AMERICAN ACADEMY OF OTOLARYNGOLOGY–HEAD AND NECK SURGERY FOUNDATION

Otolaryngology–Head and Neck Surgery
2015, Vol. 152(6) 1108–1112

DOI: 10.1177/0194599815573702
http://otojournal.org
SAGE

Assessment of the Potential Ototoxicity of High-Dose Celecoxib, a Selective Cyclooxygenase-2 Inhibitor, in Rats

Bei Li, MD[1], Kaiming Su, MD, PhD[1], Guang Yang, MD, PhD[1], Yanmei Feng, MD, PhD[1], Li Xia, MD, PhD[1], and Shankai Yin, MD, PhD[1]

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract

Objective. To evaluate the potential ototoxicity of high-dose celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor.

Study Design. Prospective animal study.

Setting. Laboratory.

Methods. Twenty adult male Sprague Dawley rats were divided into 2 groups for hearing and tinnitus tests, respectively. The auditory brain-stem response (ABR) and the gap prepulse inhibition of acoustic startle (GPIAS) were used as indicators of hearing loss and tinnitus, respectively, and were measured before and at 2, 4, 6, 8, 12, 24, and 48 hours after administration of celecoxib (2 g/kg) via gavage.

Results. ABR threshold and wave III latencies did not increase significantly at any frequency following celecoxib administration, at any time point ($P > .05$). GPIAS remained below 30% after celecoxib, from a baseline of 20.03% $\pm$ 3.62%; no change was significant.

Conclusion. High-dose celecoxib (2 g/kg), a selective COX-2 inhibitor, did not cause hearing loss or tinnitus in Sprague Dawley rats within 48 hours of administration. Further studies are needed to explore the roles played by COX-related mechanisms when nonselective COX inhibitors induce ototoxicity.

Keywords

ototoxicity, cyclooxygenase, auditory brain-stem response (ABR), celecoxib, hearing loss, tinnitus

Received October 20, 2014; revised January 2, 2015; accepted January 29, 2015.

Introduction

Aspirin, a representative nonsteroidal anti-inflammatory drug (NSAID), is the most widely used anti-inflammatory agent and has multiple pharmacological effects, including inhibition of fever, pain, inflammation, rheumatism, and platelet aggregation.[1-2] However, many reliable clinical trials using large samples, as well as basic research results, confirm that acute heavy or long-term use of aspirin and other NSAIDs triggers ototoxicity that can cause temporary (or even permanent) hearing loss or tinnitus.[3-5] Much research on the physiological, histological, and morphological effects of aspirin on the auditory system suggests that these effects may be associated with biochemical or metabolic changes in the cochlea. These changes include induction of apoptosis of auditory nerve cells, electrophysiological changes in auditory nerves, and reduction of blood flow to the cochlea, but the exact mechanism of ototoxicity has not yet been defined.[3, 5-8]

The anti-inflammatory and analgesic effects of aspirin and other NSAIDs result from inhibition of the production of prostacyclin and prostaglandins from arachidonic acid by cyclooxygenase (COX).[9-11] Therefore, many previous studies relating to the ototoxic mechanism of NSAIDs have focused on their effects on arachidonic acid metabolism. Large doses of sodium salicylate (aspirin) reduce prostaglandin levels in the cochlea, indicating that COX inhibition might be a mechanism underlying NSAID-induced ototoxicity.[12-16]

Two isoforms of COX, COX-1 and COX-2, are present in humans. COX-1 maintains the normal functioning of the stomach, intestine, kidney, and cochlea in humans and other mammals.[17-19] COX-2 induces pain and inflammation.[20-21] Aspirin produces analgesic effects by inhibiting COX-2, but its inhibition of COX-1 can sometimes lead to severe adverse events such as gastric ulcers or gastrointestinal bleeding.[22]

To reduce such adverse events, in the 1990s Pfizer developed a selective COX-2 inhibitor, celecoxib, a new-

[1]Department of Otolaryngology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Otolaryngology Institute of Shanghai Jiao Tong University, Shanghai, China

Corresponding Authors:
Kaiming Su, MD, PhD, and Guang Yang, MD, PhD, Department of Otolaryngology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Otolaryngology Institute of Shanghai Jiao Tong University, 600 Yishan Road, 7th Building, Rm 220, Shanghai 200233, China.
Email: 021china@sina.com; gyang321@hotmail.com

generation analgesic that does not inhibit COX-1. Because its specificity reduces gastrointestinal side effects, it has become the treatment choice for modest to severe arthritis and postoperative pain.[23-24] More recently, its potential as a cancer therapy has also received attention.[25-26] Even though celecoxib is the only COX-2 inhibitor available in the United States, its effects on the auditory system have still not been investigated.

