Clinical Medicine Insights: Ear, Nose and Throat

ORIGINAL RESEARCH

Comparison of the Protective Effects of Radix Astragali, α-Lipoic Acid, and Vitamin E on Acute Acoustic Trauma

Min Xiong[1], Huangwen Lai[2], Chuanhong Yang[2], Weiyi Huang[3], Jian Wang[1], Xiaoyan Fu[1] and Qinglian He[4]

[1]Department of Otolaryngology, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, China. [2]Medical Research Center, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, China. [3]Department of Traditional Chinese Medicine, Guangzhou General Hospital of Guangzhou Military Command, Guangzhou, China. [4]Department of Pathology of the Second Hospital Affiliated with Guangzhou Traditional Chinese Medical University, Guangzhou, China. Corresponding authors email: dr_xiong@163.com; ebhwj@yahoo.com.cn

Abstract

Objective: Oxidative damage is a critical role which involves hearing loss induced by impulse noise. That exogenous antioxidant agents reduce noise induced hearing loss (NIHL) has been well demonstrated in both animal studies and clinical practices. Choosing a stronger and more effective antioxidant is very important for treatment of NIHL. Vitamin E, α-lipoic acid, and radix astragali are the most commonly used anti-oxidants for cochlear oxidative damage from acoustic trauma. In this study, the protective effects of radix astragali, α-lipoic acid, and vitamin E on acute acoustic trauma are investigated.

Methods: Guinea pigs in the experimental groups were intragastrically administered vitamin E, α-lipoic acid, and radix astragali. Auditory thresholds were assessed by sound-evoked auditory brainstem response (ABR) at click and tone bursts of 8, 16 and 32 kHz, 24 hours before and 72 hours after exposure to impulse noise. Cochlear malondialdehyde (MDA) concentrations were detected. Hair cell damage was analyzed by scanning electron microscopy.

Results: Vitamin E, α-lipoic acid, and radix astragali significantly reduced ABR deficits, reduced hair cell damage, and decreased the concentrations of MDA. α-lipoic acid and radix astragali were better than vitamin E, and there were no significant differences between α-lipoic acid and radix astragali.

Conclusions: α-lipoic acid or radix astragali are recommended for treatment of NIHL.

Keywords: impulse noise, cochlea, hearing loss, vitamin E, α-lipoic acid, radix astragali, malondialdehyde, reactive oxygen species

Clinical Medicine Insights: Ear, Nose and Throat 2012:5 25–31

doi: 10.4137/CMENT.S10711

This article is available from http://www.la-press.com.

Introduction

Acute acoustic trauma (AAT) is a common disease of modern life caused, among other reasons, by music, video games, and accidental explosions. Surges in noise levels due to spreading industrialization have contributed to make noise induced hearing loss (NIHL) an environmental hazard globally. Particularly in a military environment, AAT is caused by noise from weapons and equipment systems, turbine engines, and high-performance aircraft and helicopters. Controlling noise and reducing its harmful effects have been a challenge for environmental protection and safety professionals. Noise should be paid close attention because it may affect an individual's quality of life and working profession. A variety of mechanisms have been proposed to account for hearing loss after AAT. There are two main mechanisms. The first is direct mechanical trauma to the organ of Corti. The second is metabolic damage following mechanical trauma-dependent processes in the inner ear. It is well known that intense metabolic activity results in the production of excess free radicals[1] and lipid peroxidation products.[2] Noise-induced production of reactive oxygen species (ROS) in the cochlea has now been well confirmed.[3,4] Antioxidants have been confirmed to partially protect sensory cells in the organ of Corti from oxidative damage. Upregulation of the endogenous antioxidant glutathione reduces NIHL,[5] whereas the opposite occurs with reduced endogenous antioxidants.[6] That exogenous antioxidant agents reduce sensory cell death and NIHL has been well demonstrated in animal studies and clinical practices.[2,7]

Vitamin E and α-lipoic acid are the most commonly used anti-oxidants for cochlear oxidative damage from acoustic trauma.[8–12] Radix astragali is a kind of traditional Chinese herb which is widely used in treating free radical-mediated injury in various organs because of its antioxidant properties.[13,14] In addition, radix astragali has been confirmed to reduce the cochlear damage induced by impulse noise through its antioxidant property.[15–17] AAT is a common disease in our otologic clinic, particularly in army patients. As no therapy produces specific effects on AAT, comprehensive treatments have been used for it instead. Antioxidant is one of the important therapies for AAT.[17] Therefore, it is critical to treatment to select a more effective antioxidant for AAT. In this study, we investigated the antioxidant properties of vitamin E, α-lipoic acid, and radix astragali in cochlea after exposure to impulse noise.

