The Investigation of Cochlear Inflammatory Process Following Noise Trauma

Abstract
Recent studies have revealed that inflammatory responses occur in inner ear under various damaging conditions including noise-overstimulation. However, the role of involved chemokines and cytokines remains to be further clarified. In addition to the investigation of the inflammatory response-related chemokine change in noise-exposed cochlea, the exploration of a therapeutic or preventive strategy to modulate the noise-induced inflammation is also imperative. Thus, in this study, we evaluated a time-dependent expression of proinflammatory cytokines in noise-exposed mouse cochlea. CBA/J mice were exposed to white band noise, 115 dB SPL, 3 hr per day continuously for 3 days. Using Bio-Plex Suspension Assay System, cochlear lymphatic fluid of noise-exposed and control groups were collected for determining the level of cytokine and chemokine. Our data showed that IL-1a, IL-6, IL-12(p40), G-CSF, MIP-2, MCP-1 and RANTES were up-regulated after noise exposure. A small number of PMNs and lymphocytes were also observed in post noise-exposed cochlear spiral ligaments. Congestion of the stria vascularis and vessel hemorrhage in spiral ligaments are consistently observed in noise-exposed cochlea. Further experiments will be focused on evaluating whether pharmacotherapy applied in cochlea can modulate the noise-related inflammatory process.

Specific Aims
1. The expression level of cytokine and chemokine in cochleae after noise exposure
2. The histological changes of cochleae following noise exposure

Materials and Methods

Animals and Noise exposure
CBA/cd mice (4–8 weeks old, weighing 20–25 g) were randomly assigned to noise exposure or control groups. Noise exposure setting was white band noise, 115 dB SPL, 3 hours per day for 1–3 days.

Hemotxlin-Eosin stain
To make paraffin sections suitable for light microscopic examination the slides were stained with hemotxlin and eosin. After staining, the specimen is then passed through xylene to nonaqueous mounting medium and covered with a coverslip to obtain a permanent preparation.

Cochlea fluid extraction and Bio-Plex suspension assay
Cochlear fluid obtained from noise-exposed and control groups were analyzed by determining the level of cytokine and chemokine using Bio-Plex Suspension Assay.

Gene expression
Cochlear mRNA was isolated at various time point following noise-exposed groups and control groups were analyzed by determining the cytokine and chemokine transcriptional level using Real-time PCR.

Results

Figure 1: Light microscopic changes in the lateral wall seen in the upper basal turn after exposure 115 dB.
A: control cochlea reveals normal stria vascularis structure.
B: noise-exposed cochlea shows congestion of stria vascularis with increased microvascular permeability and red blood cell infiltrations.

Figure 2: Light microscopic examination shows increased microvascular permeability (A) and red blood cell extravasation (B, arrow) in the spiral ligament of noise-exposed cochleae.

Figure 3: IA-6f Surface preparations show significant hair cell loss in noise-exposed cochleae. (D) Light microscopic examination shows lymphocytes appear in spiral ligament (arrows) following noise-exposure for 3 days.

Figure 4: Cytokines and Chemokines detection by Bio-Plex Suspension Assay after noise exposure
We observed that protein level of IL-1a, G-CSF, MIP-2, MCP-1 were up-regulated after noise exposure for one time, and protein level of IL-1a, IL-6, IL-12(p40), G-CSF, MIP-2, MCP-1 and RANTES were up-regulated after 3 continuous noise exposures.

Conclusion
1. Noise exposure to cochlea induced inflammatory cytokines and chemokines up-regulation.
2. Further experiments will be focused on evaluating whether pharmacotherapy applied in cochlea can modulate the noise-related inflammatory process.

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