



Original Article

Evaluation of the Effect of Betahistine on Noise-Induced Hearing Loss Using Distortion Product Otoacoustic Emission and Scanning Electron Microscopy

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OBJECTIVE: To evaluate the protective effect of betahistine on noise-induced hearing loss (NIHL) using scanning electron microscopy (SEM) and distortion product otoacoustic emission (DPOAE).

MATERIALS and METHODS: A total of 8 adult albino guinea pigs were used in this study (study group: n=4 and control group: n=4). DPOAE measurements of both groups were performed before the procedure. Two hours before noise exposure, 0.9% NaCl solution was injected perorally to the control group and betahistine was administered through a peroral catheter to the study group. Both groups were then exposed to 105-dB sound pressure level (SPL) 4-kHz frequency-based narrow-band noise for 2 h per day for 5 days. DPOAE measurements were performed again on the 6th day and cochleae were dissected and examined by SEM on the 7th day.

RESULTS: Regarding the results of DPOAE, NIHL was observed in both groups on the 6th day ($p < 0.05$). Loss, flattening, and fusion, which are findings of permanent hearing loss, were determined in the stereocilia of the inner and outer hair cells by SEM. These findings were evaluated as signs of permanent increase in the threshold. When DPOAE measurements and SEM results were evaluated in the study group, no significant difference was observed in NIHL compared with the control group ($p > 0.05$).

CONCLUSION: In our study, it was observed that simultaneous administration of betahistine during noise had no protective effect on permanent increase in the threshold. However, further studies on noise and long-term use of betahistine can be performed.

KEYWORDS: Betahistine, noise-induced hearing loss (NIHL), scanning electron microscopy (SEM), distortion product otoacoustic emission (DPOAE)

INTRODUCTION

Noise is one of the most important occupational and environmental health hazards. Exposure to loud noise causes irreversible auditory damage, resulting in sensorineural hearing loss. In addition, it can result in temporary threshold shifts (TTSs) and/or permanent threshold shifts (PTSs). Moderate exposure over a short time period may initially cause TTSs, which may fully recover within 24-48 h. If the noise causing TTSs is continuous or frequent, PTSs may develop. Noise exposure leads to damage and loss of hair cells in the organ of Corti, which is contained in a spiral-shaped structure called the cochlea. These sensory hair cells and the surrounding structures are vibrated by incoming acoustic signals and then convert this mechanical vibration into electrical events in the form of firings of the 8th cranial nerve fibers. Chronic exposure to intense noise initially damages the outer hair cells that are responsible for high-frequency sounds ^[1,2].

Betahistine dihydrochloride (betahistine) has beneficial effects on inner ear disorders such as vertigo that affect cochlear blood flow (CoBF). It has been shown to increase CoBF in animal models and a number of hypotheses have been proposed for its mechanism and site of action. Betahistine is a strong histaminergic H3 receptor antagonist; this property may account for the increase in CoBF via an increase in histamine release and consequent activation of postsynaptic histaminergic H1 and H2 receptors. Betahistine may also have weak direct effects on these postsynaptic receptors or an effect modulated by other autonomic receptors ^[3].

In this study, the protective effect of betahistine on noise-induced hearing loss (NIHL) was evaluated for the first time using scanning electron microscopy (SEM) and distortion product otoacoustic emission (DPOAE) data.

MATERIALS and METHODS

This study was approved by the Committee for Ethics in Animal Experiments of the Current Marmara University (MUHDEK) with protocol number (44.2011.mar). Eight healthy mature male albino guinea pigs weighing between 450 and 670 g with normal

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Preyer's reflexes and normal tympanic membranes were used for these experiments. These animals were provided with free access to food and water. DPOAE was evaluated in both ears of all animals before the experiments.

