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RESEARCH ARTICLE

The Evaluation of Systemic Methylprednisolone and Intratympanic Dexamethasone Treatment Efficacy in Rats with Hearing Loss Due to Acoustic Trauma

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Abstract

Aim: To evaluate the audiological, histopathological and electron microscopic effects of systemic methylprednisolone and intra tympanic dexamethasone (IT DXM) in rats with hearing loss due to acoustic trauma.

Materials and methods: Animals were exposed to white noise at a frequency of 1-10kHz and 110 dB SPL for 8 hours in a free environment. Distortion Product Otoacoustic Emission (DPOAE) measurements were performed on the first day of the trial. Forty-two adult female albino rats with acoustic trauma were randomized into three groups as group (n=8), group II (n=11) and group III (control, n=12). In group I, 0.8 mg/day IT DXM and in group II, 1 mg/kg/day intraperitoneal methylprednisolone were administrated during seven days. Saline solution was given intraperitoneally in group III. DPOAE tests were repeated on the 7th and 21st days. After animals were sacrifiated on the 21st day, their cochleas were evaluated with immunohistochemical (caspase-3) and electron microscopic examinations. Also statistical analysis was performed and compared.

Results: Measurement of the first day to 7th and 21st days were compared in allgroups separately. Statistical significant recovery was observed in the frequencies of5000 - 6000 Hz ($p<0.05$) in group I and in the frequencies of 6000 - 8000 Hz ($p<0.05$) in group II. The measurements in all frequencies between first, 7th and 21st days were similar incontrol group ($p>0.05$). No staining was obtained with caspase–3. Group I and II showed more stable and a large number of stereocilia than group III in electronmicroscopic examination.

Conclusion: Intratympanic dexamethasone and systemic methylprednisolonetreatment is effective at certain frequencies in hearing loss acoustic trauma. Thecombined use of both methods can provide additional benefits.

Keywords: Hearing Loss; Noise-Induced; Injection; Intratympanic; Otoacoustic Emissions

Abbreviations: IT DXM: Intratympanic dexamethasone; DPOAE: Distortion Product Otoacoustic Emission; TEM: Transmission Electron Microscopy; ROS: Reactive Oxygene Species; TTS: Temporary threshold shift; PTS: Permanent threshold shift; NOS: Nitric oxide synthesis; CAP: Compound action potential IT: Intratympanic

Introduction

Acoustic trauma Noise induced hearing loss is a common problem and causes sensor neural hearing loss since the gun shots and blasts in the war. Of recent date and present day, social life has provided people noise induced hearing loss with night clubs, listening the music on earphones on high level or working in industrial increase in cost of medical expenses. This problem was the disease of the workers after the industrial revolution before the II. World War. Many soldiers have had employment etc [1].

Noise damages many cells in the cochlea, but the outer hairy cells are the most damaged cells. However, in more severe noises, pathology can progress with internal hair cell death, loss of hearing nerve fibers, and damage to the striavascularis [2]. High level noise causes acute edema in striavascularis and this edema is associated with intermediate cell loss [3]. For acute acoustic trauma such as noise induced hearing loss, glucocorticoids clearly have protective effects via the glucocorticoid receptor signalling pathway [4].

Since the glucocorticoid receptors were detected in human inner ear, corticosteroids have been in use for the inner ear diseases for a long time like; autoimmune inner ear disease, tinnitus and Meniere disease [5-7]. The first intratympanic drug injection was the lidocaine injection in 1935 by Barany and Schuknect administrated streptomycin in Meniere disease in 1956 [8,9]. Although literature knowledge shows steroid treatment in acoustic trauma, still the way of the use (systemic or intratympanic) is not certain.

Researches on acoustic trauma pathophysiology and treatment still continue. Clinical human studies are generally based on to identify risk factors in extensive groups. Animal studies are important in preclinical research.

Material and Methods

The research protocol was submitted and approved by the Kocaeli University Ethics Committee for Animal Experiments and was conducted in accordance with the ethical regulations of the Declaration of Helsinki and in adherence to Turkish law and regulations.

Animals

Forty-two female Wistar albino rats weighing 200-230 g from Kocaeli University experimental medical research and scientific training laboratory were used. The animals were maintained on a 12:12 h light: dark cycle at 22 ºC with free access to food and water.

