Med. Sci. Innov. 71(1-2):35-43, 2024

Effect of Monoclonal Antibody against TNF-alfa on Noise-Induced Inner Ear Damage

Youhei Yamamoto, Kazuma Sugahara, Junko Tsuda, Makoto Hashimoto and Hiroshi Yamashita

Department of Otolaryngology, Yamaguchi University Graduate School of Medicine, 1-1-1 Minamikogushi, Ube, Yamaguchi 755-8505, Japan
(Received October 23, 2023, accepted January 22, 2024)
Correspondence to Kazuma Sugahara, MD. Email: kazuma@yamaguchi-u.ac.jp

Abstract This study investigated the potential of adalimumab (ADA), a monoclonal antibody targeting TNF-alfa, to protect the inner ear from intense sound exposure, given that inflammatory cytokines, including TNF-alfa, are linked to hearing loss in acoustic disorders. In this study, adalimumab was administered to mice, and its effect on the inner ear was assessed. We examined the translocation of ADA to the inner ear and its ototoxicity and impact on acoustic exposure. The results showed that adalimumab partially reached the cochlea after administration but increased the susceptibility to acoustic exposure, resulting in higher hair cell loss in the inner ear. While TNF-alfa had been considered a potential therapeutic target, the results suggested that excessive TNF-alfa suppression could harm the inner ear. We acknowledged some limitations, such as the use of adalimumab instead of an anti-mouse TNF-alfa antibody and the need to explore the suppression of other cytokines for better inner ear protection. In conclusion, adalimumab administration was found to increase the inner ear's susceptibility to acoustic exposure, potentially leading to more significant hair cell damage, possibly due to excessive TNF-alfa suppression.

Key words: TNF-alfa, noise trauma, Adalimumab, mouse

Introduction

After exposure to high-intensity sound, hearing loss occurs because of decreased blood flow to the cochlea[1-3] and damage to hair cells.[4] In addition, inflammatory cytokines such as IL-1, IL-6, and TNF-alfa are expressed in the inner ear along with increased expression of the transcription factor NFkB after exposure to high-intensity sound.[5,6]

Glucocorticoids have been clinically used to treat inner ear disorders, including acoustic disorders. Glucocorticoids can suppress NFkB activity via glucocorticoid receptors in many tissues, thereby suppressing inflammation.[7] Steroids also suppress cytokine production following acoustic disturbances in the inner ear.[8] Acoustic disorders can be avoided by inhibiting inflammatory cytokines in the inner ear.[9-11] Recently, many types of antibody pharmaceuticals have been developed that potently suppress inflammatory cytokines and are used to treat many diseases. However, although these new drugs strongly suppress the activity of cytokines in the inner ear, their effects on inner ear disorders remain unclear. TNF-alfa is a major mediator of the inflammatory response whose levels increase after acoustic injury. It is possible that acoustic disturbances can be circumvented by inhibiting the activity of TNF-alfa. Animal experiments have shown that etanercept, an anti-TNF-alfa antibody, suppresses acoustic disturbances.[12] Many anti-TNF antibody preparations have been developed, including adalimumab, infliximab, golimumab, certolizumab, and ozoralizumab; however, their effects on the inner ear are unknown. This

study aimed to determine the effects of adalimumab on acoustic disturbances in the inner ear.

Materials and Methods

Animal use and care

Male CBA/N mice (4-6 weeks old, 20-25 g body weight) with normal Preyer reflexes were obtained from Japan SLC, Inc. (Shizuoka, Japan).

The experimental protocol was reviewed and approved by the Committee for Ethics on Animal Experiments at Yamaguchi University School of Medicine (43-056). This study was conducted in accordance with the guidelines of Japanese Federal Law (No. 105) and Notification No. 6 of the Japanese Government.

Inner ear translocation of adalimumab

The animals were divided into two groups, namely, Adalimumab and Control. Six animals were intraperitoneally administered adalimumab (AbbVie GK, Tokyo, Japan) or saline (10 mg/kg body weight). Twenty-four hours after the administration of adalimumab, the animals were anesthetized with an overdose of pentobarbital and immediately decapitated. Their temporal bones were quickly removed.

