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ORIGINAL ARTICLE

The Role of Different Agents in the Prevention of the Negative Effects of Immediate Hyperbaric Oxygen Therapy in Acute Acoustic Trauma

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Objective: To prevent the negative effect of immediate hyperbaric oxygen therapy (HBOT) in the treatment of acute acoustic trauma (AAT).

Study Design: Animal study.

Materials and Methods: Sixteen rats were divided into four groups. Rats were exposed to 110 dB white noise for an hour. The first group was used as a control; the second group was treated with HBOT alone; the third group was treated with steroid and HBOT; the fourth group was treated with N-Acetyl Cysteine and HBOT. The first HBOT was started at first hour. In all groups signal to noise ratios (SNRs) were recorded before the noise exposure, immediately after the post exposure and at 3rd, 5th, and 7th days.

Results: The analysis of distortion product otoacoustic emission (DPOAE) indicated that; for frequency 1kHz, there was no statistically difference between groups ($p>0.05$). For frequency 1.5 kHz, there was statistically difference between Group I – III and Group II – III ($p<0.05$). For frequency 2 kHz, statistically significant difference between control group and groups III and IV were found ($p<0.05$). For frequency 3 kHz, there was statistically difference between Group I and the other groups (Group II, III, IV) ($p<0.05$). For frequency 4 kHz, 5 kHz and 6 kHz there was statistically difference between Group I – II; Group I – IV and Group II – IV; Group III – IV ($p<0.05$). Additional for frequency 6 kHz, there was statistically difference between Group II – III ($p<0.05$).

Conclusion: Immediate HBOT after AAT has a negative effect on healing process. This negative effect can be prevented by steroid therapy in addition to HBOT partially.

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Introduction

Acute acoustic trauma (AAT) is a sudden onset of sensorineural hearing loss due to exposure of the hearing organ to acoustic overstimulation [1, 2]. AAT causes temporary or permanent hearing loss. It may occur in occupational (military gunshots etc.) or recreational activities (rock concerts etc). The effect of the AAT on the hearing sensitivity is generally observed in the higher frequencies, however all frequencies may be affected [3]. Degeneration of outer and inner hair cells, massive destruction of the dendrites of the primary auditory neurons, rupture of cell membranes are the ultra-structural changes in the inner ear, moreover decrease of cochlear blood flow and oxygen tension as well as heavy production of reactive oxygen species have been observed [3-9]. Eventually, acoustic trauma can lead to oxidative stress and apoptotic cell death of the hair cells [1, 9].

Hearing loss at AAT can be reversible [1-3]. However, many therapeutic approaches including hyperbaric oxygen, low molecular weight dextran, steroid, vasoactive and anti oxidative agents have been used for several years. In general all of them are directed to the repair of the microcirculation [1-3, 5-7, 10, 11].

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Hyperbaric oxygen therapy (HBOT) is a treatment which is applied 100% oxygen by a mask or a hood in a sealed chamber, above 1 atmosphere pressure. The air pressure inside a HBOT chamber is about two and a half times greater than the normal pressure in the atmosphere. Indications for HBOT in Otorhinolaryngology are; sudden sensorineural hearing loss, malignant otitis externa, bone infections and tinnitus[5]. In AAT, HBOT can be used after unsuccessful treatment with medical management or can be used combined with medical treatment. Providing adequate oxygen supply and preventing the oxidative stress secondary to cochlear hypoxia are the possible mechanisms of HBOT[2, 3]. Generally, it believes that the use of HBOT in the treatment of acute hearing loss improves the results of conventional treatment if it started early after the onset of deafness [5]. However according to Cakir et al. [3] and D'aldin et al.[4], early HBOT could cause a possible additional injury to cochlear hair cells and had a negative effect in acute acoustic trauma [3].

The aim of the present study was to determine whether it is possible to prevent the negative effect of immediate HBOT by using anti-inflammatory or antioxidant drugs in rats exposed to intense sound.

