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# Mechanism of alpha-lipoic acid in attenuating kanamycin-induced ototoxicity☆

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## Abstract

In view of the theory that alpha-lipoic acid effectively prevents cochlear cells from injury caused by various factors such as cisplatin and noise, this study examined whether alpha-lipoic acid can prevent kanamycin-induced ototoxicity. To this end, healthy BALB/c mice were injected subcutaneously with alpha-lipoic acid and kanamycin for 14 days. Auditory brainstem response test showed that increased auditory brainstem response threshold shifts caused by kanamycin were significantly inhibited. Immunohistochemical staining and western blot analysis showed that the expression of phosphorylated p38 mitogen-activated protein kinase and phosphorylated c-Jun N-terminal kinase in mouse cochlea was significantly decreased. The experimental findings suggest that phosphorylated p38 and phosphorylated c-Jun N-terminal kinase mediated kanamycin-induced ototoxic injury in BALB/c mice. Alpha-lipoic acid effectively attenuated kanamycin ototoxicity by inhibiting the kanamycin-induced high expression of phosphorylated p38 and phosphorylated c-Jun N-terminal kinase.

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## Key Words

alpha-lipoic acid; kanamycin; mouse; cochlea; p38 mitogen-activated protein kinase; c-Jun N-terminal kinase; auditory brainstem response; phosphorylation; hearing loss; western blot; immunohistochemistry

## Research Highlights

(1) The possible mechanism of alpha-lipoic acid in attenuating kanamycin-induced ototoxic injury was verified in accordance with the antioxidant properties of alpha-lipoic acid.
(2) Phosphorylated p38 mitogen-activated protein kinase and c-Jun N-terminal kinase mediate kanamycin-induced ototoxic injury in BALB/c mice. Alpha-lipoic acid can effectively attenuate kanamycin ototoxicity by inhibiting the kanamycin-induced high expression of phosphorylated p38 and c-Jun N-terminal kinase.
(3) The pathway of alpha-lipoic acid for neuronal ototoxicity protection was investigated to provide a theoretical basis for the treatment of drug-induced deafness in clinic.

## Abbreviations

p38 MAPK, p38 mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase

## INTRODUCTION

p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK) are major members of the MAPK family, which play important roles in inflammation, stress, cell growth and apoptosis[1-4]. Studies have demonstrated that as the key kinases in stress signal transduction, p38 and JNK can be activated by various factors such as heat shock, radiation, ischemia and oxidative stress, which in turn leads to the initiation of apoptosis. Thus, both p38 and JNK are thought to be the important signaling pathways mediating apoptotic cell death[5-6]. Recent reports indicated that p38 and JNK are involved in cochlear cell apoptosis induced by noise, gentamycin and other ototoxic drugs[7-12]. Kanamycin and gentamycin are both aminoglycoside antibiotics[13], and kanamycin can induce apoptosis of cochlear hair cells[14-16]. However, it is unclear whether p38 and JNK are involved in cochlear cell apoptosis induced by kanamycin. At present, it is thought that kanamycin ototoxicity promotes the formation of reactive oxygen species and then causes lipid peroxidation[17-18]. Thus, using antioxidants to protect against kanamycin ototoxicity has become a major research focus in recent years[19-20].

Alpha-lipoic acid is a new powerful antioxidant. It can effectively scavenge oxygen free radicals and thereby prevent the occurrence of oxidative stress *in vivo* [21-22]. Recent studies have shown that alpha-lipoic acid can protect cochlear cells of rats and guinea pigs from injury caused by various factors such as cisplatin and noise[23-25]. However, it has not been reported whether alpha-lipoic acid can also protect against kanamycin-induced cochlear cell injury, and whether the p38 and JNK pathways are involved. In this study, to explore the protective role and mechanism of alpha-lipoic acid in kanamycin-induced ototoxicity, we investigated the effect of alpha-lipoic acid on the kanamycin-induced expression of phosphorylated p38 (p-p38) and phosphorylated JNK (p-JNK) in mouse cochlea, using immunohistochemical staining and western blot analysis, combined with auditory brainstem response test.

## RESULTS

### Quantitative analysis of experimental animals

A total of 56 healthy BALB/c mice were randomly divided into control, kanamycin, kanamycin plus alpha-lipoic acid, and alpha-lipoic acid groups. Mice were given a subcutaneous injection of saline, kanamycin and/or alpha-lipoic acid, respectively, twice daily for 14 days. All mice were included in the final analysis, and no death or infection occurred.

