acetylcysteine (NAC) com-
pared with placebo. The authors found a pure-tone threshold shift at 4 kHz; however, DPOAE reductions were mainly seen at 3 kHz. Our preliminary data confirm the sensitivity of DPOAEs for frequencies higher than 3 kHz, although we did not find significant changes with PTA; this result could be due to the different noise exposure protocols used com-

Figure 1. Average DPOAE amplitude levels with error bars in 1/3 octave bands for each time point comparing Q-Ter® treated ears (n=20) and placebo group ears (n=20). 1 h after exposure, the subjects in the placebo group showed a significant decrease in DPOAE amplitude for $f_2=3174$, 4004, 5042, and 6348 Hz compared with the subjects treated with Q-Ter® ($p<0.05$). 16 h after noise exposure, a significant reduction in amplitude was recorded in the placebo group for high frequencies ($f_2=5042$, 6348, p<0.05). At 7 and 21 days after exposure, no significant differences were observed between the Q-ter-treated and placebo-treated ears (7 days: $p=0.085$, 21 days: $p=0.56$).

Figure 2. Average PTA thresholds values with error bars in the Q-Ter® and placebo groups did not differ between the placebo (A) and Q-Ter® (B) treatments at 1 h and 7 days after loud sound exposure.
pared to the previous studies. A recent study conducted in a
group of volunteers exposed to impulse noise confi rms the
sensibility of OAEs, suggesting that low-level OAEs indicate
an increased risk of future hearing loss [23].

Although DPOAEs seem to be a helpful method that pro-
vides additional information on cochlear function com-
pared with pure-tone audiometry, an additional aim of this
study was to evaluate whether DPOAEs could be effective in
monitoring the effects of an antioxidant drug (Q-Ter®) that
was previously studied in our laboratory in a guinea pig ani-
mal model of NIHL, demonstrating a signifi cant protective
effect against noise-induced hearing loss. In this study we
compared the effectiveness of CoQ10 with a soluble formul-
ation of CoQ10 (multi-composite CoQ10 terclatrate Q-Ter®)
in an animal model of acoustic trauma. Drugs were given
intraperitoneally 1 hour before and once a day for 3 days
after pure-tone noise exposure (6 kHz at 120 dB SPL for
1 hour). The treatments attenuated NIHL as measured by
ABR and decreased active caspase 3 expression and the
number of apoptotic cells. Animals injected with Q-Ter®
showed a greater degree of activity in preventing apo-
ptosis and thus in improving hearing [6]. As also confi rmed
in this animal study, the mitochondrial respiratory chain is
a powerful source of ROS in NIHL and antioxidants and
free-radical scavengers have been shown to attenuate the
damage. Coenzyme Q10 (CoQ10), or ubiquinone, has a bio-
ergetic role as a component of the mitochondrial respi-
ratory chain, inhibits mitochondrial lipid peroxidation, in-
duces ATP production, and is involved in ROS removal and
prevention of oxidative stress-induced apoptosis. However,
the therapeutic application of CoQ10 is limited by its lack
of solubility and poor bioavailability; therefore it is a chal-
lenge to improve its water solubility in order to ameliorate
the efficacy in tissue and fl uids.

The multi-composite Q-Ter® formulation is highly soluble
and spreads in water, forming a milk-like suspension. By def-
inition, in a multi-composite material the chemical moieties
of the starting materials are preserved, while physicochemi-
cal properties, such as solubility, stability, and dissolution
rate, are improved. CoQ10 is well known as a practically in-

Figure 3. Average with error bars of infl ammatory and oxidative stress markers measured before and 2 and 24 h after loud sound exposure in the Q-Ter®-treated (n=10) and placebo-treated participants (n=10). No signifi cant variations were observed between the groups for infl ammatory parameters except in the homocysteine level (signifi cance marked with *), an unspecifi c infl ammatory parameter that was increased in the Q-Ter®-treated group without signifi cant differences at each time point, indicating that the higher levels were not related to the treatment, but to the status of subjects in a small group. Lipoprotein a, C-reactive protein (CRP), serum amyloid A, and ceruloplasmin revealed no signifi cant alterations between the two groups or before and after sound exposure. Nitrates had a signifi cantly lower concentration in the volunteers treated with Q-Ter® than in those in the placebo group (signifi cance marked with *); nevertheless, no signifi cant diff erence between the groups before and after sound exposure was observed. Total antioxidant capacity (TEAC assay) of blood plasma also did not diff er among the subjects in the two groups nor before and after noise exposure. The Q-Ter® group showed an increase in CoQ10 24 h after loud sound compared with the placebo group, though not signifi cantly so. Vitamin E levels did not diff er between the two groups or before and after loud sound exposure.
soluble substance with very poor bioavailability and low stability problems; it is also difficult to handle due to its wax-like properties. In the multi-composite Q-Ter®, CoQ₁₀ has been treated in association with a suitable carrier material (cyclodextrin) and a bioactivator, the amino acid glycine. The resulting multi-composite has proven to be about 200 times more soluble and to retain its antioxidant capacity [24] more than 5 times compared to the native CoQ₁₀ [25].