The animals were assigned to 1 of 2 groups. In the study group ($n=4$), betahistine (Vasoser 24-mg tablet, Abdi Ibrahim, Turkey, 0.15 mg/kg) was administered through a peroral catheter 2 h before noise exposure. In the control group ($n=4$), the same dosage of 0.9% NaCl solution was administered perorally. All procedures were repeated every day for 5 consecutive days. Both groups were exposed to 105-dB sound pressure level (SPL) 4-kHz frequency-based narrow-band noise for 2 h for 5 consecutive days. The noise used was designed as PTSs to damage cochlear hair cells [4]. All animals were awake when they were exposed to that noise. Noise presentation was performed by a high-fidelity sound system (CD player Pioneer PD-306, amplifier Yamaha P4500, loudspeaker). The loudspeaker was centered over the animal's head at a distance of 40 cm. Sound intensity was monitored with a sound-level meter (Tromer, P.R.C.) positioned near the external auditory canal (Figure 1). DPOAE was measured again 1 day after the noise exposure experiments were complete. On the 7th day after the start of the experiment, the animals were deeply anesthetized intraperitoneally with sodium thiopental (Pentothal, Abbott, U.S.A, 100 mg/kg); the cochleae were dissected macroscopically after sacrificing the animals (Figure 2) and then perfused with glutaraldehyde [3% in 0.1 mol cacodylate buffer (pH 7.4)].

Preparation of Cochleae

The cochleae were prepared for examination. The tissues were viewed on a JEOL JSM-5200 SEM (Tokyo, Japan). Examination was performed separately for the basal, middle, and apical turns. Damage was evaluated for both inner and outer hair cells in terms of stereocilia loss, fusion, and shortening and scored as follows: 0, undamaged; 1, mild damage; 2, moderate damage; and 3, severe damage [5].

DPOAE Test Application

The animals were sedated using an intraperitoneal solution of 50 mg/kg ketamine hydrochloride (Ketalar; Eczacıbası, Istanbul, Turkey) and 7.5 mg/kg xylazine hydrochloride (Rompun; Bayer, Leverkusen, Germany). For the recording and subsequent analysis of DPOAE, a GSI Audera recorder (Grason-Stadler, Minnesota, USA) was used. The acoustic probe was hand-held at the opening of the external auditory meatus with gentle pressure. The measurements were performed in a soundproof chamber. Both ears were measured at the frequencies (f_2) of 1, 1.5, 2, 3, 4, 6, and 8 kHz, with 1 pair of stimulus tones ($f_2/f_1=1.22$, DP definition= $2f_1-f_2$; $L_1=65$, $L_2=55$) at approximately 1 measurement every 4 s. Only DPOAE were included in the analysis that was at least 3 dB above background noise. All DPOAE levels with a signal-to-noise ratio were analyzed.

Statistical Analysis

The data were analyzed using the Wilcoxon paired 2-sample test and Mann-Whitney U test variance analysis in SPSS (Statistical Package for the Social Sciences, version 15.0, IBM, New York, USA) for Windows. A p value of ≤ 0.05 was considered significant.

RESULTS

When DPOAE values before and after noise exposure were compared in the study group, a statistically significant decrease was observed



Figure 1. The equipment used to generate noise



Figure 2. An image of the cochlea after incubation in a decalcifier before being dissected to remove the bony capsule

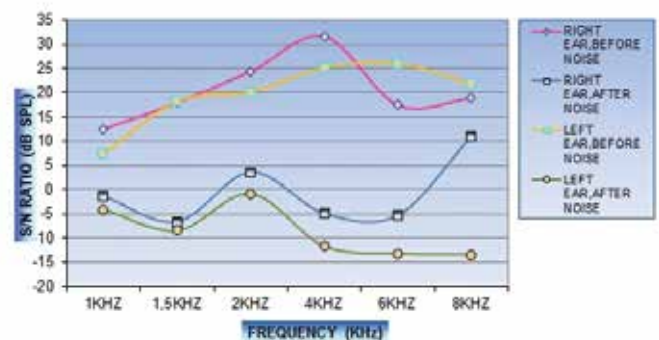


Figure 3. DPOAE results of the study group

in the post-noise values ($p<0.05$) (Figure 3). Similarly, a statistically significant decrease in the post-noise values was detected in the control group ($p<0.05$) (Figure 4). When the study and control groups were compared in terms of hearing loss after noise exposure, there were no significant differences in the DPOAE values ($p>0.05$).