Material and Methods

Animals and impulse noise exposure

Fifty albino guinea pigs weighing between 250 and 350 grams were used in this study. All experimental protocols were reviewed by the Committee for Ethics on Animal Experiments of the General Hospital of PLA Guangzhou Command (Guangzhou, China). All experiments were carried out in accordance with these guidelines. All animals were confirmed to have a positive Preyer's reflex and were free from otitis media on microscopic examination. They were free to move around inside their cages and had free access to food and water at all times. Guinea pigs were randomly assigned to five groups and each group consisted of 10 guinea pigs. Group I was the normal control group in which animals did not receive any treatments. Group II was the experimental control group, wherein animals were exposed to intense impulse noise and only treated with saline intragastrically. Animals in group III, group IV and group V were intragastrically administered vitamin E (Beijing Shuanghe Modern Medical Technology Limited Company, approved no. H11021397), α-lipoic acid (Shandong Qidu Pharmaceutical Limited Company, approved no. H20100153) and radix astragali (Yangzijiang Pharmaceutical Limited Company, approved no. Z32020370) respectively, 24 hours before and 72 hours after exposure to impulse noise. The dose of vitamin E, α-lipoic acid, and radix astragali was respectively 100 mg/kg, 200 mg/kg, 6000 mg/kg per 24 hours, intragastrically. All three drugs were delivered equally at 8:00, 14:00, and 20:00, respectively. The same volume of isotonic saline solution was administered to the animals in group II.

The animals were exposed to intense impulse noise from the 7.62 mm Chinese Army 81-1 type of assault rifle. Each animal was exposed to impulses of 15 shots delivered at an interval of 1 second so as to avoid the involvement of the stapedius reflex. During the exposure, each animal was kept with its head towards the muzzle of the rifle at a distance of 0.35 m to the direction left 15° (average impulse peak 176 dB SPL, the frequency spectrum 1.05–20.3 kHz). The variation in sound pressure was about 5 dB in

the area where the animal cage was placed. Sound peak was measured by a digital sound pressure meter (TDJ-23, Jinan, China). The average impulse peak sound pressure level was calculated from the average of 10 shots.

Auditory brainstem response (ABR) measurements

The guinea pigs were anesthetized with sodium pentobarbital (40 mg/kg i.p.). ABR recordings were obtained 24 hours before experiment and 72 hours after exposure to impulse noise by means of an electrodiagnostic system (Pathfinder I; Nicolet Biomedical Instruments). Responses were recorded using the far-field technique. The reference electrode was inserted subcutaneously (s.c.) below the ipsilateral pinna, the ground electrode s.c. below the contralateral pinna, and the active electrode into the top of the head. Acoustic stimuli were delivered by an earphone through a small tube inserted into the external ear meatus in a soundproof box. The stimuli consisted of click and tone bursts of 8, 16, and 32 kHz (sine wave pulses with a trapezoidal envelope, total duration was 10 ms, and rise and fall times were 2 ms). They were presented at a rate of 11.1 s^{-1} and duration of 0.11 ms. Responses were accumulated 500 times. The levels of stimuli was lowered from 95 to 10 dB SPL by 5 dB steps. The ABR threshold was determined as the minimum sound level giving reproducible waveforms. The recordings were repeated twice at the threshold level and the reproducibility was confirmed.