COX-1 and -2 are expressed in many rat tissues,[21,27-29] and this experiment aimed to evaluate the effect of the selective COX-2 inhibitor celecoxib on hearing loss and tinnitus in rats.

Materials and Methods

Experimental Animals and Grouping

Twenty 8-month-old adult male Sprague-Dawley rats (Shanghai Laboratory Animal Center, Shanghai, China) that weighed 280 ± 20 g, exhibited sensitive auricle responses, and did not have middle ear infections were used. After adaptive feeding for 1 week, animals were divided into 2 groups for hearing and tinnitus testing, respectively. All animals were given celecoxib (2 g/kg, Pfizer Inc, New York, New York) by gavage over less than 30 minutes. All animal procedures were performed with the permission of the Ethics Committee of Animal Care and Experimentation, University of Shanghai Jiaotong University.

The dose administered was determined by gradually increasing the dose in pilot experiments from that recommended for treatment of arthritis, 0.2-0.4 g/d, until severe adverse effects were observed. At 3 g/kg, 2 rats died at 2 and 4 hours after administration, so the dosage was reduced until all animals survived. The experimental medication dose was equivalent to 300- to 600-fold the normal dosage (calculated for adults of 60 kg body weight).

Auditory Brainstem Response Test

The auditory brainstem response (ABR) was tested in the open field. Ten animals were individually tested, in a sequential manner, using a TDT system (System III; TDT, Alachua, Florida) 1 day before and 2, 4, 6, 8, 12, 24, and 48 hours after drug administration. The basis for running the closely spaced time points was due to the negative results of the preliminary experiment; we ran tests at those points to rule out the possibility that hearing loss could have been in play for short periods of time.

Rats were anesthetized via intraperitoneal injection of a mixture of 75 mg/kg ketamine and 5 mg/kg xylazine (Sigma-Aldrich Co LLC, St. Louis, Missouri) and placed on a warm blanket in a soundproof room. We generally gave each animal only 1 injection per ABR test. Depending on the extent of recovery from that injection, a dose 33% to 66% that of the initial dose was given upon ABR testing 2 hours after the first injection of ketamine.

Tone bursts of 2-, 4-, 8-, 12-, 16-, and 32-kHz frequencies were presented through a loudspeaker placed 10 cm from the animals' ears. The recording electrode was placed at the median of the calvaria, while the reference electrode and the ground electrode were placed in the mastoid skin of the sound-exposed ear and the contralateral ear, respectively. Normally, the intensity levels of the sound gradually decreased from 100 dB SPL, and the averaging numbers and decibel step size varied with sound intensity. At high sound levels, when ABR amplitudes were larger, averaging was terminated when a clear waveform was seen. When the signal amplitude was obvious, the sound level was decreased by more than 5 dB. Use of these 2 methods minimized test time. However, when approaching the threshold, we used 1024 averages and a decibel step of 5. The previous sound pressure level just prior to disappearance of wave III was considered the ABR threshold. Wave III latencies at 80 dB SPL were also recorded.

Gap Prepulse Inhibition of Acoustic Startle

The gap prepulse inhibition of acoustic startle (GPIAS) paradigm for testing whether rats are experiencing tinnitus has been reported in detail by us[30] and others.[31] Briefly, after training, the rats' startle responses triggered by an acoustic stimulus during relatively quiet intervals (albeit with continuous noise) decrease. The trained animal is then placed in a metal cage with a pressure sensor that converts physical movements (part of the startle response) to a voltage signal, recorded by a TDT system. Voltage amplitude of the startle response is recorded under continuous background noise without gap (STng) and in the presence of continuous background noise with a gap (STg). Prepulse inhibition (PPI) was next calculated as $(STng - STg)/STg \times 100\%$.[30] Values less than 30% indicate normal inhibition, while those greater than 30% indicate tinnitus.[30] Twenty stimulus pairs (40 trials) were presented at 16 kHz; the intertrial interval was 20 seconds, background noise was 60 dB SPL, and gap duration was 50 milliseconds. Ten animals were tested 1 day before medication and at 2, 4, 6, 8, 12, 24, and 48 hours after medication; GPIAS was calculated individually.