After fixation with 4% PFA (4℃, 2h), the cells were washed with PBS at 4℃. After decalcification with 10% EDTA for three days, samples were washed with PBS at 4℃ and then incubated with anti-Humira (adalimumab) monoclonal antibody (Eurobio scientific, Les Ulis, France) (1:100). After washing with PBS, the samples were incubated with Alexa Fluor 488-conjugated mouse anti-mouse IgG (1:100; Sigma) for 4 h at room temperature. The specimens were washed with PBS at 4℃, dehydrated in four steps with ethanol, and embedded in Immuno-Bed® (Polysciences, Inc., Warrington, USA). After sectioning to obtain sections with a thickness of 4 μm, these sections were encapsulated and examined under a fluorescence microscope. In this study, a negative control was not established by staining with secondary antibodies only.

Ototoxicity of adalimumab

Experiments were conducted to confirm the toxic effects of adalimumab administration on inner ear hair cells.

Adalimumab and saline were administered to two groups, the adalimumab treatment and control groups (n = 2 each), respectively. Seven days later, the animals were decapitated under deep anesthesia, and the cochlea was removed. After fixation with 4% PFA (4℃, 2 h), the cells were washed with PBS at 4℃. The Organ of Corti was excised using a surface preparation technique. Hair cells were labeled with FITC-conjugated phalloidin (100 nM; Merck KGaA, Darmstadt, Germany), washed, mounted, and observed under a fluorescence microscope.

Effects of adalimumab on acoustic exposure

This experiment was conducted to confirm the effect of adalimumab on acoustic disturbance.

The animals were divided into two groups: adalimumab and control (n = 8 each). Hearing functions of all animals were evaluated with ABR thresholds measured with System 3 auditory brainstem response ABR system (Bioresearch Center, Nagoya, Japan) after the administration of intraperitoneal anesthesia [a mixture of medetomidine (0.3 mg/0.3 mL/kg), midazolam (4 mg/0.8 mL/kg), and butorphanol (5 mg/mL/kg)].

Adalimumab (10 mg/kg, AbbVie LLC) or saline was administered intraperitoneally 1 h before acoustic exposure.

The animals were exposed to octave-band noise with a center frequency of 4 kHz and 130 dB for 5 h under intraperitoneal anesthesia.

The ABR was measured seven days after acoustic loading, and changes in the ABR threshold before and after loading were compared. Subsequently, the animals were quickly decapitated under deep anesthesia to remove the cochlea. After fixation with 4% PFA (4℃, 2 h), the cells were washed with PBS at 4℃. The Organ of Corti was excised using a surface preparation technique to evaluate the hair cell loss rate. Hair cells were labeled with FITC-conjugated phalloidin, washed, mounted, and observed under a fluorescence microscope.

Statical Analysis

All data were analyzed using StatView version 5.0 J for Macintosh (SAS Institute Inc., Cary, NC, USA). The Mann-Whitney U test was used to compare hair cell densities and significance values. Statistical significance was set at $P < 0.05$.

Results

Effect of the administration of adalimumab on the cochlea

The results of indirect immunofluorescence analysis 24 h after adalimumab administration are shown in Figure 1. After the administration of adalimumab, signal enhancement was observed in the Organ of Corti and lateral wall of the cochlea. Signals were also observed in the blood vessels within the bone wall. By contrast, no signal enhancement was observed in the control group. This finding indicated that the intraperitoneally administered adalimumab was partially distributed in the cochlea. Figure 2 shows the surface preparation of the Organ of Corti seven days after the administration of adalimumab. In the adalimumab-treated group, no phalloidin-labeled inner or outer hair cells were damaged. This suggests that adalimumab was not toxic. Based on the aforementioned results, some intraperitoneally administered adalimumab migrated to the cochlea after 24 h, but adalimumab alone was unlikely to damage the hair cells.