Malondialdehyde (MDA) detection

All animals were sacrificed 72 hours after exposure to impulse noise. In each animal, left cochlea was for MDA detection, and right cochlea was for scanning electron microscopy. The tissues were fixed via cardiac perfusion with 4% (w/v) paraformaldehyde (pH 7.4) after flushing out the blood with 0.1 M PBS. Left cochleae for MDA detection were stored at −70 °C until biochemical analysis could be completed. Tissue (350–000 μg) was dissected from each cochlea immediately after removal from cold storage. Cochlear tissue was homogenized in 1 mL of 50 mM phosphate buffer (pH 7.4) and centrifuged at 1,000 rpm for 10 min. The supernatant (tissue extract) was used for biochemical analysis. The end product of lipid peroxidation, MDA, was measured by an MDA assay kit (Nanjing Jiancheng Biological Techniques Institute). Measurement of MDA was determined with a chemical method according to the manufacturer's instructions. 100 μL of tissue homogenate was added to 50 μL of 8.1% sodium dodecyl sulfate, vortexed and incubated for 10 minutes at room temperature. 375 μL of 20% acetic acid and 375 μL of thiobarbituric acid (0.6%) were added and the mixture was placed in a boiling water bath in sealed tubes for 60 minutes. The samples were allowed to cool at room temperature and 1.25 mL of butanol pyridine was added. The samples were then vortexed and centrifuged at 1,000 rpm for 5 minutes. 500 μL of the pink-colored layer was measured at 532 nm on a spectrophotometer using 1,1,3,3-tetra-ethoxy-propane as standard. The concentration of MDA was expressed as nanomoles per milligram protein. The protein content in the cochlear samples was estimated using Coomassie dye and bovine serum albumin as a standard. All analysis of MDA was carried out in a randomized double-blind manner.

Scanning electron microscope

After right cochleae were dissected, specimens were perfused immediately with 2.5% glutaraldehyde at 4 °C in 0.1 mol/L cacodylate (Cac) buffer (pH 7.4). A straight pick was used to create small openings into the round window, oval window, and apex of the cochlea through which the perfusions were performed. The specimens were then immersed in the glutaraldehyde solution and refrigerated overnight. The following day, the specimens were carefully rinsed with Cac buffer, then post fixed with 1.5% osmium tetroxide in 1.0 mol/L Cac buffer. After 15 minutes of rotation the specimens were again infused with Cac buffer. The lateral wall was exposed under magnification by removing the bony capsule and lateral wall of the cochlea with a dental drill. The organ of Corti was then exposed using a razor to dissect the spiral ligament. The specimens were dried, mounted, and sputter-coated with gold palladium alloy. Photographs were taken using a Hitachi S-3000N scanning electron microscope.

Statistical analyses

t-tests were performed using a commercial statistical software package (SPSS 19.0).

Results

Auditory testing

The changes in ABR thresholds for the five groups are shown in Figure 1. Impulse noise exposure caused a significant increase in ABR thresholds at all stimuli tested, when compared to pre-impulse noise exposure threshold values. The threshold shifts (mean ± SEM) in group II were 35.7 ± 8.2 dB SPL for clicks, 32.6 ± 6.9 dB SPL at 8 kHz, 31.7 ± 9.1 dB SPL at 16 kHz, and 37.6 ± 7.2 dB SPL at 32 kHz. There were no mean threshold shifts from baseline in group I. The threshold changes for group I were 4.8 ± 2.6 dB for clicks, 5.6 ± 2.9 dB for 8 kHz, 6.4 ± 1.9 dB for 16 kHz, and 5.7 ± 2.3 dB for 32 kHz. In group III, group IV and group V, where animals had been administered vitamin E, α-lipoic acid and radix astragali respectively, there were statistically significant reductions in the mean threshold shifts for clicks, 8 kHz tone bursts, 16 kHz tone bursts, and 32 kHz tone bursts compared with group II. There were no significant differences in the mean threshold shifts between group IV and group V ($P > 0.05$). The mean threshold shifts in both group IV and group V were better than that in group III ($P < 0.01$).

MDA assay

The changes in cochlear MDA levels for the 5 groups are shown in Figure 2. The MDA concentration (mean ± SEM) in group I was 2.76 ± 0.35 nmol/mg protein. The mean MDA concentration in group II was significantly higher (10.29 ± 2.47 nmol/mg, $P < 0.001$). Compared with group II, there were significant reductions in the mean MDA concentrations in group III (7.14 ± 1.89 nmol/mg, $P < 0.01$), group IV (5.23 ± 2.2 nmol/mg, $P < 0.01$) and group V (5.17 ± 2.4 nmol/mg protein, $P < 0.01$). There was no significant difference in the mean MDA concentration between group IV and group V ($P > 0.05$). The mean MDA concentration in both group IV and group V were less than that in group III ($P < 0.05$).