### Alpha-lipoic acid attenuated kanamycin-induced auditory brainstem response threshold shifts

Auditory brainstem response test showed that mice in the control group maintained stable thresholds throughout the experiment. In contrast, the auditory brainstem response threshold shifts in the kanamycin group were significantly elevated after continuous injections for 14 days ($P < 0.01$), and the functional deficit was greater at the higher frequency; the threshold shifts average was about 43 dB at 24 kHz, and 36 dB at 8 kHz (Table 1). After concurrent treatment with alpha-lipoic acid, the auditory brainstem response threshold shifts were reduced to about 17 dB at 24 kHz, and 9 dB at 8 kHz ($P < 0.01$; Table 1). Alpha-lipoic acid alone had no effect on the auditory brainstem response threshold shifts (Table 1).

Table 1 Auditory brainstem response threshold shifts in mice under different stimulation frequencies

| Group | Auditory brainstem response threshold shifts (dB SPL) | | |
|---|---|---|---|
| | 8 kHz | 12 kHz | 24 kHz |
| Control | 1.03±1.37 | 1.07±1.15 | 1.36±1.44 |
| Kanamycin | 36.61±1.46[a] | 40.43±1.83[a] | 43.54±1.53[a] |
| Kanamycin + alpha-lipoic acid | 9.64±1.34[ab] | 13.25±1.24[ab] | 17.32±1.28[ab] |
| Alpha-lipoic acid | 1.07±1.27 | 1.00±1.15 | 1.50±1.17 |

[a]$P < 0.01$, *vs*. control group; [b]$P < 0.01$, *vs*. kanamycin group. Data are expressed as mean ± SD ($n = 28$ ears). One-way analysis of variance followed by least significant difference *t*-test was used to compare the data of different groups.

### Alpha-lipoic acid reduced the kanamycin-induced expression of p-p38 and p-JNK in mouse cochlea

The immunohistochemical staining results showed that the expression of p-p38 and p-JNK in outer hair cells, spiral ganglion and stria vascularis of mouse cochlea was markedly increased in the kanamycin group compared with the control group ($P < 0.01$). Concurrent subcutaneous injection with alpha-lipoic acid significantly reduced the kanamycin-induced high expression of p-p38 and p-JNK in mouse cochlea ($P < 0.01$), while alpha-lipoic acid alone had no effect on the expression of p-p38 and p-JNK in mouse cochlea (Figures 1, 2).

Western blot analysis confirmed our immunohistochemical results (Figure 3).

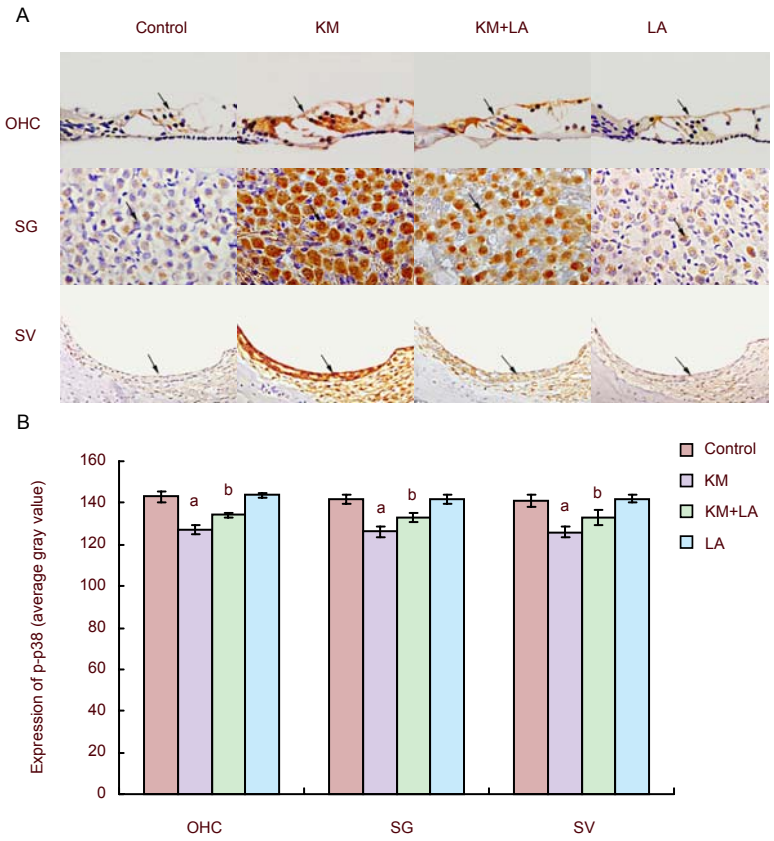



Figure 1 Effect of alpha-lipoic acid (LA) on kanamycin (KM)-induced expression of p-p38 in mouse cochlea.

(A) Immunohistochemistry showed the effect of LA on KM-induced expression of p-p38 in mouse cochlea (× 400). The positive p-p38 expression product exhibited brown yellow granules (arrows) in the cytoplasm and nucleus of OHC, SG and SV.