Experimental Procedures

All the animals were anesthetized with Xylazine (10 mg/kg) and ketamine (80 mg/kg) by intraperitoneally. Otoscopic examination and distortion product otoacoustic emission (DPOAE) assessment was performed andthose with normal data obtained were included in the trial. After animals wake upwithin cages were subjected to 110 dB SPL white noise by a sound stimulator and audio amplifier for 8 hours. Exposure levels measured at four position within each cage varied by <1 dB.

Twenty-four hours after the noise exposure, all the animals had the 2nd DPOAE measurement and data showed that they had had hearing loss. Then they were randomly assigned to the intratympanic dexamethasone (group I), systemic methylprednisolone (group II), and control group (%0.9 NaCl-group III). In group I; under ether anaesthesia the tympanic membrane of the right ear was visualized under an operating microscope and 0,8 mg dexamethasone(Onadron® 8mg, İ.E Ulagay) in 0,2 cc volume was injected trough inferior the rear quadrant using a dental needle once a dayfor 7 days. In group II animals received 1mg/kg methylprednisolone (Prednol®, Mustafa Nevzat) intraperitoneally without anesthesia once a day for 7 days. Twenty mg methylprednisolone was diluted with 40 cc with saline and 1cc was injected by insulin injector. Group III received the same volume saline solution intraperitoneally, for comparing with the group II and masking any effect of intraperitoneal inflammation for seven days. On the day 7 and 21 DPOAE assessment was repeated for all the animals and hearing results were recorded.

Determination of Distortion Product Otoacoustic Emissions

After an otoscopic examination to rule out possible middle ear pathologies, distortion product otoacoustic emissions (DPOAEs) were recorded using the smallest probe (Otodynamics Ltd, London, United Kingdom). Cubic difference distortion products ($2F_1 - F_2$) were performed in the General Diagnostic mode and the F1/F2 frequency ratio was set as 1.22 to obtain most powerful responses. In the Input -Output (IO) modality, both measurements of threshold and over threshold of I/O functions were performed using primary sound tones decreasing from 80 dB. Measurements were performed in 4004, 4358, 4761, 5188, 5652, 6165, 6726, 7336, 7996 Hz frequencies. DPOAE amplitude above 3 dB noise thresholds was considered significant. DPOAE amplitudes were analyzed statistically.

Dissection and Histologic Analysis of the Cochlea

On the day 21, animals were sacrificed by decapitation under anaesthesia inducted by intraperitoneal injection of 100 mg/kg ketamine. Temporal bones were dissected and right cochle as of the animals were harvested. Only two samples of each group were fixed in 4% glutaraldehyde and then decalcificated with formic acid and then prepared for transmission electron microscopy (TEM) using standard procedures. These samples were examined with Leica LEO 906E TEM.

Statistical Analysis

The SPSS statistical software program (SPSS, version 13.0 for windows; SPSS Inc, Chicago, Illinois, USA) was used to perform statistical calculations. Audiological results were compared with nonparametric 2 related (Wilcoxon) and 2 independent samples (Mann Whitney U) tests. Differences were accepted statistically significant at a p value < 0.05.

Results

During the study all the animals were examined every day and group 1 was planned as n=16 against the possibility of otitis. On average 2nd day five had otitis because of perforation or licking each other's injection site and two died.In group II one animal died. We think the cause of death was anesthesia in group 1 andperitonitis in group II. So eight animals were excluded from the study. Thereby the number of groups were; n=8 in group 1, n=11 in group II and n=12 in group III.

DPOAE Measurements

DPOAE measurement levels were recorded in each animal before the noise exposure and after 1,7 and 21st days. All the animals had hearing loss in all frequencies after the noise exposure. All the groups were statistically similar in Mann whitneyu test before the acoustic trauma and after the 1st day (Table 1 and 2).