The cochlear tissue was divided into 3 segments for SEM: basal, middle, and apical. The results were evaluated separately for both groups. In both groups, there was shortening of the stereocilia of the outer hair cells, irregular placement, stereocilia damage, stereocilia

loss, and outer hair cell loss due to the noise. In the control group, basal segment was 77% mildly damaged and 23% undamaged (Figure 5a); middle segment was 56% mildly damaged, 25% moderately damaged and 18% undamaged (Figure 5b). Apical segment was 30% mildly damaged, 30% moderately damaged, 20% severely damaged and 20% undamaged (Figure 5c). In the study group, basal segment was 80% mildly damaged and 20% undamaged (Figure 6a); middle segment was 44% mildly damaged, 22% moderately damaged and 33% undamaged (Figure 6b). Apical segment was 11% mildly damaged, 33% moderately damaged, 33% severely damaged and 22% undamaged (Figure 6c). When SEM results of the study and control groups were compared, there was no statistically significant difference between these two groups ($p > 0.05$) (Figure 7).

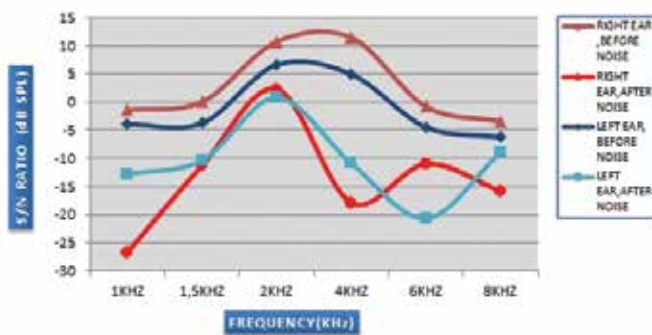


Figure 4. DPOAE results of the control group

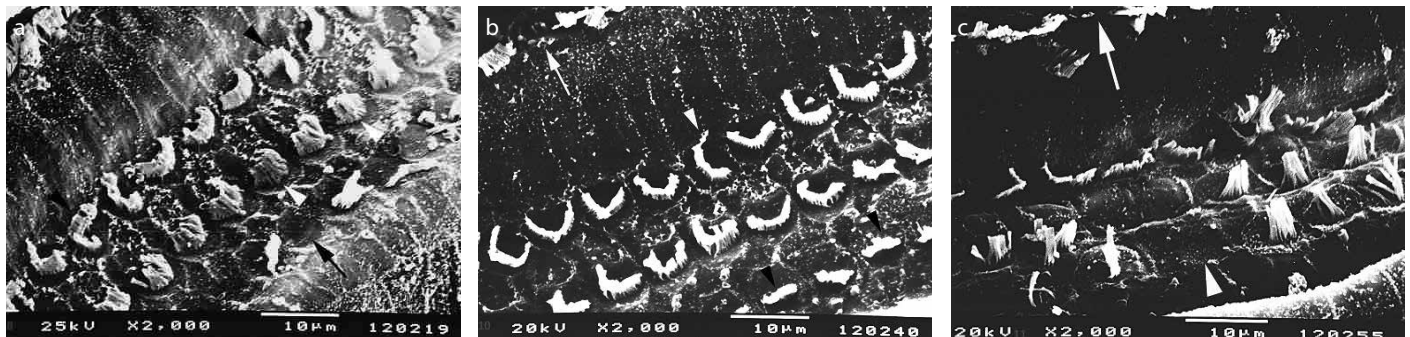


Figure 5. a-c. (a) Disorganization (black arrowhead), fusion (white arrowhead) and loss (black arrow) of stereocilia of the outer hair cells in the basal turn of the control group. SEM micrograph, x2000. (b) Stereocilia loss in the inner hair cells (arrow), disorganization in the stereocilia of the outer hair cells (white arrowhead) and played stereocilia (black arrowhead) in the middle turn of the control group. SEM micrograph, x2000. (c) The apical turn of the control group. Stereocilia loss in the inner (arrow) and outer (arrowhead) hair cells. Fusion and shortening in the stereocilia of the outer hair cells are evident. SEM micrograph, x2000.