Materials and Methods

Twenty four male Wistar albino rats (weighing 300-350 g) were used in this study. Study was performed at Eskisehir Osmangazi University, Experimental Research Center Eskisehir, Turkey. Rats were housed in polycarbonate cages at a temperature (21 ± 1°C) and humidity (45-55%) controlled room that was maintained on a 12/12 reversed light cycle. The rats were fed with a standard rat chow and allowed to drink water. This study protocol was approved by Eskisehir Osmangazi University Institutional Local Animal Care and Use Committee (Date 26.04.2011, No: 209/2011). Ears were evaluated under general anesthesia with 15 mg/kg xylazine and 100 mg/kg ketamine by otoscope. Any animal with acute otitis media was excluded. Distortion product otoacoustic emission (DPOAE) was preferred as an investigation method for hearing evaluation. The rats in which the DPOAEs were not present were also excluded from the study.

Distortion Products Otoacoustic Emission

DPOAEs were recorded with a newborn probe using Otodynamics ILO v6 system (Otodynamics Ltd., Hatfield, Herts, U.K.). Recordings were performed before the noise exposure, immediately after the noise exposure and at 3rd, 5th, and 7th days of post-exposure.

The DPOAE data were collected by means of the geometric mean of the primary frequencies (f1 and f2) at 1, 1.5, 2, 3, 4, 5 and 6 kHz. The amplitudes of 2f1-f2 distortion product were measured. The DPOAEs at 3 dB above the noise floor were defined as present. The analysis of the results is based on the signal-to-noise ratio (SNR) values that indicate the difference between the OAE response and the noise level at a particular frequency.

Noise exposure

The rats were exposed to a white noise between 1-12 kHz bands bilaterally. MATLAB (MathWorks, Natick, MA, U.S.A.) program was used to produce noise with one unit variance. The noise level was set at a distance of 2 cm from the loudspeaker with the help of sound level meter with a 110 dB sound pressure level intensity for an hour. Background noise level in the research laboratory was below 35 dB during the collection of DPOAEs and the other times.

Experimental Groups

Eight rats in which the DPOAEs were not present were excluded from the study. Sixteen rats were divided into four groups: (I) A group of 4 rats (8 ears) did not receive any treatment and was used as a control; (II) A second group of 4 rats (8 ears) was treated with HBOT alone; (III) A third group of 4 rats (8 ears) was treated with steroid in association with HBOT; (IV) A fourth group of 4 rats (8 ears) was treated with N-Acetyl Cysteine in association with HBOT. One rat from group IV dead during the 2nd day.

HBOT, Steroid and N-Acetyl Cysteine Treatments

An experimental hyperbaric chamber directly pressurized with 100% oxygen was used for HBOT. Before pressurization, 100% oxygen was flushed through the chamber for 5 min to displace ambient air. The hyperbaric chamber was pressurized slowly and reached 2.5 ATA in 10 min. The chamber was ventilated during HBOT to avoid carbon dioxide accumulation. After 60 min at 2.5 ATA the chamber

was decompressed to the normal atmospheric pressure in 10 min. The HBOT, steroid and N-Acetyl Cysteine treatments were started at first hour after the noise exposure. All treatments were given one time per day and lasted for 7 days. Methylprednisolone 10 mg/kg/day was given once a day by intramuscular injection. N-Acetyl Cysteine 300 mg/kg/day was given once a day by intraperitoneal injection.

Scanning Electron Microscopy

One rat from each group was selected randomly for histological examination. After the last DPOAE measurement, the rats were deeply anesthetized, decapitated and their temporal bones were prefixed in 2.5% glutaraldehyde solution in phosphate buffer (PBS), pH 7.3 for 12 h. The temporal bones were washed with phosphate buffered saline (0.1M PBS) for 24 h and decalcified in 0.1 M Na-EDTA (Sigma-Germany), pH 7.3 at room temperature for 2 weeks. After 2 weeks their cochleae were removed from the bullae of temporal bone. Decalcification of the cochleae was started with 0.1M PBS. After 24 h cochleae were kept in 0.1 M Na-EDTA, pH 7.3 for a week at room temperature. The otic capsules were opened, and the cochlea were dissected out and kept in 0.1M PBS for 3 days.