	DPOAE amplitudes (dB) (mean±SD)			*p Value*	
Frequency (Hz)	**1st day**	**7th day**	**21st day**	**1st - 7th**	**1th - 21st**
5138	24.8 ± 5.49	33.6 ± 4.10	37.8 ± 4.82	0.012	0.012
5652	21.1 ± 6.02	31.7 ± 4.90	37.5 ± 6.47	0.012	0.012
6175	20.1 ± 5.42	31.1 ± 4.78	35.3 ± 5.75	0.012	0.012

Table 1: In group I, at 5000 - 6000 Hz average and standard deviation results and 1st – 7th day and 1st -21st day comparison p values respectively

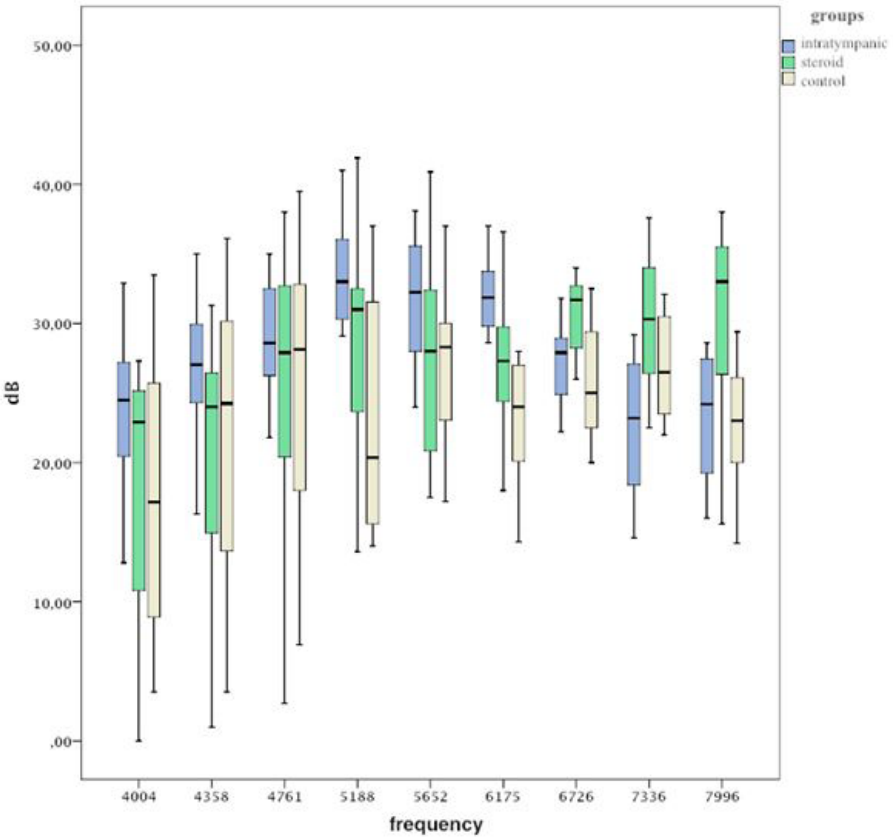
	DPOAE amplitudes (dB, mean±SD)			*p Value*	
Frequency (Hz)	**1st day**	**7th day**	**21st day**	**1st - 7th**	**1th - 21st**
6175	22.7 ± 5.72	27.4 ± 5.18	28.2 ± 5.50	0.005	0.037
6726	24.9 ± 4.16	29.9 ± 4.11	28.8 ± 3.06	0.013	0.008
7336	26.7 ± 4.00	30.2 ± 4.87	31.2 ±4.50	0.028	0.026
7996	23.8 ± 8.83	30.5 ± 6.87	31.0 ± 4.89	0.013	0.016

Table 2: In group II, at 6000 - 8000 Hz average and standard deviation results and 1st – 7th day and 1st -21st day comparison p values respectively