Cochlear morphology

No change in hair cell morphological appearance was encountered in group I animals when observed by SEM. Outer hair cells with normal-appearing stereocilia were seen at every turn of the cochlea (Fig. 3A). However, a lot of OHCs with stereocilia loss were observed at the basal turn and the second turn of the cochlea of group II animals. The IHCs appeared to retain their morphological integrity throughout the cochlea (Fig. 3B). A lesser degree of OHC damage was seen in group III, group IV and group V as compared with group II (Fig. 3C–E respectively). Under SEM observation, 10 different areas were chosen randomly in the basal turn, second turn, and apical turn, respectively. Magnification of every area was by a

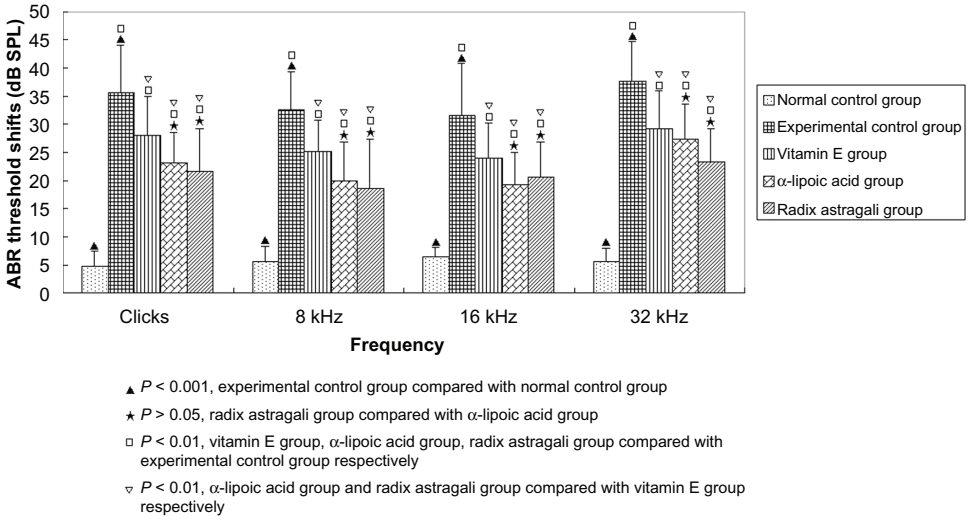

Figure 1. Mean ABR threshold changes of five groups.
Notes: In vitamin E group, α-lipoic acid group and radix astragali group ABR threshold shifts were significantly reduced in clicks, 8 kHz, 16 kHz and 32 kHz tones when compared with experimental control group ($P < 0.01$). ABR threshold shifts of α-lipoic acid group and radix astragali group were lesser when compared with vitamin E group ($P < 0.01$). There was no significant differences in ABR threshold shifts between α-lipoic acid group and radix astragali group ($P < 0.05$).