The subsequent procedures were performed in the Electron Microscopy Laboratory at Eskisehir Osmangazi University. The cochleae specimens were washed three times for 5 min with the same buffer and they were fixed with 2.5% (vol/vol) glutaraldehyde in 0.15 M PBS for 1 h at room temperature. They were then treated with 1% (wt/vol) osmium tetroxide for 1 h. Samples were subsequently washed in distilled water and dehydrated in a series of upgrading ethanol concentrations, up to 100%. After critical-point drying in carbon dioxide, they were mounted on aluminium stubs, with silver paste, coated with gold by using Polaron SC7620 Sputter Coater and examined in a scanning electron microscope (JEOL JSM-5600LV).

Only Group II and IV SEM images were used to determine the destruction of the hair cells in this study. The quality of the image produced in Group I and III SEMs were not good enough to present in this study.

Statistical Analysis

The statistical analysis was performed by IBM SPSS 19.0 for Windows (SPSS, Inc., Chicago, IL). Shapiro Wilk's test was used to test the normality of data, which was fulfilled in all measurements. Differences between 4 groups were tested by using ANOVA followed by post hoc Tukey's test. Differences between before and after AAT within group were tested by using paired t test. We used repeated measures ANOVA to compare measurements made before, after, 3rd day, 5th day and 7th day between the groups followed by post hoc Tukey's test. Values are expressed as mean ± SD. Statistical significance was accepted for p values less than 0.05.

Results

The analysis of DPOAEs indicated that the pre-exposure recordings were normal for each rat and there was no difference between the groups (I, II, III, IV) ($p>0.05$). The analysis of DPOAEs after the noise exposure show that all rats had AAT and significant differences with the pre and post exposure results ($p<0.05$). Post-exposure recordings between the treatment groups (I, II, III, and IV) were not significantly different ($p>0.05$).

A repeated measures ANOVA, in which treatments served as a between group factor and frequency was a within group factor, was used to determine the significantly difference between the overall progress of measurements. For frequency 1, there was no statistically difference between groups ($p>0.05$) (Figure 1 A). For frequency 1.5, there was statistically difference between Group I – III and Group II – III ($p<0.05$) (Figure 1 B). For frequency 2 kHz, statistically significant difference between control group and groups III and IV were found ($p<0.05$) (Figure 1 C). For frequency 3, there was statistically difference between Group I and the other groups (Group II, III, IV) ($p<0.05$) (Figure 1 D). For frequency 4 kHz, 5 kHz and 6 kHz there was statistically difference between Group I – II; Group I – IV and Group II – IV; Group III – IV ($p<0.05$). Additional for frequency 6 kHz, there was statistically difference between Group II – III ($p<0.05$) (Figure 2 A, 2 B and 2 C).

Control Group

The post exposure SNR at higher frequencies (4, 5 and 6 kHz) was decreased. A recovery in DPOAE was observed during 3rd and 5th day after noise exposure (Figure 3 A).