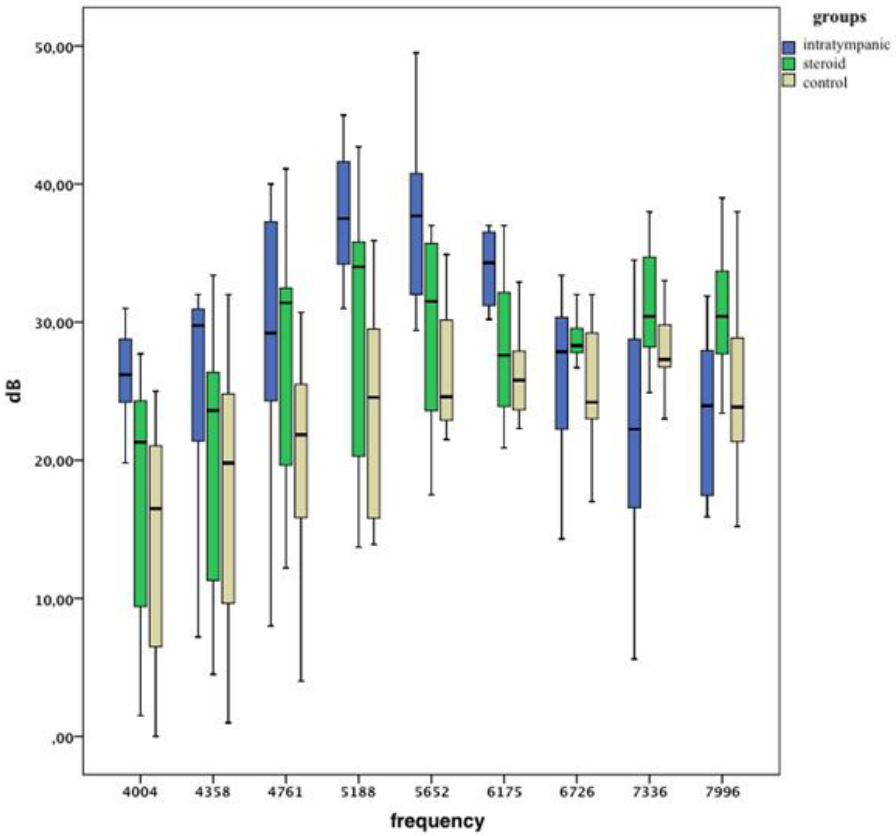
Recovery in the hearing threshold levels were observed in all groups between the 1st and 7th days (Graph 1 and 2). Also between 1st and 21st days DPOAE values showed an increase. These values were not statistically significant in control group (group III).

Group I showed statistically significant recovery in frequencies between 5000 Hz – 6000 Hz between the days 1st and 7th also 1st and 21st with Wilcoxon test ($p<0.05$) (Graph 3).

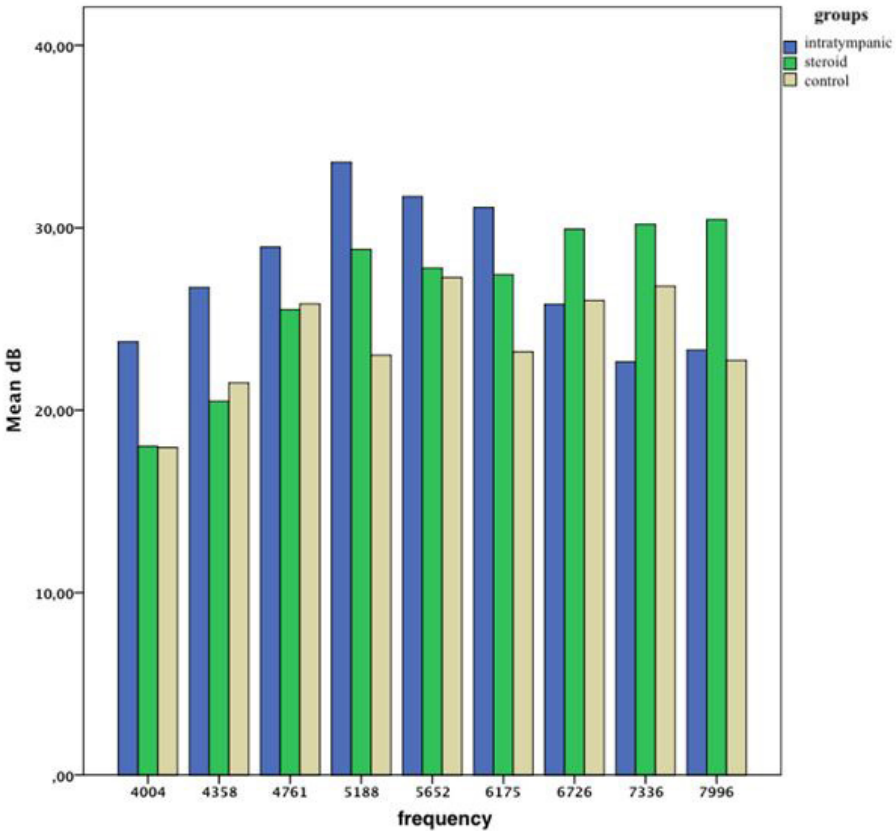
DPOAE values were statistically significant in group II at frequencies of 6000 - 8000 Hz between the days 1st and 7th also 1st and 21st ($p<0.05$) (Graph 4).