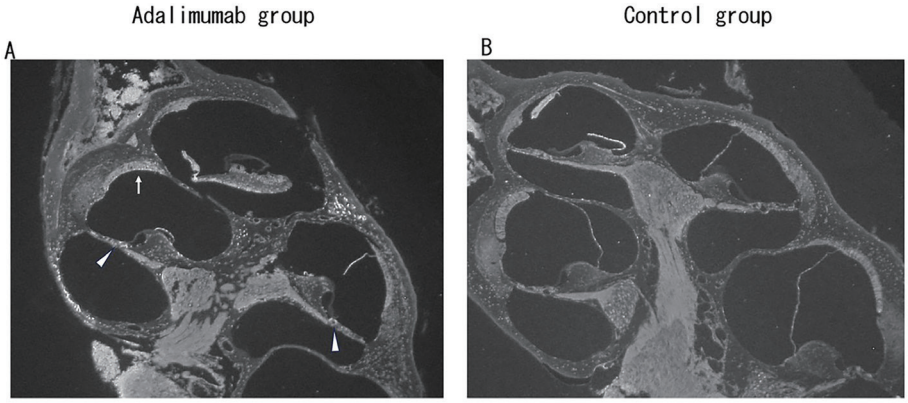

Fig. 1 Inner ear translocation of adalimumab
Following adalimumab administration, signal enhancement was observed in the Organ of Corti (arrowhead) and the lateral wall (arrow) of the cochlea (A). No signal enhancement was observed in the control group (B). (n=3, each)

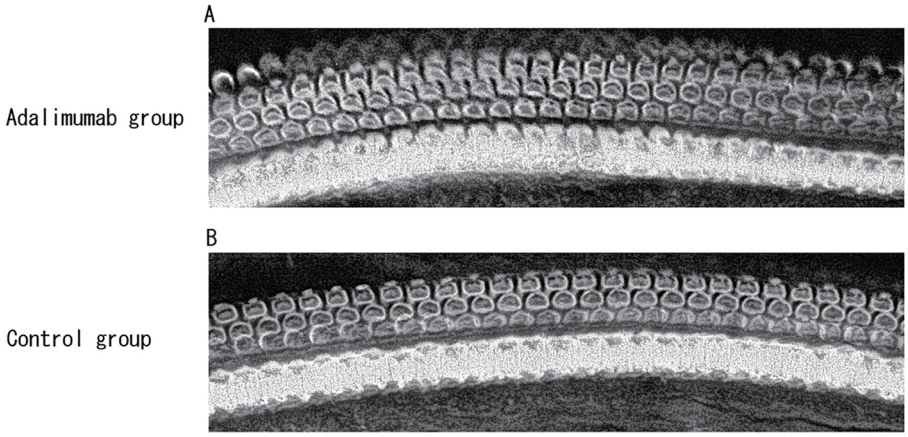

Fig. 2 Surface structure of the organ of Corti after the administration of adalimumab
Neither the adalimumab group (A) nor the control group (B) exhibited degeneration of inner and outer hair cells. (n=2, each)

Effect of the administration of adalimumab on acoustic trauma

The ABR thresholds were evaluated before and seven days after intense noise exposure (Fig. 3). Little difference was observed in the ABR thresholds between the adalimumab and control groups before noise exposure.

The ABR thresholds increased by 20-50 dB at each frequency after noise exposure compared with before noise exposure. Under the acoustic exposure conditions used in this study, the threshold elevation seven days after noise exposure reached a maximum of 16 kHz for both groups. The ABR threshold shifts seven days after noise exposure are shown in Figure 4. There was no significant difference in the ABR threshold shifts ($p = 0.089$ at 16 kHz); however, the threshold tended to be higher in the adalimumab group at each frequency (Fig. 4).

The surface structures of the Organs of Corti after seven days of noise exposure are shown in Figure 5. In both the control and adalimumab groups, hair cell loss was observed seven days after sound exposure. However, the adalimumab-treated group exhibited a higher rate of hair cell loss than the control group.

Outer hair cells were significantly lost on the basal rotation side in the adalimumab