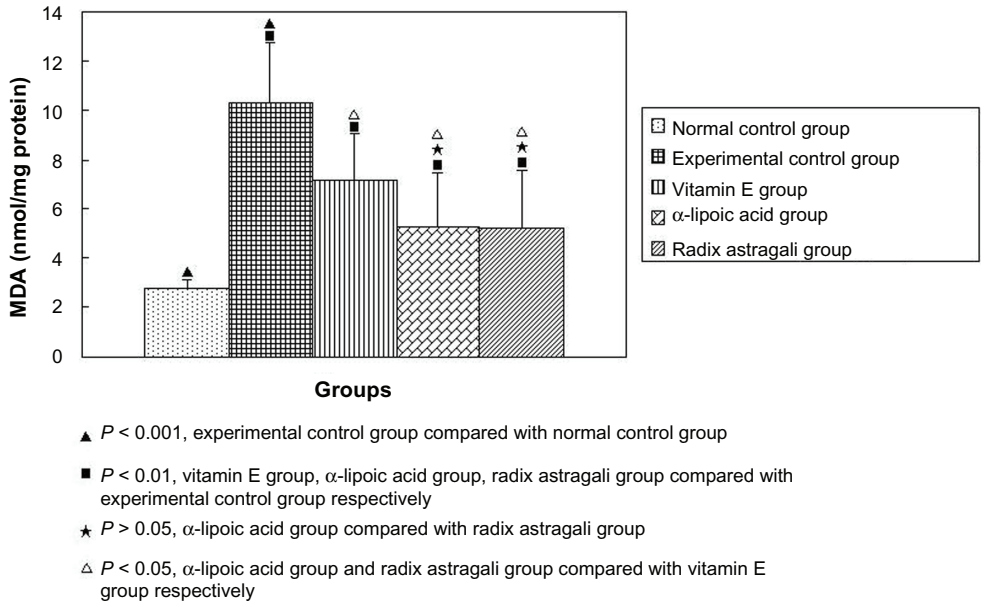

Figure 2. Mean cochlear MDA concentrations of five groups.
Notes: MDA concentrations of vitamin E group, α-lipoic acid group and radix astragali group were significantly reduced when compared with experimental control group ($P < 0.01$). MDA concentrations of α-lipoic acid group and radix astragali group were much lesser than that of vitamin E group ($P < 0.05$). There were no significant differences in MDA concentrations between α-lipoic acid group and radix astragali group ($P > 0.05$).

factor of 1200. The number of OHCs with stereocilia loss was counted in every area; the rate of OHCs with stereocilia loss was then calculated. The rate of OHCs with stereocilia loss in the cochlear turn was the average of 10 different areas. The rate of OHCs with stereocilia loss in the cochlear turn of each group was the mean of 10 cochleae. Figure 4 summarizes the rates of OHCs with stereocilia loss in three cochlear turns of the five groups. It indicates that OHCs at the basal and second turns were more vulnerable than those at the apical turn. There were no OHCs with stereocilia loss in group I. In group II, the mean rate of OHCs with stereocilia loss was 24.9% ± 6.8% at the basal turn, 18.9% ± 6.6% at the second turn, and 9.1% ± 3.7% in the apical turn. Compared with group II, there was a significant reduction in the mean rate of OHCs with stereocilia loss in group III, group IV and group V. There was no significant difference in the mean rate of OHCs with stereocilia loss in all cochlear turns in group IV and group V ($P > 0.05$). The mean rate of OHCs with stereocilia loss of both group IV and group V was less than that of group III ($P < 0.05$).

Discussion

It is well known that free radicals are produced following acoustic trauma. Hydroxyl (OH) radicals have been seen to increase nearly 4 times within 1–2 hours of noise exposure,[18] and a significant early increase in superoxide (O_2^-) with reaction products has also been evident after 5 minutes following noise exposure.[19] Additionally, significant formation of ROS and reactive nitrogen species (RNS) also appear 7–10 days after noise trauma.[20] Free radicals can induce lipid peroxidation, free radicals are produced following the acoustic trauma, and significant noise-induced lipid peroxidation has been described 15–30 minutes post-noise. Therefore, this study was designed to determine the changes in concentrations of the end product of lipid peroxidation (MDA) in the cochleae, compared to changes in ABRs and stereocilia of OHCs in guinea pigs treated with vitamin E, α-lipoic acid and radix astragali, and to evaluate the otoprotective efficacy on NIHL of these three antioxidants. This study demonstrated vitamin E, α-lipoic acid and radix astragali were all effective on NIHL; α-lipoic acid and radix astragali were considered more effective than Vitamin E. However, there was no significant difference in otoprotective efficacy on NIHL between α-lipoic acid and radix astragali.

