Research Article

Effect of Ischemia-Induced Cochlear Inflammation on Auditory Responses in Male Rats

Hamed Fanaei[1,2], **Akram Pourbakht**[3,4], **Sadegh Jafarzadeh**[5*]

[1.] *Pregnancy Health Research Center, Zahedan University of Medical Sciences, Zahedan, Iran*
[2.] *Department of Physiology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran*
[3.] *Rehabilitation Research Center, Iran University of Medical Sciences, Tehran, Iran*
[4.] *Department of Audiology, School of Rehabilitation Sciences, Iran University of Medical Sciences, Tehran, Iran*
[5.] *Department of Audiology, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran*

Use your device to scan and read the article online

Citation: Fanaei H, Pourbakht A, Jafarzadeh S. Effect of Ischemia-Induced Cochlear Inflammation on Auditory Responses in Male Rats. Aud Vestib Res. 2022;31(1):53-9.

https://doi.org/10.18502/avr.v31i1.8135

Highlights

- Cochlear oxidative stress affects hearing sensitivity
- ABR showed increased thresholds on day14th, 21th, and 28th
- TNF-α and CRP levels concentrations increased after ischemia

Article info:
Received: 26 Jun 2021
Revised: 09 Aug 2021
Accepted: 22 Aug 2021

*** Corresponding Author:**
Department of Audiology, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran.
jafarzadehs@mums.ac.ir

ABSTRACT

Background and Aim: Ischemic injury is a major cause of hearing loss and oxidative stress is an important part of ischemic injury. The goal of this study was to evaluate the cochlear oxidative stress effect on auditory responses in male rats.

Methods: Cochlear oxidative stress was induced by bilateral carotid artery occlusion for 20 minutes. The rats were evaluated by biochemical inflammatory factors tumor necrosis factor-α [TNF-α] and C-reactive protein (CRP) in the day before and 1st, 4th, and 7th days following surgery. The auditory brainstem response (ABR) and electrocochleography (ECochG) were evaluated on the day before surgery and 14th, 21th and 28th days after surgery.

Results: TNF-α and CRP levels concentrations increased one day after ischemia and subsequently decreased on the 7th day. The click and tone burst evoked ABR showed increased thresholds on day14th, 21th, and 28th. The highest threshold was recorded on day14th. The ECochG results also were abnormal for 55%, 70%, and 45% of cases on day 14th, 21th, and 28th, respectively.

Conclusion: Cochlear oxidative stress affects hearing sensitivity. The ABR shows elevated thresholds and abnormal ECochG was found in many cases.

Keywords: Oxidative stress; auditory brainstem response; electrocochleography; rat

Introduction

The ischemic injury could cause hearing loss [1]. Previous studies have shown that a major part of the ischemic effect on hair loss is caused by oxidative stress and inflammation [1, 2]. In the auditory system, the cochlea is the key organ that converts auditory stimuli into nerve impulses. The cochlea is very sensitive to reduced blood flow or disruption of blood flow. Ischemia damages the cochlea by various mechanisms [1-3]. In general, inflammation is one of the key factors that ischemia damages tissues [3].

Damaged cells after ischemia release stimulant factors to trigger the inflammatory process and aggregation of immune cells [2, 3]. Past studies have shown that ischemia causes permanent damage to the cochlea [2]. Recent evidence demonstrated that cochlear inflammation can cause vascular vasospasm [3] which in theory can lead to exacerbation of ischemia and vicious cycle. Tumor necrosis factor- α [TNF-α] is a proinflammatory factor released from ischemia-damaged cells [3, 4]. An increase in TNF-α in the cochlea initiates and expands the inflammatory process [4].

After ischemic damage, the cochlear nerve and the hair cells would have irreplaceable damage. Knowing the effect of ischemia through TNF-α and cochlear inflammation is important for successful treatment [2].

In this study, we studied the cochlear ischemia effect induced by occlusion of carotid arteries on cochlear TNF-α inflammation and hearing sensitivity of rats. The auditory brainstem response (ABR) was used for hearing evaluations and electrocochleography (ECochG) for evaluating cochlear endolymphatic hydrops. A previous study showed the fluctuating behavior of auditory thresholds in the first week after surgeries [1]. This might relate to abnormal endolymphatic hydrops and cochlear function. Therefore, a wider period of time was selected for evaluation.