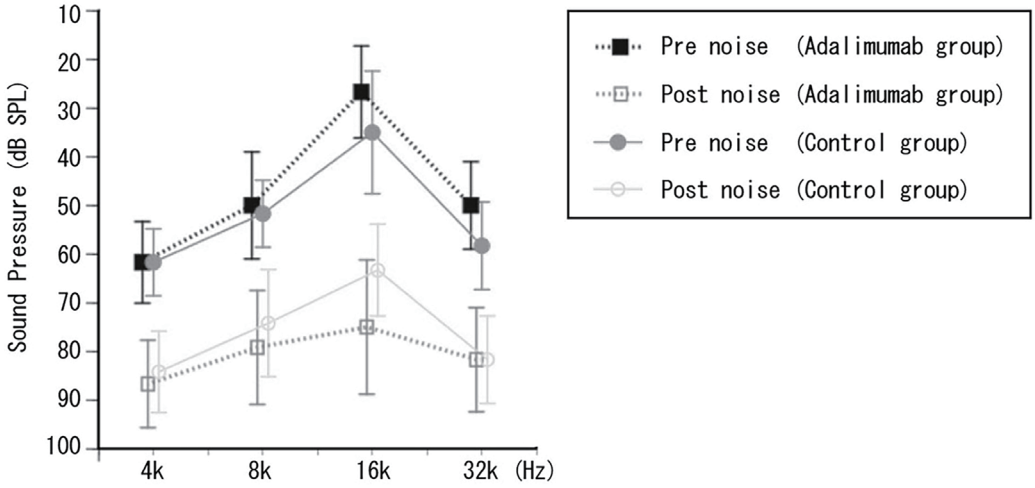

Fig. 3 The ABR thresholds before and seven days after exposure to intense noise. There was minimal difference in the ABR thresholds between the adalimumab and control groups before noise exposure. Error bars indicate ± I.s.d. (n=8, each)

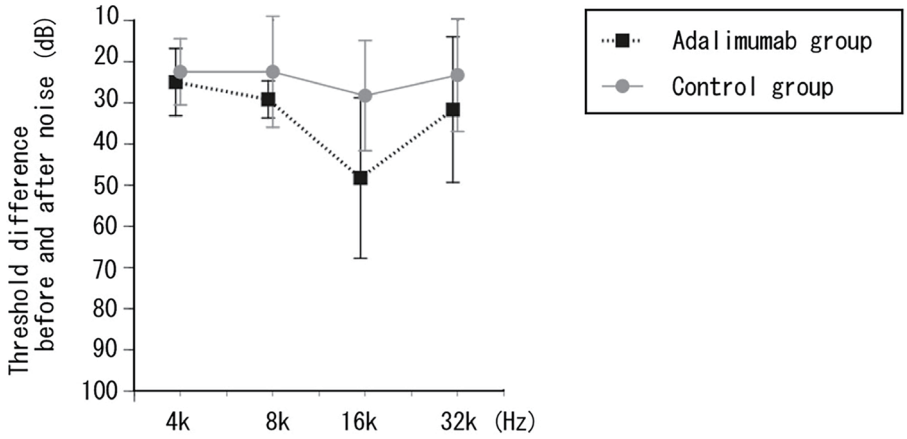

Fig. 4 Shifts in the ABR threshold in both adalimumab group and control group. At each frequency, the adalimumab group exhibited larger threshold shifts than the control group. Error bars indicate ± I.s.d. (n=8, each)

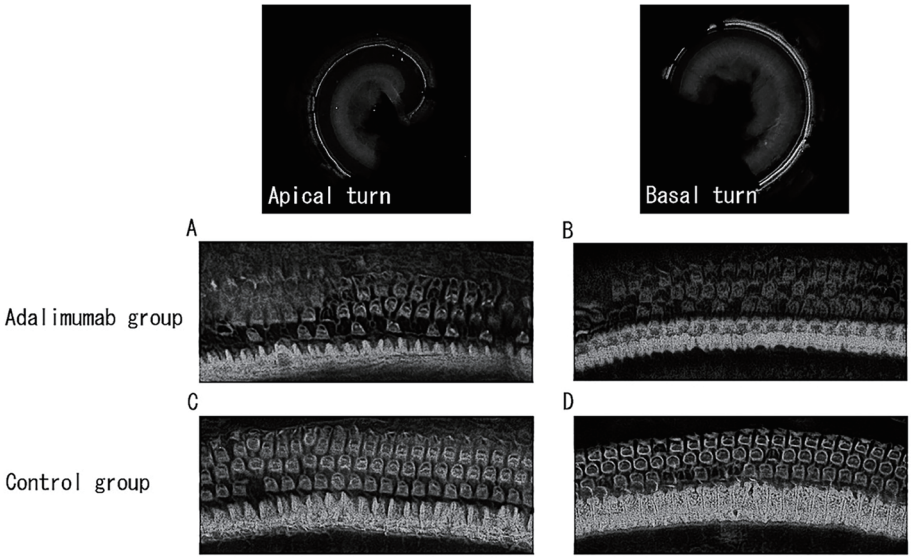

Fig. 5 Surface preparation of the cochlear hair cells seven days after sound exposure. A higher number of outer hair cells were degenerated in the adalimumab-treated group (A, B) than in the control group (C, D). (n=8, each)