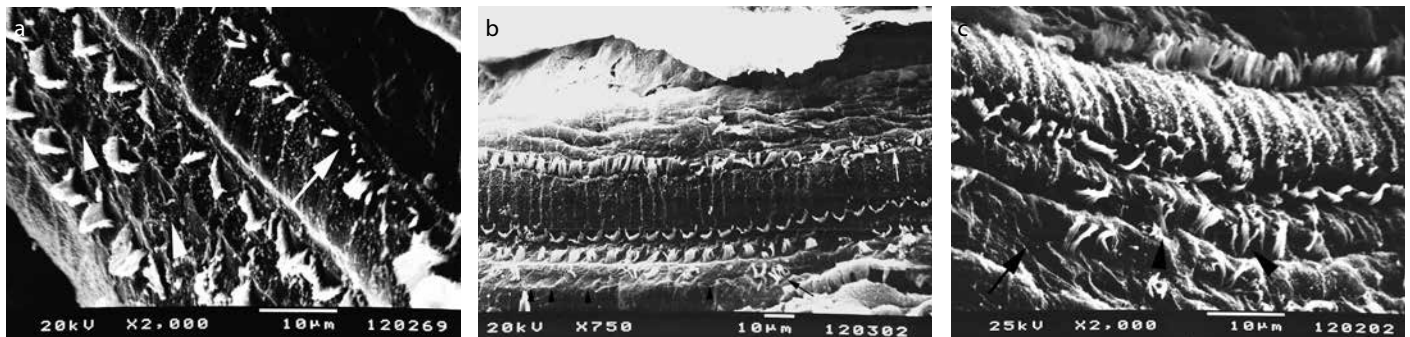


Figure 6. a-c. (a) Stereocilia loss in the outer (arrowhead) and inner (arrow) hair cells is shown in the basal turn of the study group. SEM micrograph, x2000. (b) Stereocilia damage in the inner (white arrow) and outer (black arrow) hair cells in the middle turn of the study group. Stereocilia loss in the outer hair cells (arrowhead) is evident. SEM micrograph, x750. (c) Stereocilia loss (arrow) and damage (arrowhead) in the outer hair cells in the apical turn of the study group. SEM micrograph, x2000.

DISCUSSION

Noise is defined as unwanted and unpleasant sound, whereas NIHL is defined as the damage in the cochlea due to noise. Sound causing damage to the inner ear does not have musical quality or source; it is an acoustic energy to the inner ear. Some parameters of noise must be known to determine the risk of hearing loss that may occur after noise exposure. These include noise intensity, frequency spectrum, and exposure duration. Noise intensity is the level of sound pressure reaching the inner ear and is measured according to the dBA scale. Legally permissible exposure times (h) with the corresponding noise intensity (dBA) are as follows: 90 dBA: 8 h; 95 dBA: 4 h; 100 dBA: 2 h; 105 dBA: 1 h; 110 dBA: 30 min; 115 dBA: 15 min [6].

In adults, the 2 most common reasons of sensorineural hearing loss are presbycusis and NIHL. Both result in damage to the outer hair cells, particularly in the basal turn of the cochlea. NIHL is one of the 10 most common occupational diseases and causes labor and economic loss [7].

NIHL results in both mechanical and metabolic injuries. Sound pressure levels higher than 125 dB cause severe mechanical damage, whereas lower acoustic stress causes micro lesions in the cell membrane and results in calcium ion entry into the cell by disturbing ion homeostasis. Excessive stimulation of the cochlea leads to excessive secretion of glutamate. Glutamate, the main neurotransmitter of primary afferent neurons, causes intense calcium entry into cells along with oxidative and metabolic stress. Continuous acoustic stress reduces the partial pressure of oxygen and causes tissue hypoxia on

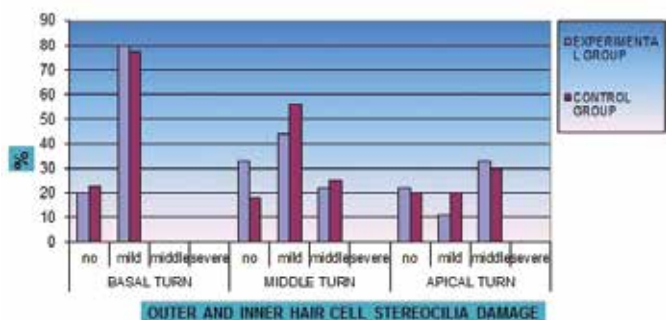


Figure 7. The damage scores of the stereocilia of inner and outer hair cells in the scanning electron microscopy