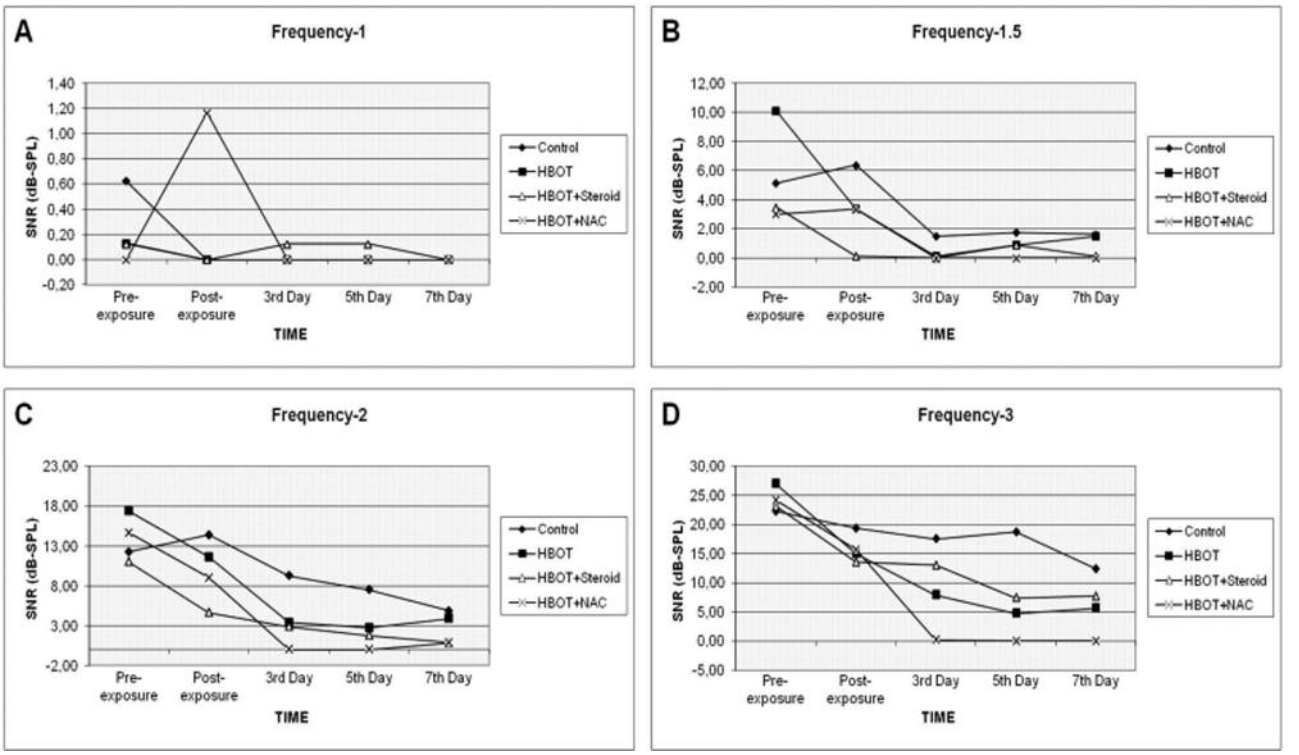

Figure 1. A: Frequency 1 kHz (SNR values, dB-SPL), **B:** Frequency 1.5 kHz (SNR values, dB-SPL), **C:** Frequency 2 kHz (SNR values, dB-SPL), **D:** Frequency 3 kHz (SNR values, dB-SPL)