The aim of this study was to assess the effect of the ischemia-induced cochlear oxidative stress on auditory responses in male rats.

Methods

Animals

In this study, 20 male Wistar rats (200–220 g) were used. Animals provided from Pasteur Institute, Tehran, Iran. The animals were adapted to the laboratory setting two weeks prior to the experiment. Rats were kept under standard conditions (temperature: 22–24 °C, humidity of 40–45%, 12-hour lighting cycle). They had unlimited access to water and food.

Experimental design

The rats were categorized into two groups (10 in each group):

Control group for biochemical evaluations: common carotid arteries (CCAs) were surgically prepared for the occlusion without any CCAs occlusion or induced ischemia.

Ischemic group: the rats were subjected to both CCA occlusion for biochemical evaluations and auditory evaluations.

Surgery

For cochlea ischemia induction in the ischemic group, the animals were anesthetized (ketamine [100 mg/kg] and xylazine [10 mg/kg]). Then, the both CCA of animals were separated from the vagus nerve and occluded by atraumatic arterial clamps. After 20 minutes the clamps were detached and reperfusion was visually confirmed [1].

After the surgeries, the evaluation consisted of two parts: biochemical and auditory evaluations. The biochemical evaluation was used in the first week after surgery for showing the presence of inflammatory factors. The auditory evaluations covered a longer period until day 28th after surgeries and were used for showing the change of hearing sensitivity in this period.

Biochemical evaluations

Biochemical evaluations included measuring the level of inflammatory factors (TNF-α and C-reactive protein [CRP]) in the day before and 1st, 4th, and 7th days after surgery [5]. TNF-α and CRP were measured using specific kits (all kits come from Zellbio, Germany). The results of Biochemical evaluations were used to show the inflammatory process in the first week after surgery.

The blood samples were taken on 0, 1st, 4th, and 7th days after ischemia from the tail and centrifuged (4° C, 3000 g for 15 min) to separate the serum. One month later, inflammatory factors (TNF-α (Zellbio, Germany), C-reactive protein (CRP) (Zellbio, Germany)) were measured using special kits in the physiology department.

Tumor necrosis factor-α measurement

TNF-α level was performed by a commercial chemical colorimetrical assay kit based on the manufacturer's protocol (Zellbio, Germany).

The 50 µL of all samples and standards were added to appropriate wells. Afterward, to each well 50 µL of the Antibody Cocktail was added. The plate was sealed and incubated for one hour at room temperature on a plate shaker. Each well was washed with 3 x 350 µL Wash Buffer. 100 µL of tetramethylbenzidine (TMB) substrate was added to each well and incubate for 10 minutes in the dark on a plate shaker. 100 µL of Stop Solution was added to each well. The plate was on a plate shaker for one minute to mix. Finally, optical density (OD) at 450 nm was recorded.

C-reactive protein measurement

CRP level was performed by a commercial chemical colorimetrical assay kit based on the manufacturer's protocol (Zellbio, Germany). All reagents, samples, and standards were done as instructed in the manual. 100 µl of standard or sample were added to each well. Incubated 2.5 h at room temperature (RT). 100 µl of prepared biotin antibody was added to each well. Then incubated one hour at RT. 100 µl of prepared Streptavidin solution was added to each well. Incubated 45 min at RT. 100 µl of TMB One-Step Substrate Reagent was added to each well. Incubated 30 min at RT. 50 µl of stop solution was added to each well. Finally, optical density at 450 nm was recorded.

Auditory evaluations

The rats were tested by ABR and EcochG on the day before surgery and 14th, 21th and 28th days after surgery. The selected rats must have a normal hearing before surgery. Therefore, all cases with ABR thresholds over 20dB nHL for any stimuli were excluded.

