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Research article

Minocycline attenuates noise-induced hearing loss in rats

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HIGHLIGHTS

- Minocycline attenuates the elevation of ABR thresholds induced by noise exposure in rats.
- Minocycline attenuates hair cell loss induced by noise exposure.
- Minocycline is otoprotective in an experimental model of NIHL.

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ABSTRACT

Noise-induced hearing loss (NIHL) is a serious health concern and prevention of hair cell death or therapeutic intervention at the early stage of NIHL is critical to preserve hearing. Minocycline is a semi-synthetic derivative of tetracycline and has been shown to have otoprotective effects in ototoxic drug-induced hearing impairment, however, whether minocycline can protect against NIHL has not been investigated. The present study demonstrated elevated ABR (auditory brainstem response) thresholds and outer hair cell loss following traumatic noise exposure, which was mitigated by intraperitoneal administration of minocycline (45 mg/kg/d) for 5 consecutive days. In conclusion, the present study demonstrated that minocycline, a clinically approved drug with a good safety profile, can attenuate NIHL in rats and may potentially be used for treatment of hearing loss in clinic.

1. Introduction

Noise is pervasive in daily life and noise-induced hearing loss (NIHL) has become a serious public health concern [1]. According to the WHO, it is estimated that about 10% of the word population is exposed to high noise levels that could potentially cause NIHL. The loss of hair cells is one of the major causes of hearing loss in NIHL. As mammalian hair cells cannot regenerate, the prevention of hair cell death or therapeutic intervention at the early stage of NIHL is critical to preserve hearing. It has been suggested that oxidative stress plays an important role in the NIHL [10,15], and both caspase-dependent and -independent pathways are involved in the impairment of cochlear hair cells induced by noise trauma [9,15,30].

Minocycline is a semi-synthetic derivative of tetracycline and has both anti-inflammatory and neuroprotective properties [7]. Minocycline can cross the blood-brain barrier [5], and protect neurons through antioxidant actions [4,12,17]. Minocycline, which exerts no ototoxic effects [11], has also been shown to have protective effects in ototoxic drug-induced hearing impairment, as minocycline can attenuate gentamycin-induced hair cell loss in vivo and in vitro [6,26]. Minocycline can also protect from neomycin-induced hearing loss [16,22] and attenuate the ototoxicity of cisplatin [8,13]. However, it remains to be investigated whether minocycline had otoprotective effects in NIHL.

It has been shown that oxidative stress plays important roles in both ototoxic drugs-induced hearing loss and NIHL, and minocycline can attenuate the adverse effects of ototoxic drugs, like aminoglycosides and cisplatin, through antioxidant actions. The present study hypothesized that minocycline could attenuate NIHL. The otoprotective effects of minocycline in NIHL were determined using auditory brainstem response (ABR) and a morphological analysis of the loss of hair cells induced by a noise trauma.

2. Materials and methods

2.1. Animals

All experiments were performed on male Sprague Dawley rats (180–250 g, 8–10 weeks old) obtained from the Laboratory Ani-

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mal Center of Fourth Military Medical University (FMMU), Xi'an Shaanxi Province P.R. China. The animals were maintained under standard laboratory conditions (12 h dark/light circles, temperature 22–26 °C, air humidity 40–60%) with food and water available *ad libitum*. The experimental protocols were approved by the Institutional Animal Care and Use Committee of FMMU. The rats were randomly divided into four groups (n = 10 per group): (1) Control, experimentally naïve rats that were exposed to the ambient sound levels (50–60 dB sound pressure level, measured with sound level meter), without any noise exposure; (2) Noise, rats that were exposed to intensive noise exposure; (3) Noise + Saline, rats that received noise exposure and intraperitoneal injection of sterile physiological saline; (4) Noise + Minocycline, rats that received noise exposure and intraperitoneal injection of minocycline (45 mg/kg, dissolved in sterile physiological saline) (Sigma, USA). Minocycline or saline were administered immediately following noise exposure (within 1 h) and were injected continuously for 5 days.

2.2. Noise exposure

The noise exposure was conducted in a sound insulated room. The animals were individually placed in metal wire-cages with free access to food and water during the one week acclimation to the sound insulated room where the noise exposure was conducted. During noise exposure, pellets were provided on the mesh floor of the cage and tips of the water bottle were located on the flank of the cage, and there were no objects between the speakers and the rats' ears. The noise (4 kHz octave band, 120 dB sound pressure level) was generated from a RadioShack Supertweeter located above the cages and was amplified with a power amplifier (Yamaha AX-500U, Japan) and a loud speaker. The noise level was monitored with a sound level meter (Bruel and Kjaer, Typer 2606), with a variation less than 2 dB across the space available to animals. The animals were exposed to noise 3 h per day for 2 days.