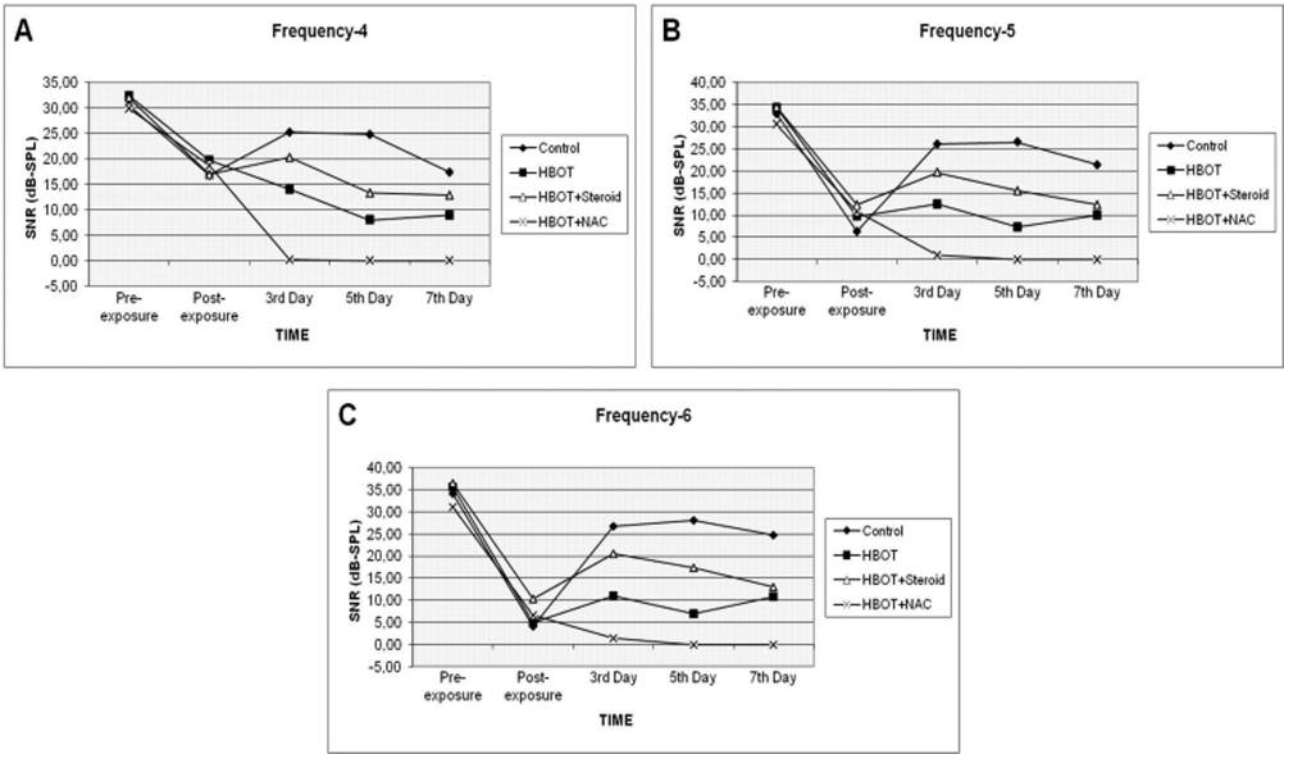

Figure 2. A: Frequency 4 kHz (SNR values, dB-SPL), **B:** Frequency 5 kHz (SNR values, dB-SPL), **C:** Frequency 6 kHz (SNR values, dB-SPL)