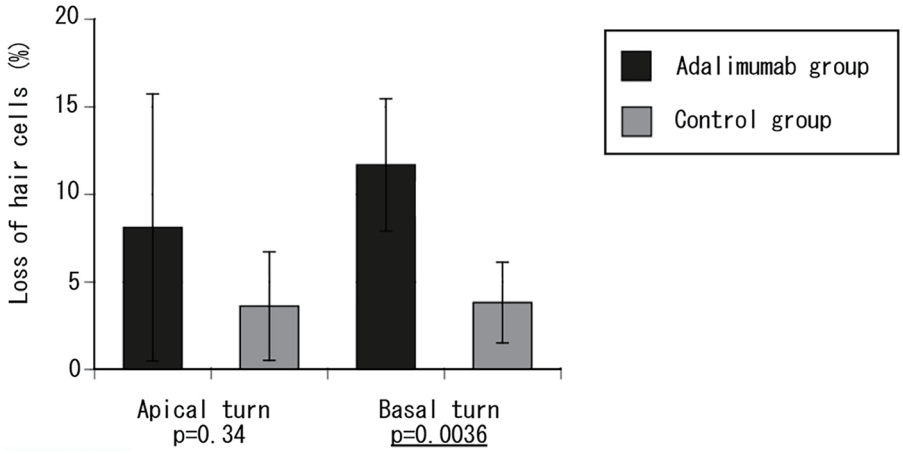

Fig. 6 The rate of degenerated outer hair cells seven days after sound exposure. The number of degenerated outer hair cells was higher in the adalimumab group than in the control group. Error bars indicate ± I.s.d. (n=8, each)

group (Fig. 6). These results suggest that adalimumab administration increases susceptibility to acoustic exposure in the inner ear.

Discussion

The immune system of the inner ear is unique because it is deficient in immunoglobulins.[13] However, it was recently revealed that immune cells also exist in the inner ear and help maintain homeostasis in the inner ear.[14,15]

Sensory cell degeneration and synaptic disturbances occur when the inner ear is exposed to loud sounds, leading to a decline in inner ear function.[16] Various inflammatory cytokines are involved in inner ear damage after sound.[5,6,17-19]

TNF-alfa is a potent inflammatory cytokine, similar to IL-1 and IL-6. It plays an important role in chronic inflammatory diseases, including Crohn's disease and rheumatoid arthritis, and has been targeted for treatment. The levels of TNF-alfa increase in the inner ear after acoustic disturbance.[5,19,20] This

study was designed to investigate the potential of TNF-alfa as a therapeutic target for inner ear disorders caused by loud sounds. The administration of Etanercept, a TNF-alfa inhibitor, suppressed the elevation of the ABR threshold after acoustic stress.[12]

If TNF-alpha is involved in hair cell damage, anti-TNF-alpha drugs may protect the inner ear from acoustic damage. However, our experimental results showed that the administration of anti-TNF-alfa antibody enhanced hair cell damage following acoustic disturbance. TNF-alfa, unlike other inflammatory cytokines, including Il-1 and Il-6, is expressed even before acoustic disturbance.[5,21] This suggests that TNF-alfa may have a functional role even when the inner ear is intact. The abnormal inner ear morphology and impaired hearing in mice with TNF-alfa knockout also indicates that TNF-alfa plays an important role in the inner ear.[22] Our results suggest that excessive suppression of TNF-alfa might have adverse effects on the inner ear. Previous animal experiments have reported inhibitory effects of etanercept on noise-induced inner ear damage.[12] Etanercept comprises TNF-alfa receptor section, which is responsible for binding to TNF-alfa, and an IgG1 Fc portion, providing circulatory stability and sustained therapeutic effects. It has an affinity not only for TNF-alfa but also for TNF-beta.[23] In contrast, adalimumab, being an antibody formulation, not only binds to the resultant TNF-alfa for inhibition but also impairs TNF-alfa-producing cells themselves.[24,25] These differences in the mechanism of action may explain why adalimumab exacerbates inner ear damage after intense noise exposure.