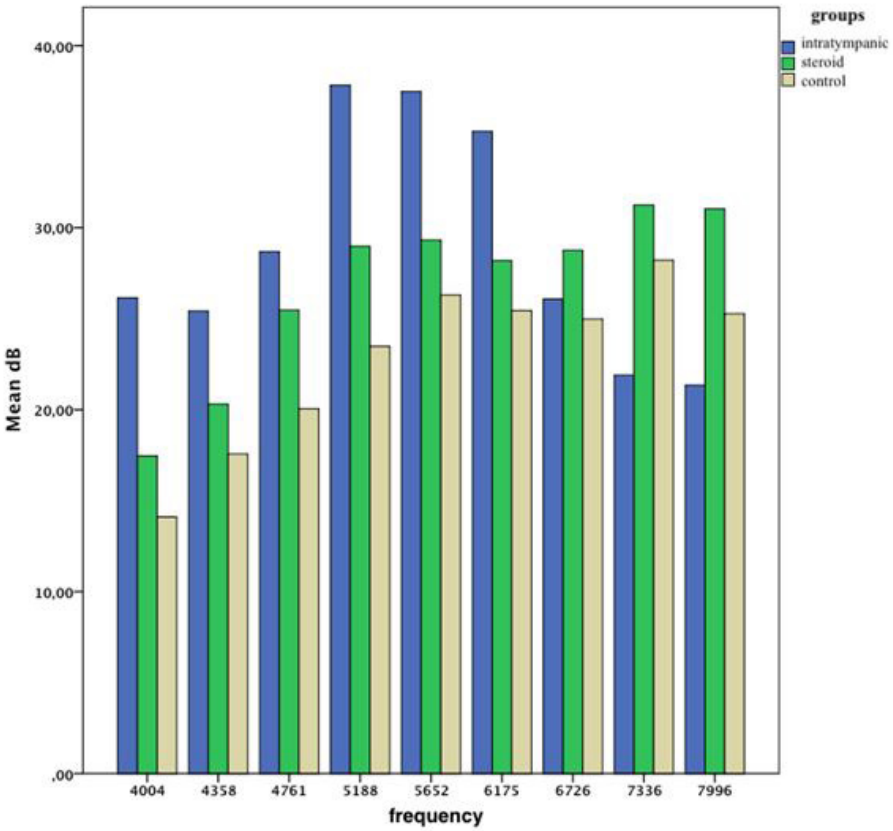
Graphic 1: Emission averages on the 7th day after trauma of each 3 groups are highest and lowest measurement, respectively



Graphic 2: Emission averages of 21 days after trauma of each 3 groups are highest and lowest measurements with distributions



Graphic 3: Bar graphic of all groups of posttraumatic day 7th. While statistical significance was observed at 5000 Hz and 6000 Hz in the non parametric Wilcoxon test performed in the intratympanic group, a significant difference was observed between 6000-8000 Hz in the systemic steroid group ($p < 0.05$). There was no significant difference in the control group ($p > 0.05$)



Graphic 4: Bar graphic of all groups of posttraumatic day 21st. While statistical significance was observed at 5000 Hz and 6000 Hz in the non parametric Wilcoxon test performed in the intratympanic group, a significant difference was observed between 6000-8000 Hz in the systemic steroid group ($p < 0.05$). There was no significant difference in the control group ($p > 0.05$)

Histomorphologic Assessment

In all the groups right ears of animals were analysed. Right temporal bone dissection was applied to all animals and stapedotomy and cochlea resection were performed. The oval window was drilled, allowing the fixative to penetrate into the inner ear. The resulting cochleas were taken to 10% neutral formalin solution for fixation. The tissues were then taken to hydrochloric acide + formic acide solution (BiodecR®, Bio-Optica) for decalcification, and then kept there for 16 hours.Histological tissue follow-up procedures were applied to the materials and embedded in paraffin blocks. Three μm thick sections were obtained with Leica microtome. Sections were passed through xylene and 3-stage alcohol, followed by hematoxylin and then with eosin. H & E stained sections were evaluated and Caspase-3 immunostaining was performed.