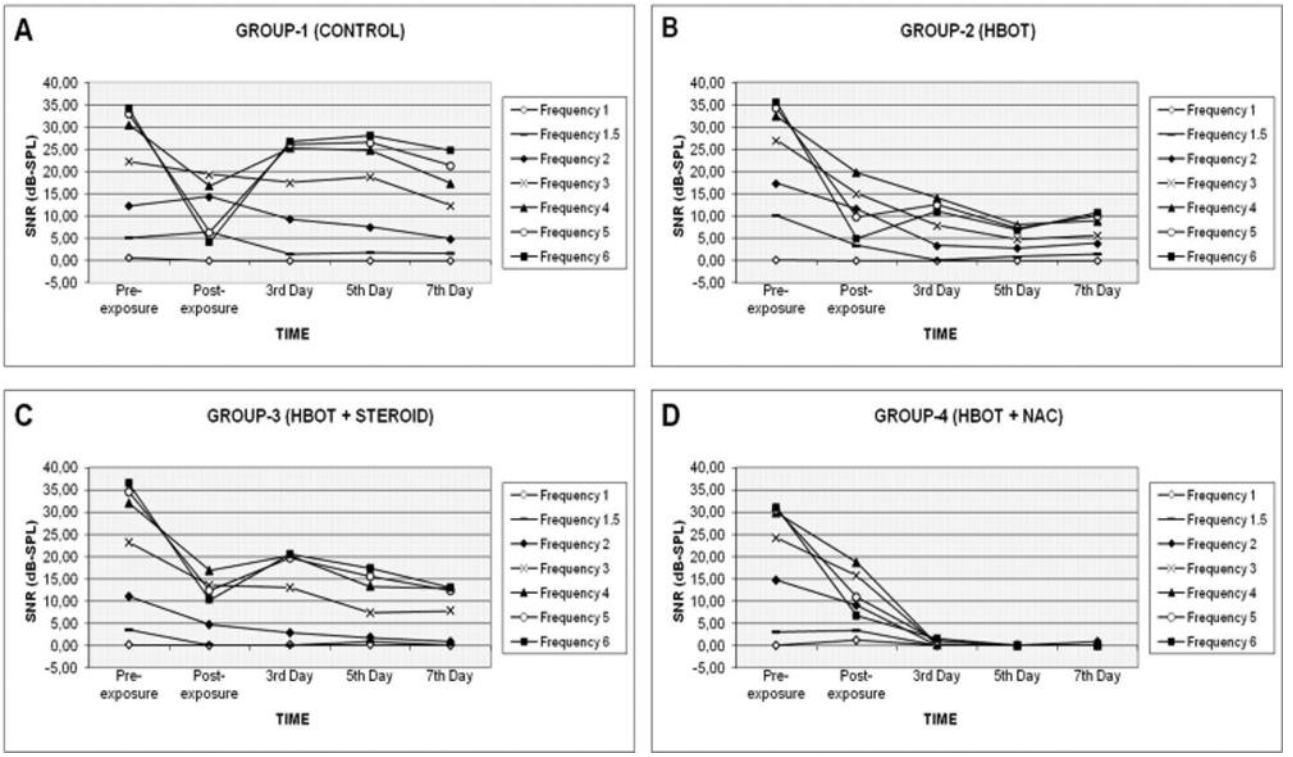

Figure 3. A: Control group (SNR values, dB-SPL), **B:** HBOT (SNR values, dB-SPL), **C:** Steroid in association with HBOT (SNR values, dB-SPL), **D:** N-Acetyl Cysteine in association with HBOT (SNR values, dB-SPL)

HBOT

SNR at 4, 5 and 6 kHz were significantly decreased after the noise exposure like control group. A recovery was observed during the 3rd day at 5 kHz and 6 kHz. This recovery was not as marked as in the Group III (Figure 3 B).

Steroid in Association with HBOT

Decreases of SNR at higher frequencies (4, 5 and 6 kHz) were also seen after the noise exposure. A significant recovery was observed at 4, 5 and 6 kHz frequencies during 3rd day after noise exposure (Figure 3 C).

N-Acetyl Cysteine in Association with HBOT

The same result as the other groups was observed after noise exposure. Moreover, 3rd day of exposure, significant decreased DPOAEs was observed at all frequencies except 4 kHz (Figure 3 D).

Discussion

High intensity sound can be found in everywhere as a result of industrial development. Moreover, noisy recreational activities and portable music devices are more popular than before. Therefore, the risk of AAT has to be seriously taken into consideration.

Experimental studies in animals have shown that a mechanical lesion with damage in the sensorial cells in the organ of Corti after the exposure to intense noise [1]. Different components of hearing organ seem to be involved in the cochlear damage such as massive swelling of the synapsis, rupture of the cell membrane in the organ of Corti, displacement and damage of hair cell stereocilia, collapse of supporting cells, rupture of the reticular lamina and degeneration of fibrocytes in spiral limbus and spiral ligament [4, 12-15]. In addition to direct mechanical damage, noise causes metabolic changes in the hair cells and supplying blood vessels

which leads to reduced oxygen content of the perilymph which is the main oxygen source of the organ of Corti. Hypoxia in the organ of Corti and increased demand for oxygen can lead to oxidative stress and production of reactive oxygen metabolites [16, 17]. Finally apoptotic cell deaths of many preserved hair cells that are initially not or only mildly damaged were revealed. Since oxidative stress and intracochlear inflammation are thought the major mechanism of the neural and apoptotic cell death, all treatment options are planned to base on these findings.

