Efficacy of Systemic Methylprednisolone and Intratympanic Dexamethasone in Rats with Noise Induced Hearing Loss

Gürültüye Bağlı İşître Kaybı Olan Sıçanlarda Sistemik Metilprednizolon ve İntratimpanik Dexametazonun Etkinliği

Sebla ÇALIŞKANa, Murat TOPDAg, Kadri İLAc, Ahmet KARAd

aDepartment of Otorhinolaryngology, Head and Neck Surgery, University of Health Sciences Derince Training and Research Hospital, Kocaeli, Türkiye
bDepartment of Otorhinolaryngology, Head and Neck Surgery, Altunizade Acibadem Hospital, İstanbul, Türkiye
cDepartment of Otorhinolaryngology, Head and Neck Surgery, University of Health Sciences Umranıye Training and Research Hospital, İstanbul, Türkiye
dDepartment of Otorhinolaryngology, Head and Neck Surgery, Sakarya University Faculty of Medicine, Sakarya, Türkiye

ABSTRACT

Objective: To evaluate the audiological and electron microscopic (EM) effects of systemic methylprednisolone and intratympanic dexamethasone (IT DXM) in rats with noise induced hearing loss.

Material and Methods: Thirty-one adult female Wistar albino rats were randomized into 3 groups as Group I (n=8), Group II (n=11) and Group III (control, n=12). The animals were exposed to white noise at a frequency of 1-10 kHz and 110 dB sound pressure level for 8 hours in a free environment. Distortion product otoacoustic emission (DPOAE) measurements were performed before and after the noise.

Results: Measurement of the first day and after the noise for the DPOAE measurements. statistical analysis was performed.

Conclusion: IT DXM and systemic methylprednisolone are effective at certain frequencies in noise induced hearing loss. The combined use of both methods may provide additional benefits.

Keywords: Hearing loss, noise-induced; injection, intratympanic; otoacoustic emissions; spontaneous

ÖZET

Amaç: Çalışmamızda, gürültüye bağlı işître kaybı gelişen sıçanlarda, sistemik metilprednizolon ve intratimpanik (IT) deksametazonun odyolojik ve elektron mikroskopik (EM) etkilerinin değerlendirilmesi amaçlandı. Gerçek ve Yöntemler: Otuz bir adet erişkin dişi Wistar albino sıçan Grup I (n=8), Grup II (n=11) ve Grup III (kontrol, n=12) olmak üzere 3 gruba randomize edildi. Tüm sıçanlara 1-10 kHz frekansında, 110 dB şiddetinde 8 saat boynucu kesinşiz, homojen karakterde gürültü verildi. Gürültü öncesi ve 1 gün sonrası “distortion product otoakustik emisyon (DPOAE)” ölçümleri yapıldı. Birinci gruba 7 gün boynucu 0,8 mg/kg IT deksametazon, 2. gruba 7 gün 1 mg/kg/gün intratimpanik metilprednizolon uygulandı. Ölünçü gruba ise 7 gün boynucu intratimpanal yolla serum fizyolojik verildi. DPOAE ölçümleri 7 ve 21. gün de tekrarlandı. sıçanlar 21. günü sakrifiye edildiler ve kolesterolu çıkarıldı. Ölüm dış analiz işitme kaybını ve EM incelenecekti. Ölülerin ise istatistiksel analyze tabi tutuldu. Bulgular: Tüm sıçan gruplarının 1. günü ölçümü ile 7 ve 21. günü ölçümü ayrı ayrı karşılaştırıldı. Grup I’de ise 5,000 ile 6,000 Hz frekanslarında istatistiksel anlamlı değişiklik gözlemdi (p<0,05). Grup II’de ise 6,000 ile 8,000 Hz frekanslarında istatistiksel anlamlı değişiklik gözlemdi (p<0,05). Kontrol grubunda ise 1. gün ile 7 ve 21. günü ölçümü benzer olarak izlenmişti (p>0,05). Elektron mikroskopide Grup I ve Grup II’de Grup III’de ise stereosilya sayısı ve bünyeliği kaliteli olarak daha fazla izlenmişti. Sonuç: IT deksametazon ve sistemik metilprednizolon gürültüye bağlı işître kaybı tedavisinde belirli frekanslarda etkili olabilir. Her iki metodun kombin kullanılması ek yarar sağlayabilir.
Noise induced hearing loss is a common problem, causing sensorineural hearing loss and labor loss. Unfortunately, social life has recently and nowadays brought people the problem of noise induced hearing loss, such as nightclubs, listening to music with high-end headphones or working in an industrial area. This problem most affected the workers of the industrial revolution before World War II. Occupational noise, in particular is a significant risk factor for workers of many ages, ranging from 7% to 21% (average 16%) of the adult-onset hearing loss worldwide.\(^1\)