(B) Quantitative analysis of p-p38 expression. Data are expressed as mean ± SD, five mice in each group. One-way analysis of variance followed by least significant difference *t*-test were used to compare the data of different groups. [a]*P* < 0.01, *vs*. control group; [b]*P* < 0.01, *vs*. KM group.

OHC: Outer hair cell; SG: spiral ganglion; SV: stria vascularis; p-p38: phosphorylated p38 mitogen-activated protein kinase.

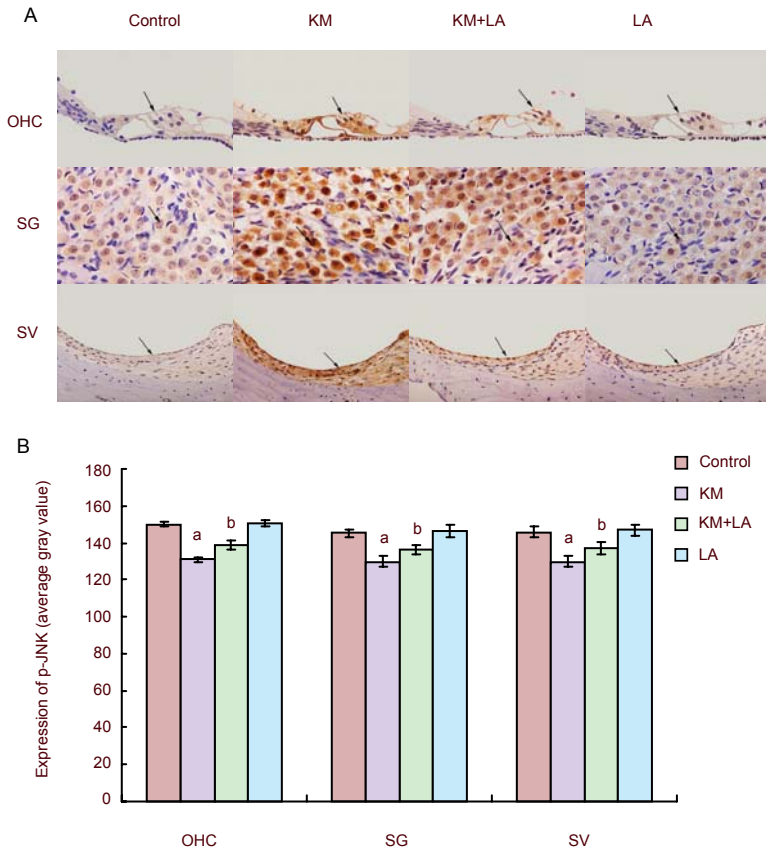



Figure 2 Effect of alpha-lipoic acid (LA) on kanamycin (KM)-induced expression of phosphorylated c-Jun N-terminal kinase (p-JNK) in mouse cochlea.

(A) Immunohistochemistry showed the effect of LA on KM-induced expression of p-JNK in mouse cochlea (× 400). The positive p-JNK expression product exhibited brown yellow granules (arrows) in the cytoplasm and nucleus of OHC, SG and SV.

(B) Quantitative analysis of p-JNK expression. Data are expressed as mean ± SD, five mice in each group. One-way analysis of variance followed by least significant difference *t*-test were used to compare the data of different groups. [a]*P* < 0.01, *vs*. control group; [b]*P* < 0.01, *vs*. KM group.

OHC: Outer hair cell; SG: spiral ganglion; SV: stria vascularis.

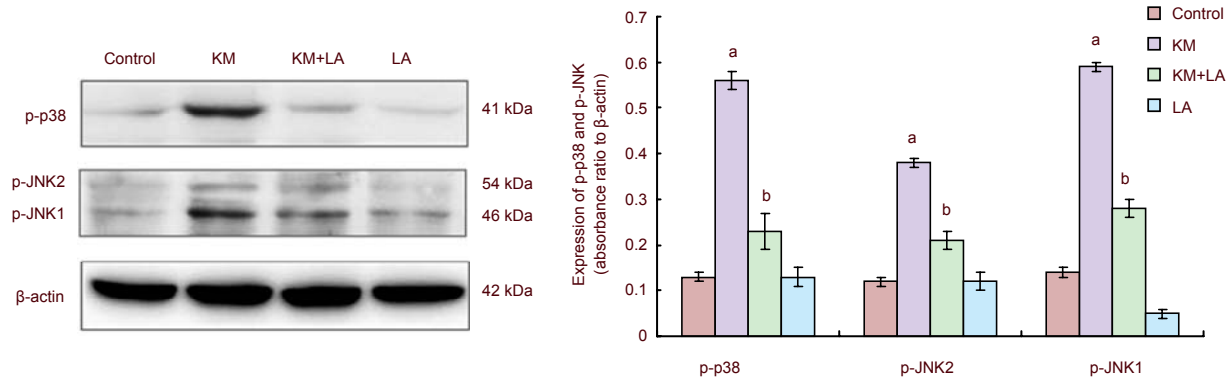



Figure 3 Expression of phosphorylated p38 (p-p38) and phosphorylated c-Jun N-terminal kinase (p-JNK) in mouse cochlea (western blot assay).