Prior to each test, rats were put under anesthesia and their body heat was controlled with a blanket. The stimulus was presented monaurally for all cases. ABR and ECochG were tested by Eclipse (EP25 software, Intracoustic) and needle electrodes. The impedance was kept low (under 5kΩ) and balanced (under 2kΩ between electrodes). The non-inverting, inverting, and ground electrodes were placed at the forehead, mastoids, and tail, respectively. For ABR evaluation, the alternate polarity click and tone burst stimuli (4 kHz) with default rise, fall, and plateau time presented via insert phone. The stimuli rate was 37.7 Hz. The response was amplified at 100000X and filtered at 100 to 3000Hz. Wave II was selected for threshold estimation. At least, a 2000 sweep was used for intensities near the threshold.

The ECochG responses were recorded with similar settings and electrode arrays. The stimulus was click stimuli that presented at 11.3Hz and 95–100 dB nHL. The response filtered at 0 to 3000 Hz. At least, two replicated waves were recorded for each ear.

Data analysis

The analyses were performed by SPSS version 19 software. The normal distribution of data was checked by the Kolmogorov-Smirnov test. The paired t-test was used for showing the difference between the results of the right and left ear. The repeated-measures ANOVA and paired t-test were used for showing the difference between ABR threshold changes on days 14th, 21th, and 28th after surgery relative to the day before surgery. For ECochG, the summation potential (SP) and action potential (AP) amplitude ratio were calculated.

Results

Biochemical evaluations

As shown in Figure 1, TNF-α and CRP levels concentrations increased markedly one day after ischemia and subsequently decreased on the 7th day. The mean level of TNF-α in the ischemic group was significantly elevated relative to the control group at 1st, 4th, and 7th days ($p<0001$). CRP levels also were significantly higher than the control group at 1st, 4th, and 7th days ($p<0001$).

Auditory evaluations

The data had a normal distribution. The results of the right and left ears didn't show any significant difference. Therefore, in further analysis results of both ears were presented to gather.

The ABR responses were evaluated in 5 dB steps near thresholds (Figure 2). The ABR thresholds for the day before surgery were 16.39 (±4.13) and 19.44 ((±2.35) for click and 4 kHz tone burst, respectively. Table 1 shows the difference between the ABR thresholds at days 14th, 21th and 28th relative to the day before surgery.

The ABR thresholds changed remarkably after surgery. There is no significant difference between days the 14th, 21th and 28th for click stimuli ($p>0.05$). This difference was significant for tone burst stimuli on 14th day

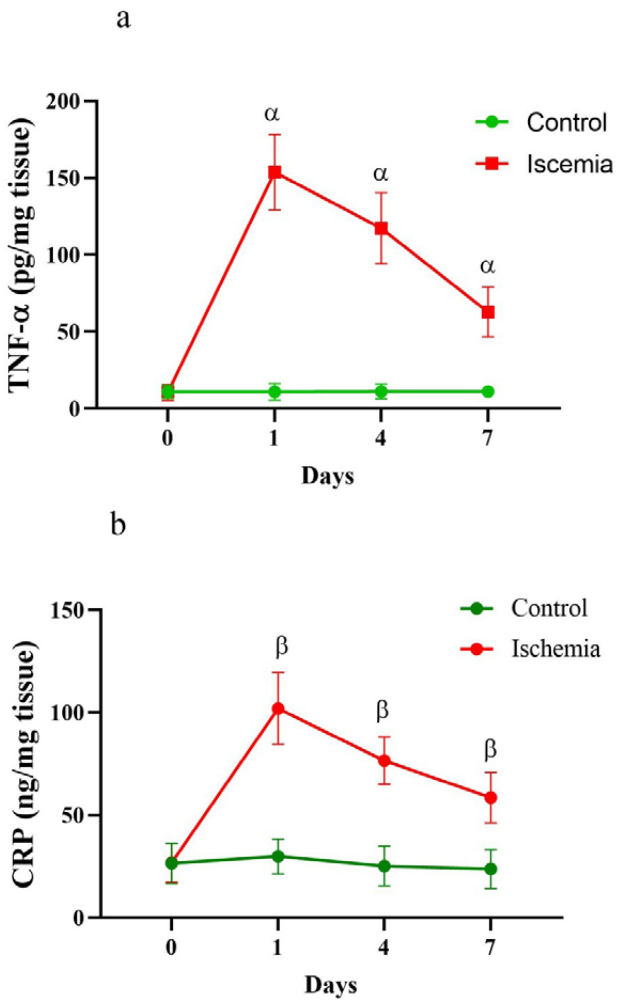

Figure 1. Tumour necrosis factor-α (a) and C-reactive protein (b) levels of cochlea in the control and ischemic groups one day before ischemia (0 Day) and on the first, fourth, and seventh days after ischemia (Mean±SD). p<0.0001, control versus ischemic group on the first, fourth, and seventh days after ischemia. TNF-α; tumor necrosis factor-α, CRP; C-reactive protein

(p=0.010). The difference between day 21th and 28th was not significant for both stimuli (p>0.05).