Statistical Methods

We calculated the required sample size using Gpower[32]; we set a mean ABR threshold difference of 5 dB, an SD difference of 4 dB, an α error probability of 0.05, and a power of 0.90. All experimental data were processed using the statistical software SPSS, version 13.0. ABR response threshold, latency, and the percentage of GPIAS were presented as mean ± SD. Values before and after treatment were compared using 1-way analysis of variance and Dunnett's t test, where $P < .05$ was considered to indicate statistical significance.

Results

All 20 animals survived, and all experiments were completed successfully.

ABR

The ABR thresholds before and 2, 4, 6, 8, 12, 24 and 48 hours after drug administration are shown in **Figure 1**. The

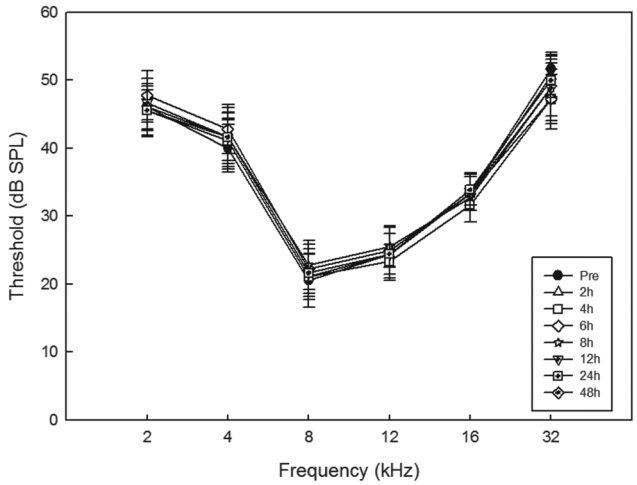

Figure 1. Effect of celecoxib on the auditory brainstem response threshold at various frequencies in rats.

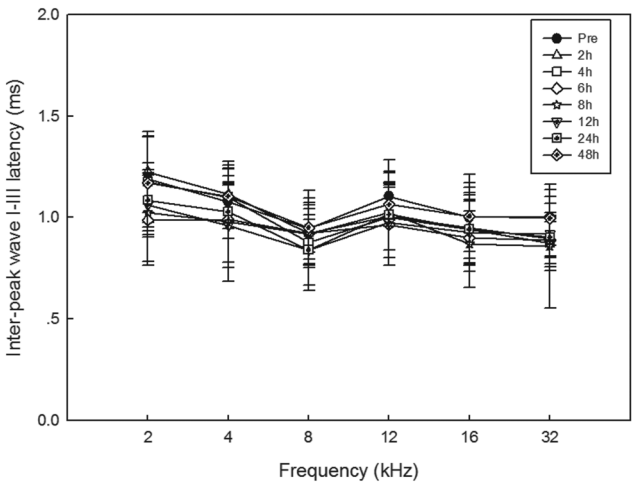

Figure 3. Effect of celecoxib on interpeak wave I to III latencies (milliseconds), at various frequencies, in rats (80 dB SPL).

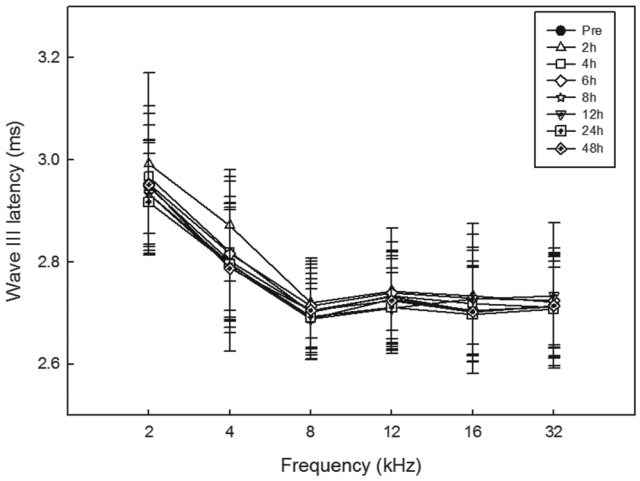

Figure 2. Effect of celecoxib on wave III latencies, at various frequencies, in rats (80 dB SPL).