The expression of p-p38 and p-JNK in the control group was weak, while in the kanamycin (KM) group it was significantly strong. The expression of p-p38 and p-JNK in the KM + alpha-lipoic acid (LA) group was significantly weaker than in the KM group.

Data are expressed as mean ± SD, nine mice in each group and six cochleae as a specimen. One-way analysis of variance followed by least significant difference *t*-test were used to compare the data of different groups. $^{a}P < 0.01$, *vs*. control group; $^{b}P < 0.01$, *vs*. KM group.

## DISCUSSION

Kanamycin, which is a common aminoglycoside antibiotic for clinical use, can produce ototoxicity in mice. In the present study, after healthy BALB/c mice aged 4 weeks were given subcutaneous injection of kanamycin for 14 days, the auditory brainstem response threshold shifts were significantly elevated, and the threshold shifts at 24 kHz were higher than at 8 kHz. These results were consistent with previous findings[26-27], indicating that kanamycin-induced hearing injury in mice followed the common characteristics of aminoglycosides' ototoxicity, and developed in the order of high frequency to low frequency. After concurrent injection with alpha-lipoic acid, the auditory brainstem response threshold shifts were significantly decreased, demonstrating that alpha-lipoic acid could effectively protect mice from kanamycin-induced hearing injury. In addition, we found that alpha-lipoic acid alone had no effect on auditory brainstem response threshold shifts in mice, indicating that alpha-lipoic acid is safe and non-toxic, and thus may serve as a potential effective drug protecting against aminoglycoside-induced ototoxicity.

Jiang *et al* [28] reported that after treatment with kanamycin, the expression of the lipid peroxidation product, 4-hydroxynonenal, in the mouse cochlea was significantly increased, indicating that kanamycin can induce production of excessive reactive oxygen species. Kanamycin-induced production of reactive oxygen species causes reactive oxygen species accumulation, which then attack the cochlear hair cells, inducing cell membrane lipid peroxidation, and eventually leading to irreversible injury or death of hair cells, resulting in hearing disorders[28]. Alpha-lipoic acid is a naturally occurring dithiol compound, which possesses powerful antioxidant roles. It can scavenge reactive oxygen species and prevent lipid peroxidation[29-31]. Alpha-lipoic acid has both hydrophilic and hydrophobic properties, which allow it to reach any part of the body. Recent research showed that noise exposure caused a decrease in total antioxidant capacity in serum and an increase in nitric oxide levels in guinea pig cochlea, while alpha-lipoic acid could exert a protective effect against hearing loss in acoustic trauma through its antioxidant effects[32]. In addition, alpha-lipoic acid inhibited cisplatin-induced high expression of nicotinamide adenine dinucleotide phosphate hydratenucleotide oxidase 3 to reduce reactive oxygen species production in the rat cochlea, thus attenuating cisplatin-induced hearing loss[33]. In this study, we also observed that alpha-lipoic acid could effectively protect mice from kanamycin- induced hearing impairment, suggesting that alpha-lipoic acid may clear excessive accumulation of reactive oxygen species *in vivo* through its powerful antioxidant action, thus reducing kanamycin-induced hair cell damage in the mouse cochlea to protect auditory function.

Reactive oxygen species can directly attack the cochlear hair cell membrane to damage the hair cell. Furthermore, reactive oxygen species can serve as an initiating factor to activate multiple signal transduction pathways of apoptotic cell death, ultimately resulting in apoptosis of cochlear hair

cells and impairment of auditory function[15, 28, 34]. As important signaling pathways mediating apoptotic cell death, p38 and JNK play critical roles in cochlear hair cell apoptosis induced by noise and ototoxic drugs[5-6]. After intense noise exposure for 4 hours, p-p38 expression significantly increased in the mouse cochlea, and the auditory brainstem response threshold shifts elevated markedly at the same time, suggesting the involvement of the p38 pathway in noise-induced hearing loss[7]. Gentamycin can induce significant hair cell loss in cochlear explants from postnatal rats, and induce high expression of p-p38, indicating that the p38 pathway is also involved in gentamycin ototoxicity[9]. Moreover, gentamycin can also activate the JNK pathway to damage cultured cochlear hair cells from postnatal rats[11]. In addition, amikacin can induce high expression of p-JNK in guinea pig cochlea, and p-JNK expression is highly correlated with hearing loss[12]. These studies demonstrated that the JNK pathway participates in gentamycin and amikacin ototoxicity. In the current study, using immunohistochemistry and western blot analysis, we observed that the positive expression of p-p38 and p-JNK was significantly increased after continuous subcutaneous injection of kanamycin for 14 days, accompanied by a concurrent significant elevation in auditory brainstem response threshold shifts. These results indicate that p38 and JNK also mediate kanamycin-induced ototoxic damage in BALB/c mice. After concurrent treatment with alpha-lipoic acid, the expression of p-p38 and p-JNK was significantly decreased, and the auditory brainstem response threshold shifts attenuated significantly at the same time, suggesting that alpha-lipoic acid can effectively attenuate kanamycin ototoxicity by inhibiting the high expression of p-p38 and p-JNK. This may be one of the mechanisms by which alpha-lipoic acid protects against kanamycin ototoxicity.

