The Effect of *Gingko Biloba* on Hearing in Mice with Noise-Induced Temporary Threshold Shift

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**Background and Objectives:** *Gingko biloba* extract is known for enhancing blood circulation, scavenging free radicals, and antagonizing against platelet-activating factor. This study evaluated the effect of *Gingko biloba* on the noise-induced temporary threshold shift of hearing. **Materials and Methods:** Temporary threshold shift was induced by exposing mice to 110 dB SPL sound for 1 hour. The experimental group consisted of mice fed *Gingko biloba* [3 mg/kg, 6 mg/kg, and 12 mg/kg in 0.5% carboxymethyl cellulose (CMC)] for 7 days before noise exposure. CMC solution without *Gingko biloba* was fed to control mice. Hearing threshold was measured by auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE). **Results:** The hearing threshold increased after noise exposure and recovered to normal within 5 days in all groups. Compared to control mice (fed CMC solution only), mice fed *Gingko biloba* showed more rapid recovery of ABR threshold at 16 kHz in all three experimental groups. At the other frequencies, there was no significant change in hearing recovery in the *Gingko biloba* groups. There was no difference in DPOAE between groups. **Conclusions:** Temporary threshold shift of hearing after noise exposure was partly affected by oral *Gingko biloba*. **KEY WORDS:** Hearing loss · Noise-induced · Temporary threshold shift · *Gingko biloba* · Hearing.
Laboratory equipment

The booth to generate noise was soundproof. We connected a speaker (290-8L, ALTEC LANSING, Oklahoma City, OK, USA) and an amplifier (R-399, INTER M, Seoul, Korea) with input and output resistances of 8 Ω, respectively. The amplifier was placed in the left corner of the noise booth with a speaker on it, and the horn was attached at an angle of 45 degrees.

Experimental animal anesthesia

Zolazepam/tiletamine (Zoletil) 25 mg/kg and xylazine (Rompun) 10 mg/kg were administered intraperitoneally for anesthesia, and half of the above amounts were added if necessary.

Temporary threshold shift (TTS) induction experiment

We exposed mice with normal hearing to broad band white noise of 110 dB SPL for 1 hour to induce TTS. We administered Gingko biloba once a day for 7 days before noise exposure, and the dose was set to the adult standard dose, i.e., 3 mg/kg powder. The animals were divided into four groups, a control group that did not receive Gingko biloba, a standard dose group, a double (6 mg/kg) dose group, and a quadruple (12 mg/kg) dose group. For each experiment, we used one animal as a control and three animals in each remaining group. Then, we repeated the experiment three times using the same experimental protocol; therefore, we used three control animals and nine animals in each of the other groups.

Gingko biloba

We used SK Chemical’s Gingko leaf extract powder products and mixed them with 0.5% carboxymethyl cellulose solution.

Protection effect for the temporary threshold shift model

For measurement of hearing level, we used ABR and distortion-product otoacoustic emission (DPOAE) with an auditory evoked potential workstation (Tucker-Davis Technologies, Alachua, FL, USA). Tone burst stimulation was used to measure frequency-specific ABR. The frequencies measured were 4, 8, 16, and 32 kHz, and we gained the waveform by repeatedly decreasing the stimulation tone by 5 dB from the strength of 90 dB HL. The most uniformly generated waveform was used for analysis. We judged a waveform with an amplitude over 0.2 μV that appeared to be similar to the reaction from the previous stimulation tone as a significant waveform. We determined the minimum stimulation level with a significant signal as the hearing threshold and then calculated the hearing threshold shift before and after noise exposure. DPOAE was measured using an amplifier system that could provide two stimulation tones. We generated f1 and f2 primary stimulation tones (f2/f1=1.2) by using a dual channel synthesizer. Sounds were introduced to a mouse through an acoustic probe (ER-10B+) attached to the external auditory meatus. The emission sound from the microphone attached to the probe was collected at the speed of 44100 Hz and analyzed with a 4096 point fast Fourier transform. For the measurement of frequency-specific responses, we set the F2 stimulation tone to 4, 5.6, 8, 11.3, and 16 kHz. Threshold was determined when DPOAE response exceeded the noise level on the measured graph at each frequency.

All the measured values were statistically validated with the t-test. A p value less than 0.05 was considered significant.

Results

Both the experimental groups and the control group showed a threshold increase of hearing level immediately after noise exposure, and began to slowly recover. The ABR threshold in the control group increased at all frequencies after noise exposure, showing a large difference from the resting hearing threshold, but it recovered to the resting hearing level by day 5. In the Gingko biloba groups, the hearing level increased after noise exposure and recovered to the resting level at 4 kHz. The hearing levels at both 8 kHz and 16 kHz of the 6 mg/kg and 12 mg/kg groups were better than the hearing level of the control group at day 3 after noise exposure. The hearing threshold at 32 kHz was different only in the 6 mg/kg group on day 1 after exposure. On day 7 after exposure, groups that received the drug showed no difference from the control group at all frequencies (Fig. 1).

The DPOAE thresholds of Gingko biloba groups were better than that of the control group on day 7 after noise exposure at 4 kHz. However, there were no statistical differences in hearing levels among groups at other frequencies (Fig. 2).

Discussion

Many theories have been suggested for the pathology of noise-induced inner ear damage, and drug administration experiments and treatments are conducted on the basis of such theories. A decrease of cochlear blood flow with hypoxia-induced oxidative stress and permanent hearing loss have been proposed for a long time. This concept proposes that blood flow into the inner ear is reduced by noise, and subsequently, the inner ear becomes deprived of oxygen. A mechanism involving an increase of oxygen consumption in the tissue without regard to blood flow decrease has also been suggested. Mitochondrial dysfunction, excitotoxicity due to glutamate increase, and reduction of glutathione are known to be in-
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Fig. 1. ABR thresholds are increased at all frequencies after noise exposure that recovered by 5 days to control (control n=3, Gingko n=9 at each dose). At 8 and 16 kHz, gingko-treated mice (6 mg/kg and 12 mg/kg) showed a better hearing level at 3 days after noise exposure. At 32 kHz, mice treated with gingko (6 mg/kg) showed a better hearing level one day after noise exposure. At the other frequencies, the gingko-treated group did not show a significant change of thresholds (*p<0.05 compared to the control group). ABR: auditory brainstem response.

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Fig. 2. DPOAE thresholds are increased at all frequencies after noise exposure that recovered by 5 days to control (control n=3, Gingko n=9 at each dose). The gingko-treated group shows a better threshold at 7 days after noise exposure at 4 kHz. At the other frequencies, there was no significant difference between experimental and control groups (*p<0.05 compared to the control). DPOAE: distortion product otoacoustic emission.

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