Research continues on the pathophysiology and treatment of acoustic trauma or noise induced hearing loss. Clinical human studies are generally based on the identification of risk factors in large groups. Animal studies are important in preclinical research.

Noise causes a lot of damage to the cochlea, but the outer hair cells are the most damaged. However, in more severe noises, the pathology may progress to inner hair cell death, loss of nerve fibers, and damage to stria vascularis.\(^2\) A high level noise causes acute edema in the stria vascularis, which is associated with intermediate cell loss.\(^3\) Glucocorticoids clearly have protective effects through the glucocorticoid receptor signaling pathway.\(^4\)

Since the glucocorticoid receptor was detected in the human inner ear, corticosteroids have been used for in inner ear disease such as autoimmune inner ear disease, tinnitus and Meniere’s disease.\(^5,7\) Methods of administration of drugs vary. We aimed to compare the the results obtained with the treatment options in practical clinical use and application methods of glucocorticoids by creating noise-induced hearing loss in animals.

**MATERIAL AND METHODS**

The research protocol was approved by the Kocaeli University Ethics Committee for Animal Experiments (date: June 18, 2013, number: KOU HAYDEK 6/1-3013) and was performed according to the ethical standards of the Helsinki Declaration and in adherence to Turkish law and regulations. All animals have received humane care in compliance with the Guide for the Care and Use of Laboratory Animals.

**ANIMALS**

Forty female Wistar albino rats weighing 200-230 g from Kocaeli University experimental medical research and scientific training laboratory were used because of their similarity about well defined temporal bone anatomy and hearing physiology. The animals were maintained on a 12:12 h light-dark cycle at 22 °C 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 and those with normal data obtained were included in the trial. After awakening in the cages, the animals were exposed to 110 dB sound pressure level (SPL) white (broadband) noise (1-10 kHz) with a sound stimulator and sound amplifier for 8 hours. The exposure levels measured at the 4 positions in 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. They were then randomly divided into intratympanic dexamethasone (IT DXM) (Group I), systemic methylprednisolone (Group II), control group (%0.9 NaCl-Group III). In Group I; under ether anesthesia the tympanic membrane of the right ear was visualized under an operating microscope and 0.8 mg dexametasone (Onadron® 8 mg, IE Ulagay, Turkey) in 0.2 cc (4 mg/mL) volume was injected through inferior the rear quadrant using a dental needle once a day for 7 days. In Group II animals received 1 mg/kg methylprednisolone (Prednol ®, Mustafa Nevzat, Turkey intraperitoneally without anesthesia once a day for 7 days. Twenty mg methylprednisolone was diluted with 40 cc with saline and 1 cc was injected by insulin injector. Group III received the same volume saline solution intraperitoneally for 7 days to compare Group II and to mask any effects of intraperitoneal inflammation.

During the study all the animals were examined every day and Group I was planned as n=16 against the possibility of otitis. On the 2nd day 5 of them de-
veloped otitis media due to licking or perforation of
the injection site and also 3 of them died. In Group II
one animal died. We think the cause of death was
anesthesia in Group I and peritonitis in Group II. So
8 animals were excluded from the study. Thereby the
number of groups were; n=8 in Group I, n=11 in
Group II, n=12 in Group III.

On the day 7 and 21 DPOAE assessment was re-
peated for all the animals and hearing results were
recorded.