Table 1. Effect of Celecoxib on Prepulse Inhibition of GPIAS in Rats.

Time Point	Prepulse Inhibition, %
1 day before	20.03 ± 3.62
2 h after	20.06 ± 5.58
4 h after	23.90 ± 8.22
6 h after	23.00 ± 6.24
8 h after	19.61 ± 5.89
12 h after	19.76 ± 8.19
24 h after	22.78 ± 7.83
48 h after	25.48 ± 10.35

Abbreviation: GPIAS, gap prepulse inhibition of acoustic startle.

ABR threshold did not increase significantly at any frequency following celecoxib gavage ($P > .05$). Neither the wave III nor the interpeak wave I-III latencies of ABR at 80 dB SPL differed significantly from those before drug administration at any time after administration of celecoxib ($P > .05$) (**Figures 2** and **3**).

GPIAS

Mean PPI before celecoxib was 20.03% ± 3.62%, and it remained below 30% following drug administration. PPI values did not differ significantly ($P > .05$) before versus after celecoxib (**Table 1**).

Discussion

This is the first study to examine the effects of a selective COX-2 inhibitor on the electrophysiology of the auditory system. We observed no hearing loss or tinnitus in rats within the 48 hours following gavage of 2 g/kg celecoxib as assessed by ABR and GPIAS. These results suggest that the tinnitus and hearing loss induced by NSAIDs may be associated with inhibition of COX-1, but not COX-2.

Previous studies have found that high-dose or long-term use of nonselective COX inhibitors such as aspirin can cause tinnitus and hearing loss in patients.[33] In animal electrophysiological studies, large doses of aspirin decrease the function of outer hair cells and the eighth cranial nerve as reflected by otoacoustic emissions and ABR and compound action potential recordings, respectively, but do not affect stria vascularis function as measured by endocochlear potentia threshold.[3,34-35] Similar results have been found in humans; otoacoustic emissions decline in patients taking aspirin long-term, indicating that the mechanism of aspirin ototoxicity is directly associated with the impaired function of outer hair cells in the cochlea.[36]

COX-1, COX-2, and endogenous prostaglandin are widely present in the hair cells and spiral ganglion cells in the cochlea of normal hearing animals,[15,37-38] whereas COX-1 and COX-2 expression and prostaglandin levels are decreased in the cochlea of salicylic acid–treated animals.[15] Thus, the mechanism underlying large-dose NSAID ototoxicity would

seem likely to be associated with suppression of COX activity and subsequent reduction of cochlear endogenous prostaglandin production.

Celecoxib inhibits COX-2 but does not inhibit COX-1. The lack of celecoxib ototoxicity suggests that tinnitus and hearing loss associated with the use of nonselective NSAIDs[3,5] may be linked to inhibition of COX-1. The use of selective COX-1 inhibitors as well as examination of any direct effect of COX-2 inhibition on cochlear function (eg, in COX-2 knockout rats) might further illustrate the roles played by COX-related mechanisms in ototoxicity induced by NSAIDs.

Some limitations in this study should be mentioned. First, although we used 300- to 600-fold the suggested therapeutic dose, these drugs may not be completely absorbed by the digestive tract. As the dose of celecoxib that yields maximal plasma concentrations in rats is unknown and we did not measure plasma concentrations, the effective dose may be lower than that administered. Second, assessments were limited to the first 48 hours after gavage; the potential of ototoxicity at a later time remains. Third, a medicine that lacks acute toxicity does not necessarily lack chronic toxicity and an animal study cannot absolutely exclude the possibility that celecoxib is ototoxic in humans. Fourth, aspirin ototoxicity may be associated with functional impairment of the outer hair cells of the cochlea.[36] Evaluation of hearing loss using otoacoustic emissions would have enriched our data. However, limitations of our experimental setup prohibited collection of such information.

In summary, the results in this study indicate that high-dose celecoxib (gavage of 2 g/kg), a COX-2 inhibitor, does not lead to tinnitus or hearing loss in rats within 48 hours. Further studies are needed to explore the roles played by COX-related mechanisms in the ototoxicity caused by nonselective COX inhibitors.