It is understood that pre-treatment with a variety of antioxidants reduces the early formation of free radicals in cochlear noise damage. Daily treatment with antioxidants post-noise presumably reduces the late forming radicals. Noise reduces blood vessel diameter

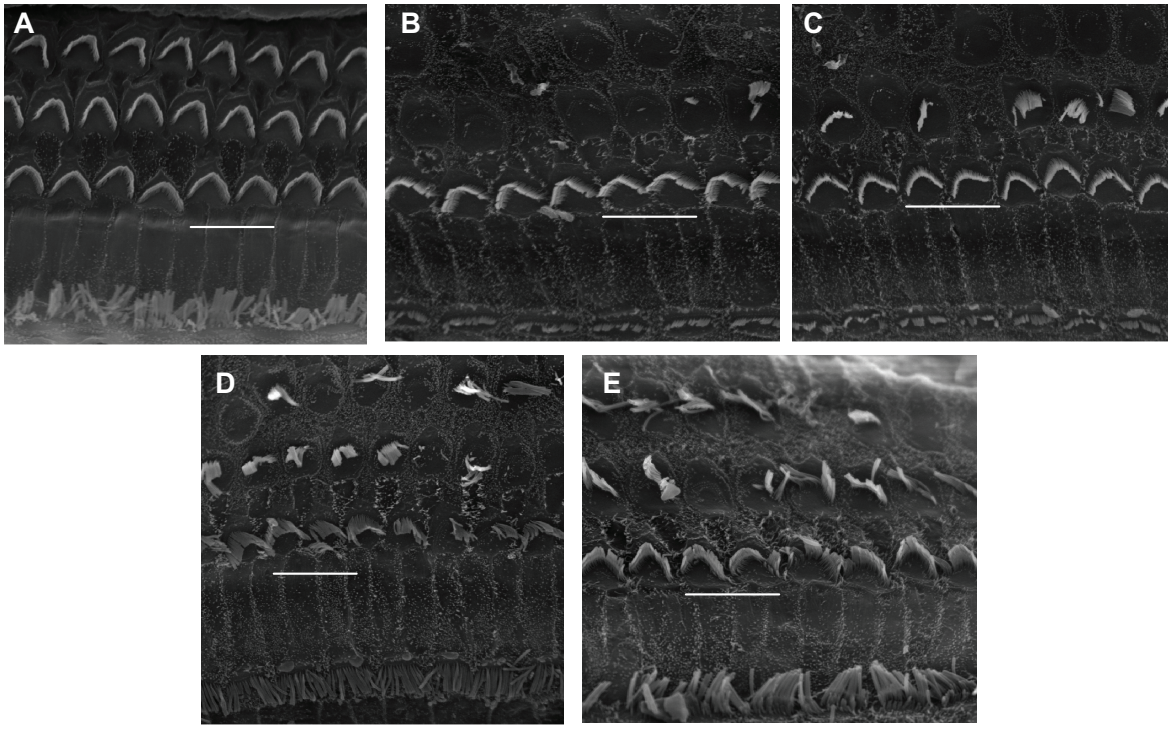

Figure 3. Scanning electronic microscopy (SEM) of cochleae. (**A**) No change in hair cell morphological appearance was encountered in normal control group animals when observed by SEM. (**B**) A large number of outer hair cells (OHCs) with stereocilia loss were observed at the basal turn and the second turn of the cochlea in experimental control group animals. Most of the damage appeared to affect the first to second rows of hair cells, with distortion and twisting of the stereocilia along with complete absence of hair cells. (**C**–**E**) A lesser degree of OHC damage was seen in vitamin E group, α-lipoic acid group and radix astragali group as compared with experimental control group.
Note: Scale bars: 10 μm.