**DETERMINATION OF DPOAEs**

After an otoscopic examination to rule out possible
middle ear pathologies, DPOAEs were recorded
using the smallest probe (Otodynamics Ltd, London,
United Kingdom). Cubic difference distortion products
(2F1-F2) 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
(I/O) 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 4,004, 4,358,
4,761, 5,188, 5,652, 6,165, 6,726, 7,336, 7,996 Hz
frequencies. DPOAE amplitude above 3 dB noise
thresholds was considered significant. DPOAE am-
plitudes were analyzed statistically.

**DISSECTION AND HISTOLOGIC ANALYSIS OF THE
COCHLEA**

On the day 21, animals were sacrificed by decapita-
tion under anaesthesia inducted by intraperitoneal
injection of 100 mg/kg ketamine. Temporal bones were
dissected and right cochleas of the animals were har-
vested. Only 2 samples of each group were fixed in
4% glutaraldehyde and than decalcified with
formic acide and than prepared for transmission elec-
tron microscopy (TEM) regarding the assessment of
outer hair cell structure using standard procedures.
These samples were examined with Zeiss Leo 906 E
TEM (Germany)

**STATISTICAL ANALYSIS**

The SPSS (SPSS, version 13.0 for windows; SPSS
Inc, Chicago, Illinois, USA) was used to perform sta-
tistical calculations. Audiological results were com-
pared with nonparametric 2 related (Wilcoxon) and
2 independent samples (Mann-Whitney U) tests. Dif-
fences were accepted statistically significant at a p
value <0.05.

**RESULTS**

**DPOAE**

DPOAE levels were recorded in each animal before
the exposure to noise and on days 1, 7 and 21 after
noise. All the animals had hearing loss in all fre-
quencies after the noise exposure. All the groups
were statistically similar in Mann-Whitney U test per-
formed before the acoustic trauma and after.

Hearing threshold levels improved in all groups
between the 1st and 7th days (Figure 1). There was also
an increase in DPOAE results between days 1 and 21
(Figure 2). These results were not statistically signif-
icant in the control group (Group III).

Group I showed statistically significant im-
provement at frequencies between 5,000 Hz-6,000
Hz between the days 1 and 7 and between days 1 and
21 with Wilcoxon test (p<0.05) (Figure 3) (Table 1).

DPOAE results were statistically significant at
of 6,000-8,000 Hz frequencies between 1st and 7th
days and between 1st and 21st days in Group II
(p<0.05) (Figure 4) (Table 2).

**HISTOMORPHOLOGIC ASSESSMENT**

Right ears of animals were analysed in all groups. All
animals underwent right temporal bone dissection,
and stapedotomy and cochlea resection were per-
formed. The oval window was drilled to allow the
fixative to enter the inner ear. The obtained cochleas
were placed in 10% neutral formalin solution for fix-
ation. The tissues were then taken to hydrochloric
acide+formic acide solution (Biodec R® Bio-Optica
Milano, Italy) for decalcification, and then kept there
for 16 hours. Histological tissue follow-up proce-
dures were applied to the materials and embedded in
paraffin blocks. Three μm thick sections were ob-
tained with Leica Microtome, (United States of
America). 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 demonstrate apoptosis with caspase-3 staining, but caspase-3 expression was not present in any group. Consequently, we did not consider 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, TEM procedure was followed. One mm³ 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 ultramicrotome 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.

In the transmission electron microscopic examination, it was observed that stereocilia formation was degenerated and the number of stereocilia decreased in control group. Since the budget of the study was not sufficient, only 2 samples could be sent. Therefore, it is not sufficient to reach a precise definition of 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 5, Figure 6, Figure 7).

DISCUSSION

Noise induced hearing loss has been the subject of many studies and still has uncertain aspects. Although numerous studies have been done and proven,
researchers are still interested in. The mechanisms of noise induced hearing loss are diverse. Noise can cause both apoptotic cell death and necrotic cell death in the organ of corti. It increases the production of free radicals by triggering mitochondrial activity, and causes exitotoxic neuronal swelling. This process causes reactive oxygen species to cause cell damages in outer hair cells, stria vascularis and spiral ganglia. They stimulate apoptotic pathways by damaging proteins, cellular lipids and DNA. Selective outer hair cell loss most often occurs within 24 hours and continues for 2 weeks.