Author Contributions

Bei Li, data analysis, drafting, final approval, accountability for all aspects of the work; **Kaiming Su**, data analysis, drafting, final approval, accountability for all aspects of the work; **Guang Yang**, data analysis, drafting, final approval, accountability for all aspects of the work; **Yanmei Feng**, data analysis, drafting, final approval, accountability for all aspects of the work; **Li Xia**, data analysis, drafting, final approval, accountability for all aspects of the work; **Shankai Yin**, data analysis, drafting, final approval, accountability for all aspects of the work.

Disclosures

Competing interests: None.

Sponsorships: None.

Funding source: This study was supported by the Fund of Shanghai Municipal Health Bureau (Grant No. 20124450), and the Project of Shanghai Committee of Science and Technology (14DZ1940704).

References

1. Wang X, Lu J, Khaidakov M, et al. Aspirin suppresses cardiac fibroblast proliferation and collagen formation through downregulation of angiotensin type 1 receptor transcription. *Toxicol Appl Pharmacol*. 2012;259:346-354.
2. He F, Xia C, Zhang JH, et al. Clopidogrel plus aspirin versus aspirin alone for preventing early neurological deterioration in patients with acute ischemic stroke. *J Clin Neurosci*. 2015;22:83-86.
3. de Almeida-Silva I, de Oliveira JA, Rossato M, et al. Spontaneous reversibility of damage to outer hair cells after sodium salicylate induced ototoxicity. *J Laryngol Otol*. 2011;125:786-794.
4. Im GJ, Choi J, Chang JW, et al. Expression of insulin-like growth factors in a mouse model of salicylate ototoxicity. *Clin Exp Otorhinolaryngol*. 2010;3:115-121.
5. Peleg U, Perez R, Freeman S, et al. Salicylate ototoxicity and its implications for cochlear microphonic potential generation. *J Basic Clin Physiol Pharmacol*. 2007;18:173-188.
6. Arruda J, Jung TT, McGann DG. Effect of leukotriene inhibitor on otoacoustic emissions in salicylate ototoxicity. *Am J Otol*. 1996;17:787-792.
7. Jung TT, Hwang AL, Miller SK, et al. Effect of leukotriene inhibitor on cochlear blood flow in salicylate ototoxicity. *Acta Otolaryngol*. 1995;115:251-254.
8. Stypulkowski PH. Mechanisms of salicylate ototoxicity. *Hear Res*. 1990;46:113-145.
9. Sostres C, Gargallo CJ, Lanas A. Aspirin, cyclooxygenase inhibition and colorectal cancer. *World J Gastrointest Pharmacol Ther*. 2014;5:40-49.
10. Lordkipanidze M, Pharand C, Schampaert E, et al. Heterogeneity in platelet cyclooxygenase inhibition by aspirin in coronary artery disease. *Int J Cardiol*. 2011;150:39-44.
11. Kowalski ML, Borowiec M, Kurowski M, et al. Alternative splicing of cyclooxygenase-1 gene: altered expression in leucocytes from patients with bronchial asthma and association with aspirin-induced 15-HETE release. *Allergy*. 2007;62:628-634.
12. Hu SS, Mei L, Chen JY, et al. Expression of immediate-early genes in the inferior colliculus and auditory cortex in salicylate-induced tinnitus in rat. *Eur J Histochem*. 2014;58:2294.
13. Hwang JH, Chen JC, Chan YC. Effects of C-phycocyanin and Spirulina on salicylate-induced tinnitus, expression of NMDA receptor and inflammatory genes. *PLoS One*. 2013;8:e58215.
14. Chen GD, Stolzberg D, Lobarinas E, et al. Salicylate-induced cochlear impairments, cortical hyperactivity and re-tuning, and tinnitus. *Hear Res*. 2013;295:100-113.
15. Hwang JH, Chen JC, Yang SY, et al. Expression of COX-2 and NMDA receptor genes at the cochlea and midbrain in salicylate-induced tinnitus. *Laryngoscope*. 2011;121:361-364.
16. Norena AJ, Moffat G, Blanc JL, et al. Neural changes in the auditory cortex of awake guinea pigs after two tinnitus inducers: salicylate and acoustic trauma. *Neuroscience*. 2010;166:1194-1209.
17. Weng Z, Li X, Li Y, et al. The association of four common polymorphisms from four candidate genes (COX-1, COX-2, ITGA2B, ITGA2) with aspirin insensitivity: a meta-analysis. *PLoS One*. 2013;8:e78093.