The ECochG responses were recorded as described (Figure 3).

The abnormal SP/AP ratio was defined as the ratio above 0.5. The SP/AP ratios were normal on the day before surgery. The abnormal SP/AP ratio was observed for 55%, 70%, and 45% of cases on days 14th, 21th, and 28th respectively.

Discussion

This study evaluated the inflammatory factors and hearing sensitivity after induced cochlear ischemia. After ischemia, the inflammatory factors increased and the hearing loss reached the maximum level on day 14th after the surgery and then slightly decreased on 21th day and remained stable on 28th day.

The ABR is a very popular test even for animal studies [6, 7]. Wave II is the most robust and reliable wave in rats [8, 9] that can be used for threshold evaluation. The morphology variation of ABR thresholds in rats is well-evaluated in previous studies [10, 11] and this response could be effectively used for the evaluation of auditory thresholds. Also, the ECochG responses and SP/AP ratio showed a valid and reliable indicator for cochlear conditions in rats [12]. In our study, ABR thresholds showed a significant change after ischemic injury. The ABR thresholds for both stimuli decrease until 21th day and then remain in a stationary situation. Although, the difference between click thresholds are not statistically different for day 14th to 21th. The ECochG results also were abnormal and remained abnormal for most of the

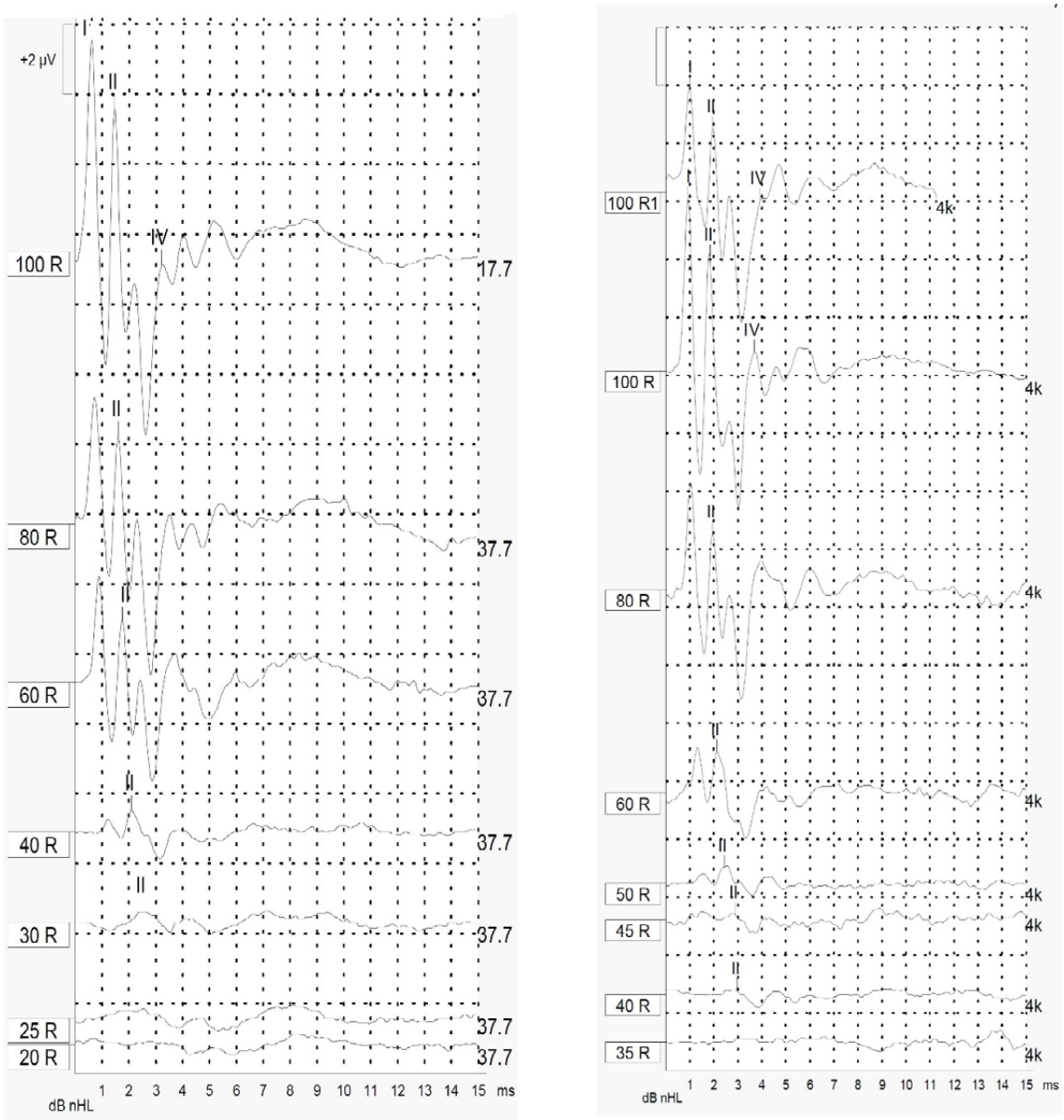

Figure 2. An example of auditory brainstem response thresholds for click (left) and 4 kHz tone burst (right) in a rat in 21th day after surgery

cases. This finding shows the continuous effects of ischemia in the cochlea.

Our results showed that the increase of inflammatory factors after cochlear ischemia can play an important role in reducing hearing sensitivity. Hearing also improves as the concentration of inflammatory factors in the cochlea decreases. In this study, TNF-α and CRP were measured because TNF-α has an important function in inflammatory damage to the cochlea, and CRP also indicates the severity of inflammation [3]. Our results showed that one day after cochlear ischemia, the severity of inflammation

Table 1. Mean and standard deviation of difference between the auditory brainstem response thresholds at days 14th, 21th and 28th relative to the day before surgery

Stimulus	14th day	21th day	28th day
Click	24.06±22.965	15.00±14.806	14.29±10.163
4 kHz tone burst	35.00±25.100	22.14±11.217	22.14±12.203