Inflammatory cytokines are produced by fibrocytes of the spiral ligament during acoustic trauma.[26,27] In the present study, no inflammatory cells were observed. However, a small number of inflammatory cells, primarily tissue macrophages, exist in the cochlea, and their number increases during acute inner ear disorders such as after acoustic trauma[28] and drug-induced damage.[29,30] We hypothesized these tissue macrophages were also affected. The fact that tissue macrophages were not observed is a subject for future investigation.

In this study, the experimental drug adalimumab was administered as an anti-human TNF-alfa antibody. Typically, an anti-mouse TNF-alfa antibody should be used. However, adalimumab was used because the manufacturer showed cross-reactivity in mice. This is a limitation of this study.

In the current study, 10 mg/kg adalimumab was administered intraperitoneally 1 h before acoustic exposure. Other studies examining adalimumab in mice administered doses of 3-10 mg/kg.[31-33] Typically, the amount of drug transferred to the inner ear is expected to be limited due to the presence of the blood-inner ear barrier.[34,35] Therefore, we conducted the experiments at a dose of 10 mg/kg. However, these experiments were not conducted at lower doses. Considering that the dosage is a crucial issue when applying these results to humans, this was a limitation of the present study.

In this study, the intense sound to which the animals were exposed consisted of an octave-band noise centered at approximately 4 kHz. According to cochleograms[36,37] previously reported in mice, sounds at 4 kHz were received at the apical turn of the cochlea. However, inner ear damage due to intense sounds occurs in frequency regions higher than the exposed frequency.[38] Therefore, under the conditions of acoustic disturbance used in this study, we believe that hair cell damage occurred in both the apical and basal turns.

In a recent study, the calcineurin inhibitor tacrolimus (TCR) was shown to protect the inner ear by suppressing the expression of cytokines (TNF-alfa and c-fos).[9] In addition, Ginkgo Biloba extract (EGb 761) inhibited TNF-alpha and protected the inner ear after acoustic injury.[10] Metformin suppresses the expression of cytokines, including TNF, in acoustic disorders and protects the inner ear.[11] In light of these findings, it appears that suppressing other cytokines might offer more robust protection for the inner ear than focusing solely on the suppression of TNF.

Conclusion

In the Adalimumab and control groups, changes in the hearing threshold at each frequency before and after the acoustic load and

the rate of hair cell depletion were compared. The rate of hair cell defects was significantly higher in the adalimumab group than in the control group.

Hence, the inner ear may be adversely affected by the extent of suppression of TNF-alfa as a cause.