This study was performed in a small number of volunteers and, although it represents a preliminary report, it also provides encouraging data for future use in a larger clinical trial. Q-Ter® administered before noise exposure prevented the initial OHC pathology as revealed by DPOAEs 1 and 16 hours after noise exposure. No significant modifications were observed between the Q-Ter® and placebo participants 7 and 21 days after sound exposure; this could be explained by the lower dosage used in the days following exposure, which could be insufficient for long-term protection. In addition, many experimental data demonstrated that the therapeutic window for a successful antioxidant approach in NIHL occurs within the first ten days after noise exposure. For these reasons, future studies are recommended to test higher Q-Ter® doses for a longer time.

In the last decade, extensive literature confirms that increasing antioxidant levels in the organ of Corti represents a rational approach against NIHL. This can be done by increasing the endogenous antioxidant response or by administering antioxidant molecules systemically or locally [3]. Many antioxidant agents have been successfully tested in numerous experimental models, such as glutathione, GlutRhenylisopropyladenosine (R-PIA) [26], D-methionine [27], ebselen [28], allopurinol [29], resveratrol [30], and dietary supplementation of vitamin C, vitamin A, idebenone, and vitamin E, which is considered one of the most effective antioxidants used in experimental models [2,31]. The efficacy of N acetyl-cysteine, a free-radical scavenger, seems to be related to the dose and schedule of administration in rats [32] and its effects seem to be enhanced by noise conditioning [33]. Kopke et al. [34] reported preliminary data on the protective effects of NAC in soldiers exposed to noise during the Iraq War (unpublished paper), while Kramer et al. [22] did not show significant NAC protection in a group of volunteers exposed to loud music in a discotheque.

Coenzyme Q has been proposed for the treatment of cardiac, neurological, oncologic, immunological, and neurogenerative diseases. Angeli et al. [35] reported that CoQ₁₀ may be helpful in delaying the progression of hearing loss in patients with the 7445A→G mitochondrial mutation. The soluble formulation of Q-Ter®, Q-Ter®, has a higher bioavailability and different pharmacokinetic properties and has been considered a safe molecule for clinical treatment.

In this preliminary study we also tested inflammatory and oxidative stress markers in all participants. The blood parameters were investigated firstly to exclude toxic effects of Q-ter during treatment and secondly to evaluate whether plasma antioxidant and inflammatory activity could be used to monitor noise-induced damage and prevention. The role of inflammation in NIHL remains controversial and the protective effects of steroids such as dexamethasone, one of the major anti-inflammatory drugs, still have to be precisely identified [2,36]. It is well known that oxidative stress is implicated in OHC damage, although there is no evidence of a correlation between oxidative damage to the cochlea and the antioxidant system in the blood. In a previous paper, lower levels of systemic CoQ were found in patients with sudden sensorineural hearing loss, thus suggesting a role as a marker of oxidative stress in the inner ear [37]. In our experimental groups, CoQ levels were significantly higher in the subjects treated with Q-Ter® 24 hours after exposure compared with the placebo group; the levels were not elevated 2 hours after exposure. However, vitamin E, another important marker of the endogenous antioxidant system, was not significantly modified in the two groups. These changes in antioxidant system-related markers suggest that a longer administration of the drug could increase the blood concentration of CoQ and thereby result in better long-term protection. Among the other blood markers studied in the two groups before and after trauma, no significant changes could be seen in inflammatory markers such as homocysteine, amyloid A, lipoprotein (a), ceruloplasmin, and C-reactive protein nor in oxidative stress markers such as nitrites and total antioxidant capacity (TEAC assay), proving that Q-Ter® did not interfere with the systemic concentrations of anti-inflammatory and antioxidant markers and that they cannot be directly related to cochlear damage or used to monitor noise-induced damage and prevention. CoQ levels were slightly but not significantly increased in the Q-Ter®-treated group 24 h after loud sound exposure. The reason why this parameter was augmented needs to be explained and more data concerning the bioavailability and pharmacokinetic of Q-Ter® could be helpful. The difference could depend on the dosage or on the tissue entrance of the agent that improves the bioenergetics in the cochlea during and after stress. In previous experimental models it was demonstrated that the “therapeutic window” for the antioxidant therapy could be within the first two weeks after trauma [6]; for this reason the authors propose to study Q-Ter® at different doses and schedule of administration in a future paper.

**Conclusions**

This pilot study confirms that DPOAEs represent a sensitive test for monitoring both the effects of noise in preclinical conditions and pharmacological treatment; however, the measurement of blood parameters of inflammation and oxidative stress cannot discriminate between untreated and treated subjects. The preliminary data presented in this study are encouraging for a larger clinical trial to collect additional evidence on the effect of Q-Ter® in preventing NIHL development in subjects exposed to noise.

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