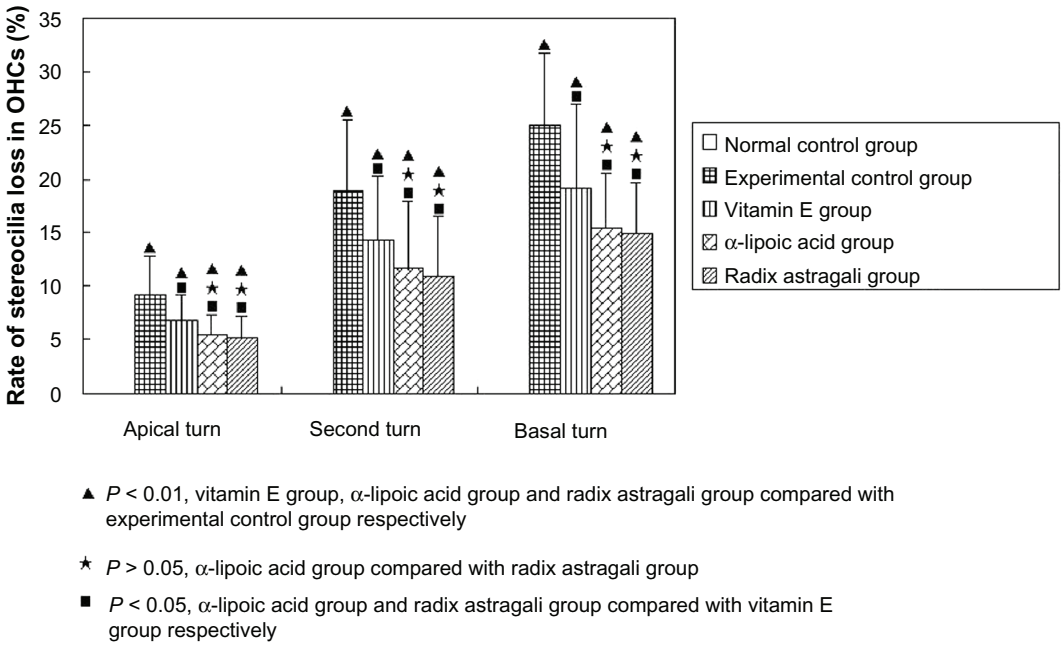

Figure 4. Rates of stereocilia loss of outer hair cells (OHCs) in the five groups.
Notes: There was no loss of stereocilia of OHCs in normal control group. The rates of stereocilia loss of OHCs in the basal turn, second turn and apical turn were much lesser in vitamin E group, α-lipoic acid group and radix astragali group when compared with experimental control group ($P < 0.05$). The rates of stereocilia loss of OHCs of α-lipoic acid group and radix astragali group were lesser than that of vitamin E group ($P < 0.05$). There were no significant differences in rates of stereocilia loss of OHCs between α-lipoic acid group and radix astragali group ($P > 0.05$).

and red blood cell velocity and decreases blood flow in the cochlea. Noise-induced vasoconstriction is a direct consequence of 8-isoprostane-$F_{2\alpha}$, a vasoconstrictor by-product of free radicals; therefore, antioxidants that reduce free radical formation may decrease noise-induced vasoconstriction. There is some suggestion that joint application of antioxidants is more effective than single agent application. We would prefer a stronger and more effective antioxidant because comprehensive treatment was recommended for NIHL, including oxygen therapy and application of dexamethasone and compound vitamin B.[7]

Dose conversion of vitamin E, α-lipoic acid, and radix astragali were applied in this study. It is common sense that the dosage of the drug affects the efficacy of the drug. The reasonable guinea pig dose is about 20 times the amount of the human dose. The doses of vitamin E, α-lipoic acid, and radix astragali in this study were 20 times the appropriate dose for humans. Therefore, the treatments with vitamin E, α-lipoic acid, and radix astragali on acoustic trauma are comparable.

Acknowledgements

This research was supported by Guangzhou Studies Abroad Personnel Service Management Center (NO.20112116).

Author Contributions

Conceived and designed the experiments: Min Xiong and Jian Wang. Analysed the data: Huangwen Lai and Chuanhong Yang. Wrote the first draft of the manuscript: Min Xiong. Contributed to the writing of the manuscript: Xiaoyan Fu. Agree with manuscript results and conclusions: Weiyi Huang. Jointly developed the structure and arguments for the paper: Qinglian He. Made critical revisions and approved final version: Min Xiong and Jian Wang. All authors reviewed and approved of the final manuscript.

Competing Interests

Authors disclose no potential conflicts of interest.

Disclosures and Ethics

This manuscript has been read and approved by the authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirms that they have permission to reproduce any copyrighted material.