Acknowledgments

We thank Ms. Kazuko Muto for assistance with animal husbandry and microscopy. We would like to thank Editage (www.editage.com) for the English language editing.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Ryan, A.F., Axelsson, A., Myers, R. and Woolf, N.K.: Changes in cochlear blood flow during acoustic stimulation as determined by 14C-iodoantipyrine autoradiography. *Acta Otolaryngol.*, **105**: 232-41, 1988.
2. Okamoto, A., Hasegawa, M., Tamura, T., Homma, T. and Komatsuzaki, A.: Effects of frequency and intensity of sound on cochlear blood flow. *Acta Otolaryngol.*, **112**: 59-64, 1992.
3. Kellerhals, B.: Acoustic trauma and cochlear microcirculation. An experimental and clinical study on pathogenesis and treatment of inner ear lesions after acute noise exposure. *Adv. Otorhinolaryngol.*, **18**: 91-168, 1972.
4. Canlon, B.: The effect of acoustic trauma on the tectorial membrane, stereocilia, and hearing sensitivity: possible mechanisms underlying damage, recovery, and protection. *Scand. Audiol. Suppl.*, **27**: 1-45, 1988.
5. Fujioka, M., Kanzaki, S., Okano, H.J., Masuda, M., Ogawa, K. and Okano, H.: Proinflammatory cytokines expression in noise-induced damaged cochlea. *J. Neurosci. Res.*, **83**: 575-83, 2006.
6. Masuda, M., Nagashima, R., Kanzaki, S., Fujioka, M., Ogita, K. and Ogawa, K.: Nuclear factor-kappa B nuclear translocation in the cochlea of mice following acoustic overstimulation. *Brain Res.*, **1068**: 237-47, 2006.
7. Catley, M.: Dissociated steroids. *Scientific World Journal*, **7**: 421-30, 2007.
8. Le Prell, C.G., Yamashita, D., Minami, S.B., Yamasoba, T. and Miller, J.M.: Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. *Hear. Res.*, **226**: 22-43, 2007.
9. Bas, E., Martinez-Soriano, F., Láinez, J.M. and Marco, J.: An experimental comparative study of dexamethasone, melatonin and tacrolimus in noise-induced hearing loss. *Acta Otolaryngol.*, **129**: 385-9, 2009.
10. Dogan, R., Sjostrand, A.P., Yenıgun, A., Karatas, E., Kocyigit, A. and Ozturan, O.: Influence of Ginkgo Biloba extract (EGb 761) on expression of IL-1 Beta, IL-6, TNF-alfa, HSP-70, HSF-1 and COX-2 after noise exposure in the rat cochlea. *Auris Nasus Larynx*, **45**: 680-85, 2018.
11. Gedik, Ö., Doğan, R., Bababemez, M.A., Karataş, E., Aydın, M., Koçyiğit, A., Eşrefoğlu, M. and Özturan, O.: Therapeutic effects of metformin for noise induced hearing loss. *Am. J. Otolaryngol.*, **41**: 102328, 2020.
12. Arpornchayanon, W., Canis, M., Ihler, F., Settevendemie, C. and Strieth, S.: TNF-alpha inhibition using etanercept prevents noise-induced hearing loss by improvement of cochlear blood flow in vivo. *Int. J. Audiol.*, **52**: 545-52, 2013.
13. Mogi, G., Lim, D.J. and Watanabe, N.: Immunologic study on the inner ear. Immunoglobulins in perilymph. *Arch. Otolaryngol.*, **108**: 270-5, 1982.
14. Hirose, K., Rutherford, M.A. and Warchol, M.E.: Two cell populations participate in clearance of damaged hair cells from the sensory epithelia of the inner ear. *Hear. Res.*, **352**: 70-81, 2017.
15. Hu, B.H., Zhang, C. and Frye, M.D.: Immune cells and non-immune cells with immune function in mammalian cochleae. *Hear. Res.*, **362**: 14-24, 2018.
16. Wood, M.B. and Zuo, J.: The contribution of immune infiltrates to ototoxicity and cochlear hair cell loss. *Front. Cell Neurosci.*, **11**: 106, 2017.
17. Wakabayashi, K., Fujioka, M., Kanzaki,