2.3. ABR

The ABR thresholds in rats were measured at 1 day and 14 days after 2 days of noise exposure. Under light anesthesia with sodium pentobarbital (40 mg/kg), the active, reference and ground needle electrodes were inserted beneath the skin at the vertex, the mastoid area of the test ear and the contralateral mastoid, respectively. The TDT III system auditory evoked potential work station was used for sound generation, presentation and data acquisition, which is controlled by SigGenRZ and BioSigRZ software (Tucker-Davis Technologies, Fort Lauderdale, FL, USA). The ABR was elicited by tone bursts (8, 16, 32 kHz; 0.5-millisecond rise/fall time, no plateau, alternating phase) or broadband clicks (10 milliseconds) presented at 21.97 s^{-1}. The stimulus was played through a high frequency speaker (model: MF1 Multi-Field Magnetic Speakers) located approximately 2 cm in front of the test ear. The intensity of stimulus was decreased in 5-dB steps until the evoked responses disappeared. The differential potential was sampled over 10 milliseconds, filtered (low-pass, 4 kHz; high pass, 100 Hz) and averaged (512 sweeps of alternated stimulus polarity) to obtain mean traces at each intensity [21]. The lowest intensity being able to elicit a two-phase waveform from 5 to 15 milliseconds after the signal onset was considered as the ABR threshold.

2.4. Hair cell counts

After receiving different treatments, the animals were deeply anesthetized with pentobarbital (50 mg/kg, i.p.) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. The cochleae on both sides were removed and then locally perfused through the open round window and the cochlear apex and post-fixed in the same fixative overnight. Following the removal of the bony capsule, the spiral ligament, stria vascularis, and Reissner's membrane were separated under a microscope. Each turn of the basilar membrane was detached from the bony modiolus. The sensory epithelium was trimmed, and the surface preparations were stained for actin using CytoPainter Phalloidin-iFluor 680 Reagent (Abcam, UK). Sensory structures of the surface epithelium were examined for missing cells under a fluorescence microscope (Olympus, Japan). The percentage of missing outer hair cells (OHCs) along the entire basilar membrane was calculated.

2.5. Statistical analysis

All data were expressed as mean ± SEM. One-way ANOVA (followed by post-hoc Fisher's LSD – least significance difference) and two-way ANOVA (repeated measures) were used to analyze mean differences when necessary. $P < 0.05$ was considered to be of statistical significance.

3. Results

3.1. Minocycline attenuates noise-induced elevation of ABR threshold

Before noise exposure, the baseline ABR thresholds across all four groups were measured and the results did not show any difference. One day following the noise exposure, the ABR thresholds of the Noise, Noise + Saline and Noise + Minocycline groups were all elevated significantly compared to Control group (n = 10 per group; $P < 0.001$) (Fig. 1A, upper panel), whereas no difference was detected among these groups, suggesting the noise exposure induced similar hearing loss in these groups.

Fourteen days after the noise exposure, the ABR thresholds of the Noise, Noise + Saline and Noise + Minocycline groups were still higher than that in the Control group (n = 10 per group; $P < 0.001$ for Noise and Noise + Saline groups; $P < 0.01$ for Noise + Minocycline group), however, the ABR thresholds of the Noise + Minocycline group were significantly lower than that in the Noise + Saline group ($P < 0.01$, e.g. the average threshold shift of Noise + Saline group was 14.5 ± 1.0 dB [32 kHZ] higher than that of Noise + Minocycline group.) (Fig. 1A, lower panel), implicating that minocycline can attenuate the noise-induced hearing loss.

3.2. Minocycline attenuates noise-induced loss of OHCs

In Control group, the structure of the organ of Corti of rats was well maintained (Fig. 2A). However, in all noise exposure groups (Noise, Noise + Saline and Noise + Minocycline), missing OHCs were detected along the basilar membrane (Fig. 2B–D) and with a higher percentage in the Noise and Noise + Saline groups than that in the Noise + Minocycline group in the basal turn of the cochlea (n = 10 per group) (Fig. 3), indicating that minocycline can attenuate noise-induced OHCs loss.

4. Discussion

The present study demonstrated that the ABR thresholds were elevated and the OHCs were missing following noise exposure, and intraperitoneal administration of minocycline attenuated noise-induced elevation of ABR thresholds and loss of OHCs, indicating that minocycline has otoprotective effects in NIHL and could potentially be used as a therapeutic drug for treatment of noise-induced hearing loss.