Spontaneous recovery is common observation after AAT. It would be a self-defense mechanism of the cochlea and this can allow to synaptic healing, however normal hearing threshold doesn't always indicate normal inner ear structure. Slow degeneration of spiral ganglion cells without detectable change of hearing threshold may continue. Animal experiments as well as clinical observations showed us there was high degree intersubject variability in the functional and structural alterations in response to AAT as well as the possibility of spontaneous recovery. Since there is no method to predict the subjects' reaction to over exposure, theurapathic options recommend to all patients [15, 18]. Timing of the therapy is also discussed in several studies.

The results of case-control studies support the idea that HBOT is useful for patients with AAT [1]. The aim of HBOT is to provide an adequate oxygen supply and preventing oxidative stress [1, 2, 6]. Moreover, HBOT improves the production of superoxide dismutase which serves as a key antioxidant in the cells and protects them. An extreme increase in the arterial oxygen tension after HBOT causes a large increase in perilymphatic oxygen tension [6, 7]. The timing of the HBOT is very controversial. Some studies strongly recommended to immediate HBOT after noise exposure [1, 7, 19]. In contrarily, some other studies found that early HBOT had the destructive effect on the cochlea [3, 4]. In latter studies, they concluded that early HBOT may increase the level of reactive oxygen metabolites and may exceed the capacity of antioxidant defense mechanism. Our findings in SEM images also support these findings. It showed injured hair cells (Figure 4 A, B).

That is way, some anti-inflammatory and antioxidant agents used additional to HBOT may prevent negative effect of hyberbaric oxygen. Corticosteroids, N-acetyl-cysteine, acetyl-L-carnitine, glutathione monoethylester, ebselen, D-methionine and carbamathione are the some examples for antioxidant and anti-inflammatory drugs [4, 16, 17, 20, 21]. Several animal studies showed the effectiveness of the use of these agents in addition to HBOT in acute acoustic trauma[7, 9].

In our study, there was an improvement on hearing thresholds observed in non-treated group. The average recovery was significantly better at non-treated group than group treated with 1 h HBOT alone and group treated with N-Acetyl Cysteine in association with HBOT. On the other hand, we observed additional improvement in group treated with steroid in association with HBOT. Recovery at high frequencies is also prominent at control (non-treated) group. Different way of the corticosteroid was suggested in the studies. Steroid may induce a corticosteroid-responsive transcription protein called promyelocytic leukemia zinc finger protein expression which is important for cochlear protection [22]. Some studies showed that the combination of HBOT and steroid therapies led to a significant improvement in recovery from AAT [16, 21].

HBOT with NAC treatment showed a protective effect on acoustic trauma. The suggested optimum NAC dosage is 1500 mg/kg/day [16, 21]. Lower dosages do not protect the cochlea [16]. However NAC used in our study resulted in an increase in hair cell loss. The protection effect is strongly dose-dependent; maybe the low dosage of our NAC could not protect sufficiently against negative effect of immediate HBOT. SEM image demonstrated that immediate treatment with HBOT in association with NAC can cause massive destruction of hair cells (Figure 4 C).

In conclusion, immediate HBOT after AAT has a negative effect on hearing threshold. This negative effect can be prevented by steroid therapy in addition to HBOT partially. However this protective effect was not equal to non-treated group. These results suggest a negative effect of HBOT for the treatment of AAT