In conclusion, this study demonstrated that p38 and JNK mediated kanamycin-induced ototoxic injury in BALB/c mice. Alpha-lipoic acid effectively attenuated kanamycin ototoxicity by inhibiting the kanamycin-induced high expression of p-p38 and p-JNK, thus protecting against kanamycin ototoxicity and improving hearing function.

## MATERIALS AND METHODS

### Design

A randomized, controlled animal experiment.

### Time and setting

This experiment was performed at the Department of Physiology and Scientific Laboratorial Center, Liaoning Medical University, China from July to December 2011.

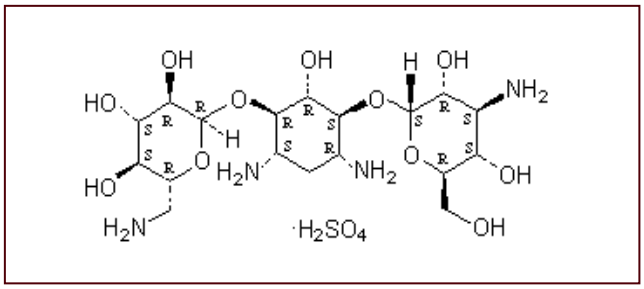
### Materials

#### *Animals*

56 healthy BALB/c mice of both genders, aged 4 weeks and weighing 18–20 g, with normal Preyer's reflexes, were supplied by the Animal Experimental Center, Dalian Medical University, China (license No. SCXK (Liao) 2008-0002). Mice were given free access to water and a regular mouse diet at room temperature, with natural light cycle and quiet conditions. Experimental procedures were performed in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, issued by the Ministry of Science and Technology of China[35].

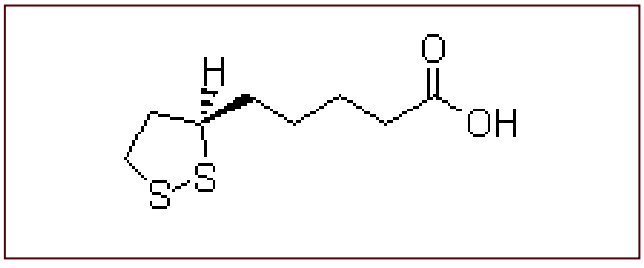
#### *Drugs*

Kanamycin sulfate chemical formula is $C_{18}H_{36}N_4O_{11}•H_2SO_4$, and the chemical structural formula is as follows:



It was purchased from Amresco Corporation (Solon, OH, USA) and dissolved in sterile physiological saline for injection.

Alpha-lipoic acid chemical formula is $C_8H_{14}O_2S_2$, and the chemical structural formula is as follows:



It was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in 0.1 M sodium hydroxide solution and then balanced to pH 7.4 by titrating with 1.0 M HCl solution[36].

### Methods

#### *Establishment of a kanamycin-induced ototoxicity model and alpha-lipoic acid intervention*

Mice in the kanamycin group were subcutaneously

injected with freshly prepared kanamycin (750 mg/kg administered in 0.02 mL/g[37]). Mice in the alpha-lipoic acid group were subcutaneously injected with freshly prepared alpha-lipoic acid at 75 mg/kg. Mice in the kanamycin plus alpha-lipoic acid group were simultaneously given subcutaneous injections of kanamycin (750 mg/kg) and alpha-lipoic acid (75 mg/kg). Control mice received sterile physiological saline (0.02 mL/g). These groups were given injections twice daily for 14 days[27].

### *Auditory brainstem response test*

Auditory thresholds were measured for each animal prior to the beginning of the study and 1 day after the end of drug treatments. Auditory brainstem response measurements were performed in a sound-isolated and electrically shielded booth. The mice were anesthetized with an intraperitoneal injection of pentobarbital sodium (90 mg/kg). The positive needle electrode was subdermally inserted at the midst vertex. The negative electrode was placed below the pinna of the test ear, and the ground electrode was inserted contralaterally[38-39]. Smart EP & OAE auditory evoked potential recording system (Intelligent Hearing Systems Co, Miami, FL, USA) was used to generate tone bursts of 8, 12 and 24 kHz, and record auditory brainstem response thresholds. Up to 1 024 responses were averaged for each stimulation frequency. The threshold was determined by reducing the sound intensity in 5 dB intervals from 95 dB SPL to the threshold. Thresholds were defined as the lowest intensity that yielded a reproducible wave I and were verified at least twice. Threshold shifts were calculated for each animal by comparison to its threshold before treatment.