The damage from noise trauma depends on many factors. The frequencies most affected are 3.4 and 6 kHz, worse at 4 kHz. Continuous and increased noise intensity can cause serious damage. Acoustic trauma is a sudden change in hearing that occurs suddenly as a result of exposure to a very loud and short-term sound. Noise-induced hearing loss is slow or delayed hearing as a result of continuous or intermittent noise. Acoustic trauma or noise induced hearing loss can lead to temporary threshold shift (TTS) or permanent 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 of exposure. In PTS cochlear hair cell destruction or damage to their mechano-sensory hair bundles are seen. So we exposed the animals to a sudden onset but long-lasting noise. In this way, we aimed to show the damage that will occur more clearly.

<table>
<thead>
<tr>
<th>TABLE 1: In Group I, at 5,000-6,000 Hz average and standard deviation results and 1st-7th day and 1st-21st day comparison p values respectively.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>5,138</td>
</tr>
<tr>
<td>5,652</td>
</tr>
<tr>
<td>6,175</td>
</tr>
</tbody>
</table>

DPOAE: Distortion product otoacoustic emission; SD: Standard deviation.

The first intratympanic drug injection was the lidocaine by Bárány in 1935 and streptomycin injection in 1956 by Schuknecht Meniere disease. Among the agents examined for noise-induced hearing loss; corticosteroids, N-acetylsystein, salicylat, melatonin, tacrolimus, resveratrol etc. To best of our knowledge, the current treatment for sudden sensorineural hearing loss is corticosteroid therapy. Therefore this agent can be used for acoustic trauma or noise induced hearing loss. Glucocorticoid receptors have been demonstrated in the human and rat inner ears. The route of glucocorticoids is unclear. Intratympanic steroid treatment has been tried. Parnes et al. studied hydrocortisone, dexamethasone, and methylprednisolone as orally, intratympanic and intravenously. They found that all 3 drugs were better than systemic application in transition to cochlear fluids with topical application and methylprednisolone was the best. Today, both methylprednisolone and dexamethasone are used in intratympanic administration. We prefer dexamethasone in daily practice because of the burning sensation of methylprednisolone. In our study, we chose to proceed based on this application.
Rat cochlea and human cochlea have some similarities and differences. 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 consists of three tubular compartments, like the scala vestibular, and the tympanic ring are larger in proportion to the size of the temporal bone. The pars flaccida is absent in the eardrum. Rats’ cochleas are convenient for neurologic studies including audiologic tests and histopathologic assessment.

We observed improvement in certain frequencies in the intratympanic group. These frequencies were 5,000-6,000 Hz. Ozdogan et al. in their study, showed that the recovery was at 6,000 Hz on 14th day after acoustic trauma either. Likewise Takemura et al. showed that direct infusion of different doses of dexamethasone into the inner ear was effective in reducing noise-induced trauma. Another finding in our study was that the measurements in the systemic steroid group improved at high frequencies and the DPOAE values were statistically significant. Takahashi et al. studied the effect of methylprednisolone intraperitoneally after the exposing animals to 2 kHz pure tone of 110, 115 or 120 dB SPL for 10 minutes. Doses were 6, 12 or 40 mg/kg for 7 days, and the compound action potential (CAP) threshold was examined on day 8. They found that the CAP threshold shift only improved after exposure to 110 dB SPL. In our study animals were exposed to 110 dB noise and

<table>
<thead>
<tr>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>6,175</td>
</tr>
<tr>
<td>6,726</td>
</tr>
<tr>
<td>7,336</td>
</tr>
<tr>
<td>7,996</td>
</tr>
</tbody>
</table>

DPOAE: Distortion product otoacoustic emission; SD: Standard deviation.
our dose for the methylprednisolone 1 mg/kg for 7 days. Calculation of methylprednisolone as 1 mg/kg is widely used for systemic treatment in many clinics. Our findings are compatible with the literature.