We aimed to show the apoptosis with Caspase-3 staining but there were no expression of Caspase-3 in any of the groups. Consequently we did not take into account the immunohistochemistry staining results for this study.

Two samples from each group were prepared for electron microscopic examination. After temporal bone dissection, tissues were fixed in 4% glutaraldehyde solution. After decalcification in formic acid, transmission electron microscopy (TEM) procedure was followed. One mm^3 trimmed tissue samples were fixed in 4% glutaraldehyde in +4 degrees for 2 hours. Then respectively after primary washing, secondary fixation and secondary washing and dehydration was applied. Samples were then stained for 1 hour at +4 degrees. Secondary dehydration was performed by removing water from the tissue in ethyl alcohol solutions at +4 °C. After infiltration and embedding, it was kept in a 60 °C oven for 24 hours and polymerization was achieved. Semi-thin sections of 1 micron thickness were taken on the slide from the plasticised tissue blocks with ultra microtome and stained with Toluidine blue.The zone was checked in the light microscope and the block was shaved for ultrathin section removal. Sections were taken to copper grids and citrate / Uranyl acetate contrasting was performed. The images in the grids were transferred to digital media after they had been examined under transmission electron microscope.

Transmission electron microscopic examination showed that stereocilia formation was degenerated and number of stereocilia was decreased in control group. Since the budget of the study was not sufficient, only 2 samples could be sent..So of course it is not enough to reach a conclusive description of the histopathological changes.

Organ of Corti, tectorial membrane, spiral ganglion, outer hair cell entirety and stereocilia formation was better in Group I and group II than control group (Figure 1, 2 and 3).

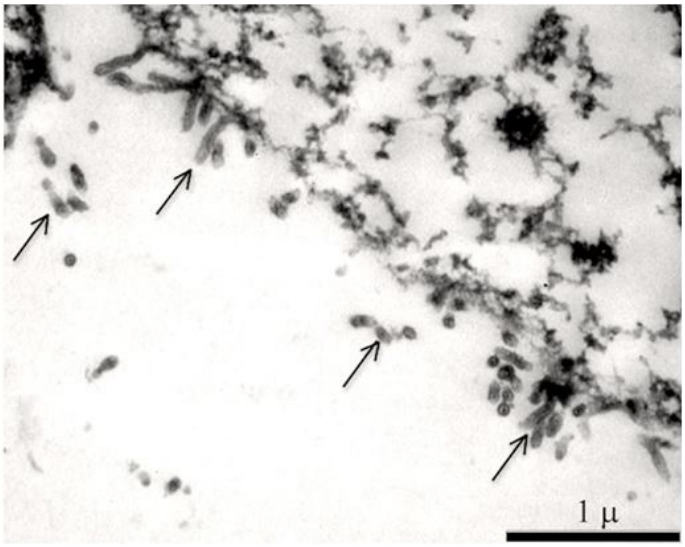


Figure 1: Transmission Electron Microscopic (TEM) image of control group. In the control group, the number of stereocilias (arrows) belonging to the outer hair cells were observed as decreased

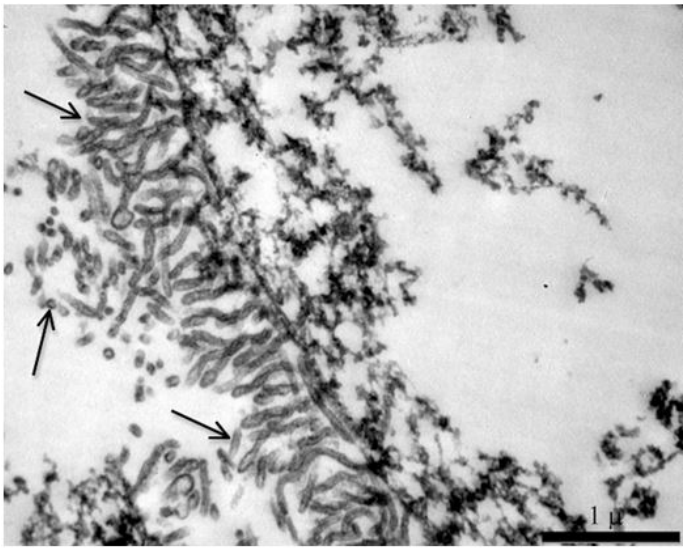


Figure 2: TEM image of the systemic steroid group. Presence of stereocilia (arrows) in the outer hair cells of the rat in the systemic steroid group. Stereocilia integrity and numbers have been preserved