S., Okano, H.J., Shibata, S., Yamashita, D., Masuda, M., Mihara, M., Ohsugi, Y., Ogawa, K. and Okano, H.: Blockade of interleukin-6 signaling suppressed cochlear inflammatory response and improved hearing impairment in noise-damaged mice cochlea. *Neurosci. Res.*, **66**: 345-52, 2010.
18. Tan, W.J., Thorne, P.R. and Vlajkovic, S.M.: Characterisation of cochlear inflammation in mice following acute and chronic noise exposure. *Histochem. Cell. Biol.*, **146**: 219-30, 2016.
19. Landegger, L.D., Vasilijic, S., Fujita, T., Soares, V.Y., Seist, R., Xu, L. and Stankovic, K.M.: Cytokine levels in inner ear fluid of young and aged mice as molecular biomarkers of noise-induced hearing loss. *Front. Neurol.*, **10**: 977, 2019.
20. Zhang, G., Zheng, H., Pyykko, I. and Zou, J.: The TLR-4/NF-κB signaling pathway activation in cochlear inflammation of rats with noise-induced hearing loss. *Hear. Res.*, **379**: 59-68, 2019.
21. Nakamoto, T., Mikuriya, T., Sugahara, K., Hirose, Y., Hashimoto, T., Shimogori, H., Takii, R., Nakai, A. and Yamashita, H.: Geranylgeranylacetone suppresses noise-induced expression of proinflammatory cytokines in the cochlea. *Auris Nasus Larynx*, **39**: 270-4, 2012.
22. Oishi, N., Chen, J., Zheng, H.W., Hill, K., Schacht, J. and Sha, S.H.: Tumor necrosis factor-alpha-mutant mice exhibit high frequency hearing loss. *J. Assoc. Res. Otolaryngol.*, **14**: 801-11, 2013.
23. Zalevsky, J., Secher, T., Ezhevsky, S.A., Janot, L., Steed, P.M., O'Brien, C., Eivazi, A., Kung, J., Nguyen, D.H., Doberstein, S.K., Erard, F., Ryffel, B. and Szymkowski, D.E.: Dominant-negative inhibitors of soluble TNF attenuate experimental arthritis without suppressing innate immunity to infection. *J. Immunol.*, **179**: 1872-83, 2007.
24. Horiuchi, T., Mitoma, H., Harashima, S., Tsukamoto, H. and Shimoda, T.: Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents. *Rheumatology (Oxford)*, **49**: 1215-28, 2010.
25. Tracey, D., Klareskog, L., Sasso, E.H., Salfeld, J.G. and Tak, P.P.: Tumor necrosis factor antagonist mechanisms of action: a comprehensive review. *Pharmacol. Ther.*, **117**: 244-79, 2008.
26. Shin, S.A., Lyu, A.R., Jeong, S.H., Kim, T.H., Park, M.J. and Park, Y.H.: Acoustic trauma modulates cochlear blood flow and vasoactive factors in a rodent model of noise-induced hearing loss. *Int. J. Mol. Sci.*, **20**, 2019.
27. Ichimiya, I., Yoshida, K., Hirano, T., Suzuki, M. and Mogi, G.: Significance of spiral ligament fibrocytes with cochlear inflammation. *Int. J. Pediatr. Otorhinolaryngol.*, **56**: 45-51, 2000.
28. Hirose, K., Discolo, C.M., Keasler, J.R. and Ransohoff, R.: Mononuclear phagocytes migrate into the murine cochlea after acoustic trauma. *J. Comp. Neurol.*, **489**: 180-94, 2005.
29. Okano, T., Nakagawa, T., Kita, T., Kada, S., Yoshimoto, M., Nakahata, T. and Ito, J.: Bone marrow-derived cells expressing Iba1 are constitutively present as resident tissue macrophages in the mouse cochlea. *J. Neurosci. Res.*, **86**: 1758-67, 2008.
30. Sato, E., Shick, H.E., Ransohoff, R.M. and Hirose, K.: Expression of fractalkine receptor CX3CR1 on cochlear macrophages influences survival of hair cells following ototoxic injury. *J. Assoc. Res. Otolaryngol.*, **11**: 223-34, 2010.
31. Catal, F., Mete, E., Tayman, C., Topal, E., Albayrak, A. and Sert, H.: A human monoclonal anti-TNF alpha antibody (adalimumab) reduces airway inflammation and ameliorates lung histology in a murine model of acute asthma. *Allergol. Immunopathol. (Madr)*, **43**: 14-8, 2015.
32. Elsakkar, M.G., Sharaki, O.A., Abdallah, D.M., Mostafa, D.K. and Shekondali, F.T.: Adalimumab ameliorates OVA-induced airway inflammation in mice: Role of CD4(+) CD25(+) FOXP3(+) regulatory T-cells. *Eur. J. Pharmacol.*, **786**: 100-08, 2016.
33. Martínez-Fernández de la Cámara, C., Hernández-Pinto, A.M., Olivares-González, L., Cuevas-Martín, C., Sánchez-Aragó, M., Hervás, D., Salom, D., Cuezva, J.M., de la Rosa, E.J., Millán, J.M. and Rodrigo, R.: Adalimumab

reduces photoreceptor cell death in a mouse model of retinal degeneration. *Sci. Rep.*, **5**: 11764, 2015.
34. Glueckert, R., Johnson Chacko, L., Rask-Andersen, H., Liu, W., Handschuh, S. and Schrott-Fischer, A.: Anatomical basis of drug delivery to the inner ear. *Hear. Res.*, **368**: 10-27, 2018.
35. Rybak, L.P., Dhukhwa, A., Mukherjea, D. and Ramkumar, V.: Local drug delivery for prevention of hearing loss. *Front. Cell Neurosci.*, **13**: 300, 2019.
36. Viberg, A. and Canlon, B.: The guide to plotting a cochleogram. *Hear. Res.*, **197**: 1-10, 2004.
37. Müller, M., von Hünerbein, K., Hoidis, S. and Smolders, J.W.: A physiological place-frequency map of the cochlea in the CBA/J mouse. *Hear. Res.*, **202**: 63-73, 2005.
38. Hickman, T.T., Hashimoto, K., Liberman, L.D. and Liberman, M.C.: Synaptic migration and reorganization after noise exposure suggests regeneration in a mature mammalian cochlea. *Sci. Rep.*, **10**: 19945, 2020.