### *Immunohistochemical staining for the expression of p-p38 and p-JNK in mouse cochlea*

After the last auditory brainstem response test, five mice from each group were decapitated under anesthesia, and the temporal bones were removed immediately. The round and oval windows were opened, then cochleae were perfused with 40 g/L paraformaldehyde (pH 7.4), and the specimens were immersed in the same fixative solution for 24 hours at 4°C. Following decalcification in 40 g/L EDTA solution (pH 7.4) for 5 days at 4°C, the cochleae were dehydrated with ethanol, cleared by xylene, and then embedded in paraffin wax. Serial sections (5 μm thickness) were prepared, dewaxed, hydrated, rinsed with distilled water and treated with high pressure to retrieve antigen. Endogenous peroxidase activity was blocked with 3 g/L $H_2O_2$ for 10 minutes at room temperature, followed by a PBS wash. After incubation with normal serum for 15 minutes at room temperature, sections were incubated with rabbit anti-p-p38MAPK polyclonal antibody (1:500; Beijing Biosynthesis Biotechnology Co., Ltd., China) and rabbit anti-p-JNK polyclonal antibody (1:200; Beijing Biosynthesis Biotechnology) overnight at 4°C and rinsed with PBS. The sections were incubated with biotinylated goat anti-rabbit IgG (1:1; Beijing Zhong Shan-Golden Bridge Biological Technology Co., Ltd., China) for 15 minutes at 37°C and rinsed with PBS. Subsequently, sections were incubated with horseradish peroxidase-labeled streptavidin-biotin complex (1:1; Beijing Zhong Shan-Golden Bridge Biological Technology) for 15 minutes at 37°C and rinsed with PBS. The chromogen- reaction was performed with diaminobenzidine at room temperature and rinsed with distilled water to stop the reaction. Finally, sections were counterstained with hematoxylin for nuclear staining. Sections were dehydrated by ethanol, cleared by xylene, mounted with neutral gum, and observed by a Zeiss A1 microscope (Carl Zeiss Inc., München, Germany). For control sections, the immunostaining procedure was the same except they were incubated with PBS. Ten sections from each group were randomly selected and the average gray value of p-p38 and p-JNK immunostaining in the outer hair cells, spiral ganglion and stria vascularis of each section was measured under identical conditions by the CIAS-1000 image analysis system (Daheng Group, Beijing Image Vision Technology Branch Company, China). The lower the average gray value, the stronger was the expression of p-p38 and p-JNK[40].

### *Western blot analysis of p-p38 and p-JNK expression in mouse cochlea*

After the last auditory brainstem response test, nine mice from each group were decapitated under anesthesia, and the temporal bones were removed immediately. Cochleae were rapidly removed and dissected in 10 mM HEPES-NaOH buffer solution. Cochlear tissues from three mice were pooled and grinded in ice-cold RIPA lysis buffer (Shanghai Beyotime Biotechnology Ltd, China) by ultrasonication for 30 seconds at 0°C. Then, tissue debris was removed by centrifugation at 12 000 r/min for 25 minutes at 4°C. The supernatants were obtained and the protein contents were measured. The protein samples (50 μg) were separated by SDS-PAGE. After electrophoresis, the proteins were transferred onto polyvinylidene difluoride membranes and blocked with 10% bovine serum albumin for 1 hour at 4°C. The membranes were incubated with rabbit anti-p-p38MAPK polyclonal antibody (1:200), rabbit anti-p-JNK polyclonal antibody (1:200), and rabbit anti-β-actin polyclonal antibody (1:500; Beijing Zhong Shan-Golden Bridge

Biological Technology) overnight at 4°C. After washes with Tris-buffered saline containing Tween-20, membranes were incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (1:1 000; Beijing Zhong Shan-Golden Bridge Biological Technology) for 1 hour at 4°C. Following a final wash with Tris-buffered saline containing Tween-20, the immunoreactive bands were visualized by enhanced chemiluminescence reagent. The iBox 600 gel imaging analysis system (UVP Co, Upland, CA, USA) was used to analyze the electrophoresis bands. p-JNK1 and p-JNK2 represented the activation products of p-JNK, and β-actin was used as an internal loading control. The absorbance ratio of p-p38 or p-JNK to β-actin represented p-p38 and p-JNK protein expression levels.

### *Statistical analysis*

Data were presented as mean ± SD, and were analyzed using SPSS 16.0 software (SPSS, Chicago, IL, USA). Intergroup differences in mean values were compared by one-way analysis of variance and least significant difference *t*-test. A $P < 0.05$ value was considered statistically significant.