account of increased oxygen consumption. This metabolic stress, caused by ion entry due to glutamate and cell membrane micro lesions, leads to the formation of free radical species. Cytochrome c, a known activator of caspase proteins in the apoptotic cascade, is released from the inner matrix of the damaged cochlea and spreads into the cytoplasm. These and other intracellular events lead to the activation of the apoptotic cascade, ultimately resulting in cell death. Oxidative damage of cellular DNA is high during and immediately after acoustic trauma; therefore, the first 8 h after exposure have been suggested to be critical for antioxidant therapy [8-11].

Histological studies have shown that two significant changes occur in the cochlea after noise exposure. These are loss of hair cells and changes in the stereocilia. Stereocilia changes are more frequent than loss of hair cells. First, death and dysfunction are seen in the stereocilia of the outer hair cells. The stereocilia can refresh them after the cessation of the noise. This situation is expressed as TTSs. TTS recovery can be within minutes, hours, or may be days. If the noise continues, the stereocilia stick together and a permanent change in hearing, termed as PTSs, occurs. Increase in the hearing threshold with PTSs is not reversible. This situation leads to the development of permanent hearing loss. In the later stages, damage to the inner hair cells and secondary neuronal degeneration may develop. Pathology is seen first in the basal turn of the cochlea where high-frequency hearing loss is found; the apical folds and low frequencies are affected with time. Noise above 85 dB leads to hearing loss and the effect is greater at higher frequencies. Severe and short-term sound stimulation, defined as acoustic trauma, is capable of causing PTSs without causing TTSs. This trauma leads to cortical organ damage and mixture of the perilymph and endolymph due to the rupture of membranes [12, 13].

In the audiogram of patients with NIHL, impairment is observed to being at 3-6 kHz and the corresponding distortion is classically described as a 4-kHz notch. With longer noise exposure, hearing loss is further amplified. Lower and higher frequencies begin to be affected. With ultrastructure examinations, damage is observed to be in the first 8-10-mm-diameter section of the cochlea and this region corresponds to the 4000-Hz area topographically [14].

DPOAE is an effective method for the detection of damage to the outer hair cells. NIHL is usually localized to the basal turn of the hair cells and DPOAE (2f1-f2) appears to be the most appropriate test in determining the integrity of the outer hair cells. DPOAE is emphasized

as a sensitive method for the evaluation of acoustic trauma-induced hearing loss in patients with abnormal hearing symptoms but normal audiogram [15-17]. Among patients subjected to acute acoustic trauma, those with undetectable DPOAE in the affected frequencies have been found to have poor prognosis compared with those with detectable DPOAE [18]. In guinea pigs, short latency and short duration of emissions constitute a technical problem in measurement. Recoding transient OAE is difficult. Transient OAE is more sensitive in detecting low frequency and minor cochlear hearing loss, whereas DPOAE can measure over 4 kHz. In a previous study, comparison of conventional audiometry (0.25-8 kHz) and extended high-frequency audiometry (9-20 kHz) in patients with acoustic injury showed that acoustic trauma exposure occurs mostly in the 4-8 kHz range and conventional audiometry is satisfactory for the evaluation of hearing. [19] In our study, DPOAE provided an adequate range for the evaluation of hearing as it can measure up to 8 kHz.

Damaged end links of fused or separated bundles of stereocilia are considered as abnormal. Fused stereocilia bundles are also seen in normal animals. In the measurements, in order to ensure maximum transmission function in mice outer hair cells, a correlation was observed between simple transmission channel number and number of end links of stereocilia [20]. Transmission channels are concluded to be at the end links. It was also shown in other studies that these end links have a mechano-electrical conduction task. Non-linear mechano-electrical conduction function of the outer hair cells caused by non-linear movements in basilar membrane is the source of DPOAE. Thus, fusion or other changes in stereocilia bundles cause loss in stereocilia function and results in decrease in DPOAE values [21, 22].