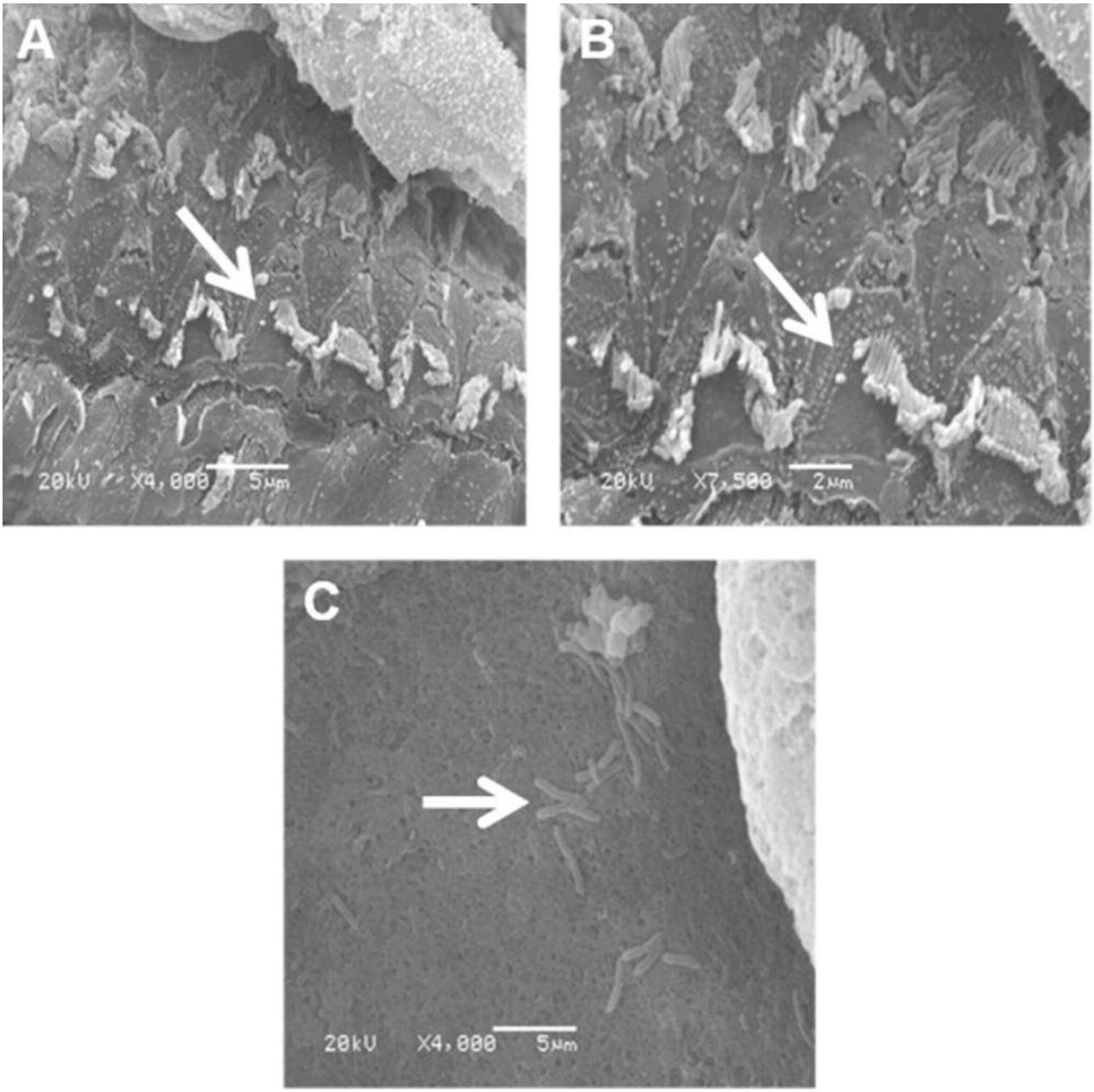

Figure 4. A, B: SEM image (After 7th day for HBOT group), **C:** SEM image (After 7th day for NAC group)

during the immediate period and if HBOT planned after AAT, it must combine with steroid. Because HBOT combined with steroid therapy could further improve recovery when compared to HBOT used alone during the immediate period. Further studies should be done in order to find out the appropriate dosage of NAC for early stage AAT.

High spontaneous recovery rate of the hearing threshold was observed in this study; however irreversible neural degeneration is a highly possible consequence of AAT. Anti-oxidative and anti-inflammatory agents may provide additional benefit to the single therapeutic option such as HBOT to prevent destruction of the auditory pathway.

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