References

1. Yamane H, Nakai Y, Takayama M, Iguchi H, Nakagawa T, Kojima A. Appearance of free radicals in the guinea pig inner ear after noise-induced acoustic trauma. *Eur Arch Otorhinolaryngol*. 1995;252(8):504–8.
2. Ohinata Y, Miller JM, Altschuler RA, Schacht J. Intense noise induces formation of vasoactive lipid peroxidation products in the cochlea. *Brain Res*. 2000;878(1–2):163–73.
3. Henderson D, Bielefeld EC, Harris KC, Hu BH. The role of oxidative stress in noise-induced hearing loss. *Ear Hear.* 2006;27(1):1–19.
4. Xiong M, He Q, Lai H, Wang J. Astragaloside IV inhibits apoptotic cell death in the guinea pig cochlea exposed to impulse noise. *Acta Otolaryngol*. 2012;132(5):467–74.
5. Yamasoba T, Nuttall AL, Harris C, Raphael Y, Miller JM. Role of glutathione in protection against noise-induced hearing loss. *Brain Res*. 1998;784(1–2):82–90.
6. Ohlemiller KK, McFadden SL, Ding DL, et al. Targeted deletion of the cytosolic Cu/Zn-superoxide dismutase gene (Sod1) increases susceptibility to noise-induced hearing loss. *Audiol Neurootol*. 1999;4(5):237–46.
7. Xiong M, He Q, Lai H, et al. Radix Astragali injection enhances recovery from acute acoustic trauma. *Acta Otolaryngol.* 2011;131(10):1069–73.
8. Le Prell CG, Hughes LF, Miller JM. Free radical scavengers vitamins A, C, and E plus magnesium reduce noise trauma. *Free Radic Biol Med.* 2007;42(9):1454–63.
9. Kapoor N, Mani KV, Shyam R, Sharma RK, Singh AP, Selvamurthy W. Effect of vitamin E supplementation on carbogen-induced amelioration of noise induced hearing loss in man. *Noise Health*. 2011;13(55):452–8.
10. Fetoni AR, Ferraresi A, Greca CL, et al. Antioxidant protection against acoustic trauma by coadministration of idebenone and vitamin E. *Neuroreport.* 2008;19(3):277–81.
11. Diao MF, Liu HY, Zhang YM, Gao WY. Changes in antioxidant capacity of the guinea pig exposed to noise and the protective effect of alpha-lipoic acid against acoustic trauma. *Sheng Li Xue Bao.* 2003;55(6):672–6.
12. Pouyatos B, Gearhart C, Nelson-Miller A, Fulton S, Fechter LD. Lipoic acid and 6-formylpterin reduce potentiation of noise-induced hearing loss by carbon monoxide: preliminary investigation. *J Rehabil Res Dev.* 2008;45(7):1053–64.
13. Toda S, Yase Y, Shirataki Y. Inhibitory effects of astragali radix, crude drug in Oriental medicines on lipid peroxidation and protein oxidative modification of mouse brain homogenate by copper. *Phytother Res*. 2000;14(4):294–6.
14. Toda S, Shirataki Y. Inhibitory effects of Astragali Radix, a crude drug in Oriental medicines, on lipid peroxidation and protein oxidative modification by copper. *J Ethnopharmacol*. 1999;68(1–3):331–3.
15. Xiong M, Lai H, He Q, Wang J. Astragaloside IV attenuates impulse noise-induced trauma in guinea pig. *Acta Otolaryngol*. 2011;131(8):809–16.
16. Xiong M, He Q, Lai H, Wang J. Astragaloside IV inhibits apoptotic cell death in the guinea pig cochlea exposed to impulse noise. *Acta Otolaryngol*. 2012;132(5):467–74.
17. Xiong M, He Q, Lai H, et al. Radix Astragali injection enhances recovery from acute acoustic trauma. *Acta Otolaryngol.* 2011;131(10):1069–73.
18. Ohlemiller KK, Wright JS, Dugan LL. Early elevation of cochlear reactive oxygen species following noise exposure. *Audiol Neurootol.* 1999;4(5):229–36.
19. Yamane H, Nakai Y, Takayama M, Iguchi H, Nakagawa T, Kojima A. Appearance of free radicals in the guinea pig inner ear after noise-induced acoustic trauma. *Eur Arch Otorhinolaryngol.* 1995;252(8):504–8.
20. Yamashita D, Jiang H, Schacht J, Miller JM. Delayed production of free radicals following noise exposure. *Brain Res*. 2004;1019(1–2):201–9.