In a long-term experimental study, DPOAE measurements were performed repeatedly over 6-8 months in 12 guinea pigs. Nine weeks after the first measurement, the guinea pigs were subjected to industrial level noise (approximately 110 dB) for 2 h. DPOAE values were measured before noise exposure and 10 min, 70 min, 1 day, 2 days, and once a week after noise exposure. Three to four months after noise exposure, the cochlea of the guinea pigs were dissected and examined using SEM. The inner hair cells were found to be complete in all guinea pigs and significant loss correlated with DPOAE amplitude changes was determined in the outer hair cells of some guinea pigs. A close relationship was indicated between decrease in DPOAE amplitude and the number of lost or changed (fused stereocilia bundles, lost end links) outer hair cell. [23].

In a study performed in 78 patients with sudden sensorineural hearing loss, hyperbaric oxygen and steroid therapy were administered in combination and it was found that DPOAE was significantly correlated to hearing improvement in the patients [24]. In another experimental study on 25 guinea pigs, impulsive style noise with an intensity of 153 dB was applied for approximately 0.1 ms. For detecting noise-induced cochlear damage, the guinea pigs were monitored before and after noise exposure. The DPOAE values of the guinea pigs had remained stable before noise exposure but decreased significantly 1 h after noise exposure. In addition, according to the DPOAE values, 3 different patterns were observed: complete restoration, partial restoration, and permanent damage. In light of these findings, DPOAE has been proposed as a suitable method for monitoring the abnormalities of outer hair cells [25].

One of the important reasons for NIHL pathophysiology is reduced CoBF. This reduction is considered to be an underlying cause for sudden or progressive sensorineural hearing loss, acute acoustic trauma, presbycusis, and other cochlear disorders. Betahistine is a relatively weak moderate selective H1 receptor agonist. H1 receptors are found in the vascular smooth muscle and cause nitric oxide production and secretion if they are activated. Nitric oxide causes rapid, short-term muscle relaxation by diffusing into the vascular smooth muscle cells. Betahistine is also a powerful H3 receptor antagonist. H3 heteroreceptors are found in the presynaptic area and cause auto inhibition of histamine release. H3 receptors are also found at the sympathetic nerve endings in the perivascular space. The stimulation of receptors results in a decrease in perivascular sympathetic neurotransmission and norepinephrine release ^[26].

Topical application of betahistine around round window did not change CoBF but it had been shown to increase following systemic infusion. In an experimental study on guinea pig for investigating the effects of betahistine on inner ear vascular mechanisms, betahistine-induced CoBF was measured using a laser Doppler flow meter and the effects on the anterior inferior cerebellar artery and stria vascularis were measured using intravital microscopy. Betahistine was found to increase CoBF and lower systemic blood pressure ^[27, 28].

In an experimental study by Lamm et al. ^[26] on the effect of betahistine on CoBF and perilymphatic PO₂ (PL-pO₂) in NIHL, significant increase was found in CoBF and PL-pO₂ values compared with the pre-drug values in the group receiving betahistine with intravenous infusion pump after noise exposure. However, the effect of the drug was also shown to terminate after the finalization of the drug. This situation was coupled to short duration of vasodilatation effect of betahistine over H1 receptors. Cochlear reperfusion and reoxygenation were shown to be discontinued. Partial recovery of cochlear microphonic and cochlear nerve action potentials and full recovery of auditory brainstem response were observed.

Thus, the literature indicates that betahistine is effective for CoBF and takes place in the treatment protocol of sudden hearing loss with vasodilation purpose. Distortion of stria vascularis blood vessels and reduction of blood flow occur in NIHL pathophysiology. Therefore, in our study, we evaluated the effectiveness of betahistine with otoacoustic emission and electron microscopic examinations in order to evaluate whether increasing the CoBF is effective in the prevention and treatment of NIHL. To the best of our knowledge, no previous study has shown the effect of betahistine on NIHL using SEM. In this regard, we have identified SEM findings related to permanent hearing loss in NIHL in our study; no statistically significant difference was seen between the 2 groups in terms of the protective effects of betahistine on hearing thresholds. However, short-term effects of betahistine were observed in this study, and other studies on long-term efficacy may be performed.

Ethics Committee Approval: Ethics committee approval was received for this study from Committee for Ethics in Animal Experiments of the Current Marmara University (MUHDEK) with protocol number (44.2011.mar).

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