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**Author contributions:** Aimei Wang designed the study and wrote the manuscript. Ning Hou and Tao Xu conducted the experiments. Dongyan Bao revised the manuscript. Shuangyue Liu analyzed the data. All authors approved the final version of the manuscript.

**Conflicts of interest:** None declared.

**Ethical approval:** This study was approved by the Animal Ethics Committee, Liaoning Medical University, China.

**Author statements:** The manuscript is original, has not been submitted to or is not under consideration by another publication, has not been previously published in any language or any form, including electronic, and contains no disclosure of confidential information or authorship/patent application/funding source disputations.

## REFERENCES

[1] Qin Q, Niu J, Wang Z, et al. Astragalus membranaceus inhibits inflammation via phospho-p38 mitogen-activated protein kinase (MAPK) and nuclear factor (NF)-κB pathways in advanced glycation end product-stimulated macrophages. Int J Mol Sci. 2012;13(7):8379-8387.

[2] Cuadrado A, Nebreda AR. Mechanisms and functions of p38 MAPK signalling. Biochem J. 2010;429(3):403-417.

[3] Li ZH. JNK signal pathway. Guoji Bingli Kexue yu Linchuang Zazhi. 2010;30(3):273-276.

[4] Chen F. JNK-induced apoptosis, compensatory growth, and cancer stem cells. Cancer Res. 2012;72(2):379-386.

[5] Vacotto M, Coso O, Fiszer de Plazas S. Programmed cell death and differential JNK, p38 and ERK response in a prenatal acute hypoxic hypoxia model. Neurochem Int. 2008;52(4-5):857-863.

[6] Jung E, Byun S, Kim S, et al. Isomenthone protects human dermal fibroblasts from TNF-α-induced death possibly by preventing activation of JNK and p38 MAPK. Food Chem Toxicol. 2012;50(10):3514-3520.

[7] Tabuchi K, Oikawa K, Hoshino T, et al. Cochlear protection from acoustic injury by inhibitors of p38 mitogen-activated protein kinase and sequestosome 1 stress protein. Neuroscience. 2010;166(2):665-670.

[8] Xue Q, Chen J, Gong S, et al. Study of the effect of JNK signal transduction pathway in intense noise-induced apoptosis in cochlea of guinea pig. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2009;23(24):1138-1142.

[9] Jeong SW, Kim LS, Hur D, et al. Gentamicin-induced spiral ganglion cell death: apoptosis mediated by ROS and the JNK signaling pathway. Acta Otolaryngol. 2010; 130(6):670-678.

[10] Nakamagoe M, Tabuchi K, Uemaetomari I, et al. Estradiol protects the cochlea against gentamicin ototoxicity through inhibition of the JNK pathway. Hear Res. 2010;261(1-2):67-74.

[11] Choi BM, Chen XY, Gao SS, et al. Anti-apoptotic effect of phloretin on cisplatin-induced apoptosis in HEI-OC1 auditory cells. Pharmacol Rep. 2011;63(3):708-716.

[12] Liu SY, Wang AM, Xu T, et al. Effect of amikacin on the expression of phosphorylated JNK in hair cells of guinea pig cochlea. Zhongguo Laonian Xue Zazhi. 2011;31(7): 1209-1211.

[13] Taleb M, Brandon CS, Lee FS, et al. Hsp70 inhibits aminoglycoside-induced hearing loss and cochlear hair cell death. Cell Stress Chaperones. 2009;14(4):427-437.

[14] Yu L, Tang H, Jiang XH, et al. Involvement of calpain-I and microRNA34 in kanamycin-induced apoptosis of inner ear cells. Cell Biol Int. 2010;34(12):1219-1225.

[15] Yu L, Tang H, Wang AM. Apoptosis pathway in mouse cochlear due to kanamycin. Guangdong Yixue. 2011; 32(1):31-33.

[16] Taylor RR, Nevill G, Forge A. Rapid hair cell loss: a mouse model for cochlear lesions. J Assoc Res Otolaryngol. 2008;9(1):44-64.

[17] Szczepanik W, Kaczmarek P, Jezowska-Bojczuk M. Oxidative activity of copper(II) complexes with aminoglycoside antibiotics as implication to the toxicity of these drugs. Bioinorg Chem Appl. 2004:55-68.

[18] Sha SH, Zajic G, Epstein CJ, et al. Overexpression of copper/zinc-superoxide dismutase protects from kanamycin-induced hearing loss. Audiol Neurootol. 2001; 6(3):117-123.