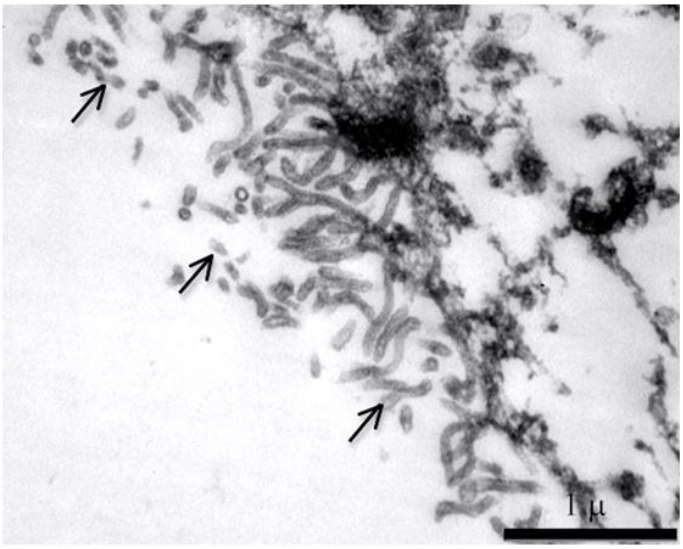


Figure 3: TEM image of the intratympanic group. Stereocilias(arrows) of the outer hair cells of the rats in the intratympanic group. As the number and structure are less than the systemic steroids, but more than the control group. Their integrity was also observed to be preserved

Discussion

Acoustic trauma has been the subject of many studies and still there are unclear aspects. Although numerous studies have been done and proven, researchers are still interested in. Mechanisms of noise induced hearing loss are various. Noise exposure drives mitochondrial activity and free radical production, reduces cochlear blood flow, causes excitotoxic neural swelling, and induces both necrotic and apoptotic cell death in the organ of Corti. Blood flow into the inner ear is reduced by noise and, subsequently, the inner ear becomes deprived of oxygen. This process leads to reactive oxygen species (ROS) making cell damages in outer hair cells, stria vascularis and spiral ganglions [10].They damage cellular lipids, proteins, and DNA, and upregulate apoptotic pathways. Selective outer hair cell loss is most commonly occurres within 24 hours and it continues for two weeks.

The damage caused by noise trauma depends on several factors. A frequency of 2000 to 3000 Hz causes more damage than lower or higher frequencies. Continuous and increased intensity ofnoise may cause severe damage [11].Acoustic trauma is a sudden change in hearing which occurs suddenly due to exposure to a very violent and short sound at a time. Noise-induced hearing loss is slow or delayed hearing as a result of continuous or intermittent noise. Hearing loss by acoustic trauma or noise induced can lead up to temporary threshold shift (TTS) or permenant threshold shift (PTS). There is no hair cell death in TTS but cochlear nerve terminals at their hair cell synapses show swelling and glutamate excitotoxicity within 24 h after exposure [12-14]. In PTS cochlear hair cell destruction or damage to their mechano-sensory hair bundles are seen [15].

Apoptosis due to noise exposure can be detected by caspase-3 staining in studies. Caspases and Bcl-2 are the main mediators for this process and caspase-3 leads to DNA fragmentation by sequentially. Active caspase-3 can be detected by immunostaining in hair cells and spiral ganglion cells [16,17]. Unfortunately in our study we could not detect sufficient caspase-3 expression by immunohistochemical staining.