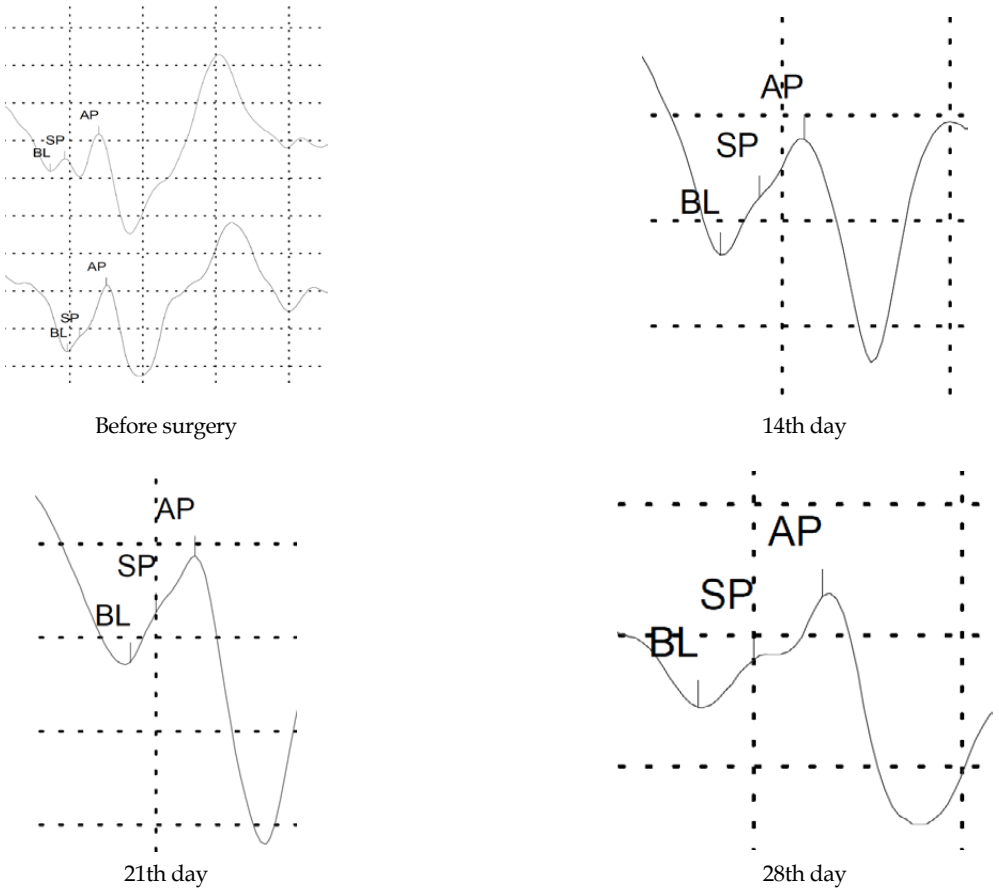

Figure 3. An electrocochleography response in an ischemic rat before and after surgery. AP; action potential, SP; summation potential, BL; baseline

reaches its maximum, and then in the following days, the severity of inflammation decreases slowly, which can be due to the activity of anti-inflammatory agents, reducing cochlear cell death and initiating repair processes. Therefore, our results show that the first 24 hours after cochlear ischemia could be important therapeutically and the use of anti-inflammatory drugs can be effective in reducing cochlear damage.

In general, proinflammatory cytokines such as TNF-α are one of the first factors upregulated in response to damages of inflammation [3]. TNF-α is produced in several tissues after damage in experimental models such as the immune response, infection, ischemia, trauma, etc, and it is created by different cell types, including residential immune-related cells, neurons, and glia in the central nervous system [3, 13].

The signaling downstream from TNF-α consists of the attraction and activation of immune cells, including leukocytes and macrophages and finally leading to necrosis or apoptosis [3]. TNF-α is also important for resistance to infection and cancers [3]. In vivo studies demonstrated after various insults to the cochlea, TNF-α was a factor that had more potency to exacerbated cochlear inflammation [14]. Blockade of TNF-α signaling pathway after ischemic damage to the cochlea using an antagonist or inhibitor (e.g. infliximab, etanercept, or tocilizumab) can be used as a therapeutic approach [15, 16].

This study had some limitations; evaluating biochemical inflammatory factors up to the 28th day after surgery could be preferable. This could show a better picture of the inflammatory process in the cochlea. Also, evaluation of different frequencies with tone burst stimuli up to 32 kHz is desirable and the intensity level delivered by insert phone also would be different in the small area of ear canal of rat. However, we didn't have access to the required instruments for better evaluation.

Conclusion

The results of this study suggested that cochlear oxidative stress could affect hearing thresholds and cause hearing loss. The auditory evaluations showed elevated thresholds and the presence of hydrops in many cases.

Ethical Considerations

Compliance with ethical guidelines

This study protocol was approved by the Ethics Committee of Iran University of Medical Sciences (IR.IUMS.REC.1393.93-02-125-24757.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

HF: Study design, performing surgeries, biochemical evaluations, analysis and interpretation of the biochemical data and writing the manuscript; AP: Study design, interpretation of the results and critically revising the manuscript; SJ: Study design, auditory evaluations, analysis and interpretation of auditory results and writing the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The grant number (930212524757) by IUMS for the auditory evaluations, and the Ethics Committee of Tehran University of Medical Sciences for biochemical evaluations (grant number 5734).

