Synaptic Mechanisms Underlying A Combinatoin Therapy for Noise-Induced Hearing Loss

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Introduction

Noise is the most common occupational and environmental hazard. Noise-induced hearing loss (NIHL) is the second most common form of sensorineural hearing deficit, after age-related hearing loss (presbycusis). Although there are promising approaches for reducing NIHL, currently there are no effective medications to prevent NIHL. Development of an efficacious treatment has been hampered by the complex array of cellular and molecular pathways involved in NIHL. We have turned this problem into an advantage by asking whether NIHL can be effectively prevented by a combination therapy targeting multiple signaling pathways. We recently found that antiepileptic drugs that block T-type calcium channels have both prophylactic and therapeutic effects for NIHL. NIHL can also be prevented by a down-regulation of glucocorticoid (GC) signaling pathways. Based on this finding, we have tested a combination therapy for NIHL that includes zonisamide, and anticonvulsant, and methylprednisolone, a synthetic GC-drug, in two mouse NIHL conditions (noise, 110 dB sound pressure level (SPL) for 30 minutes, or 81-16 kHz at 100 dB SPL for 120 minutes), which have dramatic changes for permanent threshold shifts (PTS). We first determined the dose-effect for PTS amelioration by administering each drug two hours before the noise exposure. We determined the median effective dose (ED50) for each drug, we have subsequently identified one combination with the strongest synergy against NIHL based on the combination index (CI) method. Currently, we examine whether this synergistic effect is due to the protection of synaptic connections between inner hair cells and spiral ganglion neurons. Thus, this study has not only shown the feasibility of efforts to discover effective drug combinations that act synergistically to prevent permanent NIHL, but will further our knowledge of how these drugs target different pathways involved in NIHL.

Prevention of NIHL by Low Dose Antiepileptic and Synthetic Corticosteroid Drugs: A Synergistic Effect

Figure 1: The prevention protocol used in this study.

Drug Administration Protocols

Day 1
ABR Administration (Injection)
Day 2
Noise Exposure
Day 16
ABR Administration (Histology)
Day 17
Histology

Prevention of NIHL by Antiepileptic Drugs

Figure 2: NIHL prevention by the antiepileptic drugs. (A) ABR thresholds among the control and different dosages of ethosuximide (n=8 for each group); (B) ABR thresholds among the control and different dosages of zonisamide (n=8 for each group).

Detection of Synapses between Inner Hair Cells and Spiral Ganglion Neurons

Figure 3: NIHL prevention by a combination of methylprednisolone and zonisamide at their low dosages. ABR thresholds were about 10 dB lower across four frequencies between the control (blue line) and treated mice (red line) (n=6 for each group; F(2,22)=13.57; p < 0.002). A synergistic effect was found because the Combination Index < 1.

No Protection of Outer Hair Cells

Figure 4: Drugs have on protection for outer hair cells against NIHL. Distortion product otoacoustic emissions (DPOAEs) were used to examine outer hair cells among 2-month-old control CBA/CaJ mice (n=8), noise-exposed (81-16 kHz at 100 dB SPL for 2 hours; n=7), and noise-exposed mice treated with zonisamide (ZO, 120 mg/kg, n=7), demeclocycline (DEX, 0.5 mg/kg, n=6), methylprednisolone (MP, 45 mg/kg, n=3), or both MP (20 mg/kg) and ZO (120 mg/kg) drugs (n=6).

Protection of Synapses between Inner Hair Cells and Spiral Ganglion Neurons

Figure 5: Afferent synapses in mouse cochlea. (A) Triple-staining of presynaptic ribbons (red), post-synaptic GABA-A receptors (green), and inner hair cells (pink). (B) Spatial distribution of afferent synapses within one inner hair cell.

Figure 6: The number of afferent synapses per IHC. (A) The number of presynaptic ribbons per IHC among the control (n=6), noise-exposed (n=5), and noise-exposed mice treated with zonisamide (ZO, n=6) or methylprednisolone (MP, n=6). (B) The number of postsynaptic sites per IHC among the same four groups.

Summary

The median effective dose (ED50) to prevent NIHL for drugs was determined from two different drug families: methylprednisolone (525 mg/kg) and demeclocycline (3.4 mg/kg) from the corticosteroids; and zonisamide (125 mg/kg) from the antiepileptic drug family. Due to the unpredictable nature of ethosuximide, no ED50 was found.

A low dose combination of methylprednisolone and zonisamide revealed a synergistic effect on preventing NIHL (CI=0.97). The drugs has no protections on outer hair cells based on DPOAE data.

We discovered a novel function for zonisamide: a significant protection of synapses between IHCs and SGNs after noise exposure.

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