There have been many agents studied for the noise induced hearing loss. Some of them were; corticosteroids, N-acetylsystein, salicylat, melatonin, tacrolimus, resveratrol etc. [18-21]. As our knowledge, current therapy for sudden sensorineural hearing loss is corticosteroid treatment. Therefore this agent can be used for the acoustic trauma or noise induced hearing loss. Glucocorticoid receptors were shown in human and rat inner ears [22-24]. The administration way of glucocorticoids is uncertain. Intratympanic steroid treatment has been tried [25]. Parnes, *et al.* studied hydrocortisone, dexamethasone, and methylprednisolone as oral, intratympanic and intravenously [26]. They found much higher penetration of all three drugs into the cochlear fluids following topical application as compared with systemic administration, with methylprednisolone showing the best profile. Glucocorticoids provide a protective effect against hypoxic-ischemic damage by decreasing basal metabolic energy requirements and increasing the availability or efficiency of use of energy substrates in the brain [27]. Glucocorticoids provide protection by up-regulating antioxidant enzyme activity during hypoxia-ischemia, attenuation of expression of nitric oxide synthesis (NOS) mRNA and the release of reactive nitrogen intermediates through suppression of gene expression for cytokines [28].

Rat cochlea and human cochlea have some similarities and dissimilarities. In rats the cochlea makes 3.25 or 4.25 turns. In humans, the number of turns is 2.5-2.75. The cochlea is composed of three tubular compartments, such as scala vestibular, scala tympani and scala media, as in humans [29]. The size of the tympanic membrane and the tympanic ring are larger than in proportion to the size of the temporal bone. There is no pars flaccida in eardrum. Rats' cochleas are convenient for neurootologic studies including odiologic tests and histopathologic assessment.

Intratympanic drug administration has some advantages like being an outpatient procedure, affecting only the affected ear etc. Chandrasekhar, *et al.* compared perilymph dexamethasone concentrations after systemic and intratympanic administration in 40 guinea pigs [30]. Intratympanic dexamethasone resulted in significantly higher perilymph steroid levels than intravenous dexamethasone. This higher perilymph steroid concentration feature is useful for patients who can't tolerate the systemic steroid or when it is contraindicated.

Tympanic membrane perforation, vertigo and pain can be seen in the intratympanic way due to drug concentration, type or temperature. Otitis media becomes a problem in aseptic conditions. These are also disadvantages for this method.

Otitis media due to intratympanic injection incidence is seemed seldom rarely also permanent tympanic membrane perforation does not occure generally in humans [31,32]. In animal studies; death, otitis media and permanent tympanic membrane perforation are more common. Therefore aseptic conditions need more attention, and animals must be greater number than planned in intratympanic injection groups at animal studies.

In intratympanic group we observed recovery in specific frequencies. Ozdogan, *et al.* showed in their study, recovery in 14th day after acoustic trauma was in 6000 Hz either [33]. Takemura, *et al.* showed that different doses of dexamethasone direct infusion into the inner ear are effective in attenuating noise-induced trauma [34]. Also systemic steroid group showed recovery in high frequencies and DPOAE values were statistically significant. Takahashi, *et al.* studied in animals about the effect of methylprednisolone by intraperitoneally after the exposure to 2kHz pure tone of 110, 115 or 120 dB SPL for 10 minutes [35].The doses were 6, 12 or 40 mg/kg for 7 days and the threshold of the compound action potential (CAP) was examined on 8th day. They found that the CAP threshold shift was improved in only after exposure to 110 dB SPL. In our study animals were exposed to 110 dB noise and our dose for the methylprednisolone 1mg/kg for 7 days. In many clinics it is widely used that methylprednisolone for systemic treatment is calculated as 1 mg/kg. Our findings are compatible with the literature.

Based on the data of DPOAE in our study, the audiologic improvement was obtained at 5000-6000 Hz frequencies with intratympanic steroid injection and also at 6000-8000 Hz frequencies with systemic steroid.In order to be a pilot study, two samples of cochlea from each group were analyzed with an electron microscopic examination for qualitative detection. In conclusion, both IT dexamethasone group and systemic methylprednisolone group showed that organ of corti ultra structure, outer hair cell morphology and number of stereocilias were better preserved than control group. For this reason, it may be beneficial to use both methods in the earliest period of sudden hearing loss after acoustic trauma or severe noise exposure.

Conclusion

The negative effect of acoustic trauma on hearing threshold levels can be reversed by steroid administration by systemic or intratympanic way. In our study intratympanic dexamethasone group and systemic methylprednisolone group showed recovery in different specific frequencies. It was determined at 7th day; so combined therapy of intratympanic and systemic steroid can be used in the early period of noise exposure of course this theory must be supported by another study including a combined therapy group.

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