[19] Zhuravskii SG, Aleksandrova LA, Sirot VS, et al. Natural antioxidant L-carnosine inhibits LPO intensification in structures of the auditory analyzer under conditions of chronic exposure to aminoglycoside antibiotics. Bull Exp Biol Med. 2004;138(4):361-364.
[20] Wang AM, Sha SH, Lesniak W, et al. Tanshinone (Salviae miltiorrhizae extract) preparations attenuate aminoglycoside-induced free radical formation in vitro and ototoxicity in vivo. Antimicrob Agents Chemother. 2003; 47(6):1836-1841.
[21] Gorąca A, Huk-Kolega H, Piechota A, et al. Lipoic acid - biological activity and therapeutic potential. Pharmacol Rep. 2011;63(4):849-858.
[22] Wang L, Weng CY, Wang YJ, et al. Lipoic acid ameliorates arsenic trioxide-induced HO-1 expression and oxidative stress in THP-1 monocytes and macrophages. Chem Biol Interact. 2011;190(2-3):129-138.
[23] Mukherjea D, Jajoo S, Whitworth C, et al. Short interfering RNA against transient receptor potential vanilloid 1 attenuates cisplatin-induced hearing loss in the rat. J Neurosci. 2008;28(49):13056-13065.
[24] Pouyatos B, Gearhart C, Nelson-Miller A, et al. Lipoic acid and 6-formylpterin reduce potentiation of noise-induced hearing loss by carbon monoxide: preliminary investigation. J Rehabil Res Dev. 2008;45(7):1053-1064.
[25] Husain K, Whitworth C, Somani SM, et al. Partial protection by lipoic acid against carboplantin-induced ototoxicity in rats. Biomed Environ Sci. 2005;18(3): 198-206.
[26] Wu WJ, Sha SH, Schacht J. Recent advances in understanding aminoglycoside ototoxicity and its prevention. Audiol Neurootol. 2002;7(3):171-174.
[27] Hou N, Wang AM, Bao CF, et al. Effect of α-lipoic acid on kanamycin-induced expression of phosphorylated p38 MAPK in mouse cochlea. Zhongguo Laonian Xue Zazhi. 2012;32(4):761-766.
[28] Jiang H, Sha SH, Schacht J. NF-kappaB pathway protects cochlear hair cells from aminoglycoside-induced ototoxicity. J Neurosci Res. 2005;79(5):644-651.
[29] Hussein A, Ahmed AA, Shouman SA, et al. Ameliorating effect of DL-α-lipoic acid against cisplatin-induced nephrotoxicity and cardiotoxicity in experimental animals. Drug Discov Ther. 2012;6(3):147-156.
[30] Shay KP, Moreau RF, Smith EJ, et al. Alpha-lipoic acid as a dietary supplement: molecular mechanisms and therapeutic potential. Biochim Biophys Acta. 2009; 1790(10):1149-1160.
[31] Golbidi S, Badran M, Laher I. Diabetes and alpha lipoic Acid. Front Pharmacol. 2011;2:69.
[32] Diao MF, Liu HY, Zhang YM, et al. Changes in antioxidant capacity of the guinea pig exposed to noise and the protective effect of α-lipoic acid against acoustic trauma. Shengli Xuebao. 2003;55(6):672-676.
[33] Mukherjea D, Jajoo S, Kaur T, et al. Transtympanic administration of short interfering (si)RNA for the NOX3 isoform of NADPH oxidase protects against cisplatin-induced hearing loss in the rat. Antioxid Redox Signal. 2010;13(5):589-598.
[34] Jiang H, Sha SH, Forge A, et al. Caspase-independent pathways of hair cell death induced by kanamycin in vivo. Cell Death Differ. 2006;13(1):20-30.
[35] The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30.
[36] Chaudhary P, Marracci G, Yu X, et al. Lipoic acid decreases inflammation and confers neuroprotection in experimental autoimmune optic neuritis. J Neuroimmunol. 2011;233(1-2):90-96.
[37] Li Y, Wang AM, Bao DY, et al. Effects of Kanamycin on auditory brainstem response and distortion product otoacoustic emission in Kunming mice. Zhongguo Xiandai Yixue Zazhi. 2009;19(9):1339-1345.
[38] Sun WX, Wang AM, Bao CF, et al. Effect of cisplatin on apoptosis of spiral ganglion cell and expression of caspase-3 in mouse cochlea. Zhongguo Yingyong Shengli Xue Zazhi. 2010;26(3):324-328.
[39] Sun WX, Wang AM, Bao CF, et al. Effect of cisplatin on ototoxicity and expression of caspase-3 in mouse cochlea. Xi'an Jiaotong Daxue Xuebao: Yixue Ban. 2009;30(5): 616-619.
[40] Wang AM, Tang H, Bao DY, et al. Effects of salvia miltiorrhiza injection on gentamicin-induced expression of nitric oxide synthase isoforms in guinea pig cochlea. Zhongguo Yingyong Shengli Xue Zazhi. 2011;27(2): 246-249.

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