18. Harrington LS, Lucas R, McMaster SK, et al. COX-1, and not COX-2 activity, regulates airway function: relevance to aspirin-sensitive asthma. *FASEB J*. 2008;22:4005-4010.
19. Dai M, Shi X. Fibro-vascular coupling in the control of cochlear blood flow. *PLoS One*. 2011;6:e20652.
20. Park MK, Kang YJ, Lee HS, et al. The obligatory role of COX-2 expression for induction of HO-1 in ischemic preconditioned rat brain. *Biochem Biophys Res Commun*. 2008;377: 1191-1194.
21. Seidman MD, Tang W, Bai VU, et al. Resveratrol decreases noise-induced cyclooxygenase-2 expression in the rat cochlea. *Otolaryngol Head Neck Surg*. 2013;148:827-833.
22. Nwose EU, Cann N. Whole blood viscosity issues VI: association with blood salicylate level and gastrointestinal bleeding. *N Am J Med Sci*. 2010;2:457-460.
23. Brereton N, Pennington B, Ekelund M, et al. A cost-effectiveness analysis of celecoxib compared with diclofenac in the treatment of pain in osteoarthritis (OA) within the Swedish health system using an adaptation of the NICE OA model. *J Med Econ*. 2014;17:677-684.
24. Derry S, Moore RA. Single dose oral celecoxib for acute postoperative pain in adults. *Cochrane Database Syst Rev*. 2013; (10):CD004233.
25. Ding N, Cui XX, Gao Z, et al. A triple combination of atorvastatin, celecoxib and tipifarnib strongly inhibits pancreatic cancer cells and xenograft pancreatic tumors. *Int J Oncol*. 2014;44:2139-2145.
26. Liu T, Liang X, Li B, et al. Telomerase reverse transcriptase inhibition stimulates cyclooxygenase 2 expression in cancer cells and synergizes with celecoxib to exert anti-cancer effects. *Br J Cancer*. 2013;108:2272-2280.
27. Coronel MF, Labombarda F, De Nicola AF, et al. Progesterone reduces the expression of spinal cyclooxygenase-2 and inducible nitric oxide synthase and prevents allodynia in a rat model of central neuropathic pain. *Eur J Pain*. 2014;18:348-359.
28. Tolba RH, Fet N, Yonezawa K, et al. Role of preferential cyclooxygenase-2 inhibition by meloxicam in ischemia/reperfusion injury of the rat liver. *Eur Surg Res*. 2014;53:11-24.
29. Cho JG, Lee ES, Woo JS, et al. Expressions of cyclooxygenase 1 and 2 in endotoxin-induced otitis media with effusion in the rat. *Int J Pediatr Otorhinolaryngol*. 2007;71:101-106.
30. Yang G, Lobarinas E, Zhang L, et al. Salicylate induced tinnitus: behavioral measures and neural activity in auditory cortex of awake rats. *Hear Res*. 2007;226:244-253.
31. Sun W, Lu J, Stolzberg D, et al. Salicylate increases the gain of the central auditory system. *Neuroscience*. 2009;159:325-334.
32. Faul F, Erdfelder E, Lang AG, et al. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39:175-191.
33. Zhang FY, Xue YX, Liu WJ, et al. Changes in the numbers of ribbon synapses and expression of RIBEYE in salicylate-induced tinnitus. *Cell Physiol Biochem*. 2014;34:753-767.
34. Sheppard A, Hayes SH, Chen GD, et al. Review of salicylate-induced hearing loss, neurotoxicity, tinnitus and neuropathophysiology. *Acta Otorhinolaryngol Ital*. 2014;34:79-93.
35. Zhang S, Robertson D. The electro-physiological change of guinea pig cochlea caused by intracochlear perfusion of sodium salicylate. *Lin Chuang Er Bi Yan Hou Ke Za Zhi*. 2003;17:551-553.
36. Marchese-Ragona R, Marioni G, Marson P, et al. The discovery of salicylate ototoxicity. *Audiol Neurootol*. 2008;13:34-36.
37. Heinrich UR, Brieger J, Selivanova O, et al. COX-2 expression in the guinea pig cochlea is partly altered by moderate sound exposure. *Neurosci Lett*. 2006;394:121-126.
38. Heinrich UR, Selivanova O, Schmidtmann I, et al. Noise exposure alters cyclooxygenase 1 (COX-1) and 5-lipoxygenase (5-LO) expression in the guinea pig cochlea. *Acta Otolaryngol*. 2010;130:358-365.