References

[1] Fanaei H, Pourbakht A, Jafarzadeh S. Bilateral carotid artery occlusion and cochlear oxidative stress and hearing loss in rats. Basic Clin Neurosci. 2020;11(6):821-9. [DOI:10.32598/bcn.11.6.1154.1]

[2] Tabuchi K, Nishimura B, Tanaka S, Hayashi K, Hirose Y, Hara A. Ischemia-reperfusion injury of the cochlea: pharmacological strategies for cochlear protection and implications of glutamate and reactive oxygen species. Curr Neuropharmacol. 2010;8(2):128-34. [DOI:10.2174/157015910791233123]

[3] Fujioka M, Okano H, Ogawa K. Inflammatory and immune responses in the cochlea: potential therapeutic targets for sensorineural hearing loss. Front Pharmacol. 2014;5:287. [DOI:10.3389/fphar.2014.00287]

[4] Keithley EM, Wang X, Barkdull GC. Tumor necrosis factor alpha can induce recruitment of inflammatory cells to the cochlea. Otol Neurotol. 2008;29(6):854-9. [DOI:10.1097/MAO.0b013e31818256a9]

[5] Marinović T, Bašić S, Romić D, Nevajda B, Đerek L, Maraković J, et al. Dynamics of inflammatory factors expression in ischemic brain tissue injury. Neurol Int. 2019;11(4):8282. [DOI:10.4081/ni.2019.8282]

[6] Land R, Burghard A, Kral A. The contribution of inferior colliculus activity to the auditory brainstem response (ABR) in mice. Hear Res. 2016;341:109-18. [DOI:10.1016/j.heares.2016.08.008]

[7] de Lima JP, Velasco IT, Barbeiro DF, Schochat E. Auditory brainstem response in gerbils submitted to ischemia and sepsis. Codas. 2015;27(2):155-9. [DOI:10.1590/2317-1782/20152014113]

[8] Church MW, Hotra JW, Holmes PA, Anumba JI, Jackson DA, Adams BR. Auditory brainstem response (ABR) abnormalities across the life span of rats prenatally exposed to alcohol. Alcohol Clin Exp Res. 2012;36(1):83-96. [DOI:10.1111/j.1530-0277.2011.01594.x]

[9] Church MW, Jen K-LC, Anumba JI, Jackson DA, Adams BR, Hotra JW. Excess omega-3 fatty acid consumption by mothers during pregnancy and lactation caused shorter life span and abnormal ABRs in old adult offspring. Neurotoxicol Teratol. 2010;32(2):171-81. [DOI:10.1016/j.ntt.2009.09.006]

[10] Jafarzadeh S, Pourbakht A. Morphology variations of click-evoked auditory brainstem response with low and high rate stimuli in rat. Aud Vestib Res. 2019;28(1):22-7. [DOI:10.18502/avr.v28i1.412]

[11] Alvarado JC, Fuentes-Santamaría V, Jareño-Flores T, Blanco JL, Juiz JM. Normal variations in the morphology of auditory brainstem response (ABR) waveforms: a study in Wistar rats. Neurosci Res. 2012;73(4):302-11. [DOI:10.1016/j.neures.2012.05.001]

[12] Franz B, Anderson C. Effect of static middle-ear and intracranial pressure changes on differential electrocochleographic response. Int Tinnitus J. 2008;14(2):101-7.

[13] Gorgij E, Fanaei H, Yaghmaei P, Shahraki MR, Mirahmadi H. Treadmill exercise during pregnancy decreased vulnerability to neonatal hypoxia-ischemia through reducing inflammation and increasing antiapoptotic gene expressions and antioxidant capacity in rats. Stroke Res Treat. 2021;2021:5512745. [DOI:10.1155/2021/5512745]

[14] Satoh H, Firestein GS, Billings PB, Harris JP, Keithley EM. Proinflammatory cytokine expression in the endolymphatic sac during inner ear inflammation. J Assoc Res Otolaryngol. 2003;4(2):139-47. [DOI:10.1007/s10162-002-3025-7]

[15] Wang X, Truong T, Billings PB, Harris JP, Keithley EM. Blockage of immune-mediated inner ear damage by etanercept. Otol Neurotol. 2003;24(1):52-7. [DOI:10.1097/00129492-200301000-00012]

[16] Ihler F, Pelz S, Coors M, Matthias C, Canis M. Application of a TNF-alpha-inhibitor into the scala tympany after cochlear electrode insertion trauma in guinea pigs: preliminary audiologic results. Int J Audiol. 2014;53(11):810-6. [DOI:10.3109/14992027.2014.938369]