Intratympanic drug administration has some advantages such as being an outpatient procedure, affecting only the affected ear etc. Chandrasekhar et al. studied dexamethasone concentrations in the perilymph after intratympanic and systemic administration in 40 guinea pigs. IT DXM was found to be significantly higher than intravenous dexamethasone in perilymph levels. This feature of higher perilymph steroid concentration is beneficial in patients who are intolerant or contraindicated to systemic steroid.

Depending on the concentration, type or temperature of the drug, tympanic membrane perforation, vertigo and pain may occur. Otitis media becomes a problem in aseptic conditions. These are the disadvantages of this method. While the incidence of otitis media due to intratympanic injection is rarely seen, permanent tympanic membrane perforation is not generally seen in humans. In animal studies; death, otitis media and permanent tympanic membrane perforation are more common. Therefore greater attention should be paid to aseptic conditions in animal studies, and a larger number of animals in intratympanic injection groups than planned is required. Since we encountered the disadvantages mentioned above in the intratympanic injection groups in such studies conducted in our clinic before, we kept the number of this group higher than the others and paid attention to asepsis conditions. Despite all this experiences, we lost a large number of animals in our intratympanic injection group.

In our study, the audiologic improvement was achieved with intratympanic steroid injection at frequencies of 5,000-6,000 Hz, and with systemic steroid at frequencies of 6,000-8,000 Hz, according to DPOAE data. To be a pilot study, 2 cochlea samples from each group were analyzed by transmission electron microscopic examination for qualitative detection. However, the small sample size prevented us from obtaining quantitative data. In conclusion, both IT DXM group and systemic methylprednisolone group showed better preservation of organ of corti ultrastructure, outer hair cell morphology and stereocilia number compared to the 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 exposure to severe noise.

CONCLUSION

The adverse effect of acoustic trauma on hearing threshold levels can be reversed by systemic or intratympanic steroid administration. In our study, IT DXM group and systemic methylprednisolone group showed improvement in the early period (7th day) at different specific frequencies. Therefore, combined treatment of intratympanic and systemic steroids can be used in the early phase of noise exposure. Of course, this theory should be supported by another study involving a combined therapy group.

Acknowledgments

The authors thank Professor Necdet Demir and Esma Konuk from the Akdeniz University Faculty of Medicine Department of Histology for the TEM analysis of the samples.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Sebla Çalışkan, Murat Topdağ; Design: Sebla Çalışkan, Murat Topdağ; Control/Supervision: Sebla Çalışkan, Murat Topdağ; Data Collection and/or Processing: Sebla Çalışkan, Kadri İla, Ahmet Kara; Analysis and/or Interpretation: Sebla Çalışkan, Murat Topdağ; Literature Review: Sebla Çalışkan; Writing the Article: Sebla Çalışkan; Critical Review: Murat Topdağ; References and Fundings: Sebla Çalışkan, Murat Topdağ, Kadri İla, Ahmet Kara; Materials: Sebla Çalışkan, Murat Topdağ, Kadri İla.
REFERENCES


4. Jin DX, Lin Z, Lei D, Bao J. The role of glucocorticoids for spiral ganglion neuron survival. Brain Res. 2009;1277:3-11. [Crossref] [PubMed] [PMC]


10. Spöndlin H. Primary structural changes in the organ of Corti after acoustic overstimulation. Acta Otolaryngol. 1971;71(2):166-76. [Crossref] [PubMed]


12. Robertson D. Functional significance of dendritic swelling after loud sounds in the guinea pig cochlea. Hear Res. 1983;9(3):263-78. [Crossref] [PubMed]


15. Schuknecht HF. Ablation therapy for the relief of Ménière’s disease. Laryngoscope. 1956;66(7):859-70. [Crossref] [PubMed]


20. Rarey KE, Curtis LM, ten Cate WJ. Tissue specific levels of glucocorticoid receptor within the rat inner ear. Hear Res. 1993;64(2):205-10. [Crossref] [PubMed]

21. ten Cate WJ, Curtis LM, Small GM, Rarey KE. Localization of glucocorticoid receptors and glucocorticoid receptor mRNAs in the rat cochlea. Laryngoscope. 1993;103(8):865-71. [Crossref] [PubMed]


