**Abstract.** Sensorineural hearing loss, which is limited to the cochlear basal turn, due to acute or chronic otitis media has been reported in clinical and experimental studies. In the present study, the effect of intratympanic dexamethasone on endotoxin-induced cochlear damage was investigated by measuring the cochlear blood flow and hearing.

**Materials and Methods:** Ten male Sprague-Dawley rats were inoculated with lipopolysaccharide (LPS), and divided into 2 groups of five rats each. One hour after intratympanic inoculation, the group A received 40 μl of phosphate-buffered saline (PBS) in the right middle ear cavity, group B received 40 μl of dexamethasone. The treated animals were examined 24 h after inoculation using auditory brainstem response (ABR) and cochlear blood flow (CBF). Results: The elevated threshold decreased significantly after intratympanic dexamethasone administration compared to the PBS-treated group. Intratympanic dexamethasone administration to the round window of rats led to a statistically significant increase in CBF compared to the PBS-treated group (p<0.05). The response to round window application of prostaglandin E1 (PGE$_1$) in the dexamethasone-treated group was better than in the PBS-treated group. Conclusion: Our results showed that intratympanic dexamethasone treatment was effective in protecting the function of the cochlea against endotoxin-induced otitis media.

Otitis media in both the acute and chronic form is one of the most common ear diseases in children. Otitis media induces a variety of pathological changes in the middle ear, including hyperemia and edema, granulocyte and monocyte infiltration and irreversible mucosal changes such as the generation of granulation tissue, cholesteatoma formation, tympanosclerosis, and ossicular destruction. Complications of otitis media may occur when the natural defensive barriers of the middle ear are penetrated, permitting infection to spread into adjacent structures. The sensorineural hearing loss, which is limited to the cochlear basal turn, due to acute or chronic otitis media has been reported in clinical and experimental studies (1-4). Especially in children, hearing loss leads to severe damage of development of speech, language, and cognitive functions (5).

The most likely routes of communication between the middle and inner ear are the oval and round windows, blood vessels and lymph vessels. Of these, the round window is the most important soft tissue barrier susceptible to change in its permeability properties as a result of otitis media (6-8). Cochlear disturbances following acute otitis media in animal studies have revealed hair cell loss and cochlear lateral wall damage (9, 10). Cochlear damage caused by bacterial endotoxins can be mediated by a series of events leading to the generation of nitric oxide (NO) (11-13).

Glucocorticoids are potent anti-inflammatory drugs affecting the production of a wide range of inflammatory mediators (14-16). Topical, low concentration (1 mg/mL) corticosteroid therapy has been efficacious in reducing middle ear mucosal inflammation of lipopolysaccharide (LPS) induced otitis media with effusion (14, 17-18). Several prostaglandins (PGs) are vasodilators, including PGE$_1$, PGE$_2$, PGD$_2$ and PGI$_2$. Because the cochlear blood flow (CBF) can be influenced by LPS induced cochlear lateral wall damage, the response of CBF can be inflected by topical administration of PGE$_1$ (10).

In the present study, the effect of intratympanic dexamethasone on LPS induced otitis media with cochlear damage was investigated by measuring the CBF and hearing, and the response to PGE$_1$ was monitored.

**Key Words:** Acute otitis media, sensorineural hearing loss, dexamethasone, topical treatment.
Materials and Methods

**Animals and intratympanic inoculation with LPS.** Ten male Sprague-Dawley rats weighing 250 g each were used. The animals were housed in rooms with a constant temperature of 22 °C, humidity of 50% and an ambient noise level less than 40 decibels (dB). Lyophilized LPS purified from *Pseudomonas aeruginosa* (Sigma-Aldrich Co, St. Louis, USA) was used. A single dose 30 μl of LPS (5 mg/ml) was instilled into the right middle ear cavity by Hamilton syringe with a 27-gauge needle under a surgical microscope. These animals inoculated with LPS were divided into 2 groups of five rats each. One hour after intratympanic inoculation, the group A (n=5) received 40 μl of phosphate-buffered saline (PBS) in the right middle ear cavity and group B (n=5) received 40 μl of dexamethasone (Sinil Co, South Korea). Both LPS and dexamethasone were dissolved in artificial perilymph (137 mM NaCl; 5 mM KCl; 2 mM CaCl₂; 1 mM MgCl₂; 10 mM Hepes and 11 mM glucose; pH adjusted to 7.4). The treated animals were examined 24 h after inoculation using auditory brainstem response (ABR) and CBF measurement.

**Auditory brainstem response (ABR) recordings.** The ABRs were recorded before and 24 h after inoculation under general anesthesia (ketamine 35 mg/kg and xylazine 15 mg/kg intramuscularly) using an evoked potential system (Tucker-Davis Technologies) software and presented through an insert earphone (ER-2, Etymotic Research Ltd., Illinois, USA). Acoustic stimuli consisting of click and 8, 16, and 32 kHz tone bursts were produced. Intensity was expressed in dB sound pressure level (SPL) peak equivalent. The animals were presented with a stimulus intensity series, which was initiated at 90 dB SPL and reached a minimum of 10 dB SPL. The stimulus intensity was progressively lowered in 10 dB decrements. Each average consisted of 500 stimulus presentations, with a 10 ms analysis time. Electrical activity was recorded via a platinum needle electrode inserted into the scalp at the vertex, referenced to another needle electrode in a deep neck muscle. A third needle electrode in the pinna served as a ground. The intensities that appeared to be near threshold were repeated. Threshold was defined as the lowest intensity capable of producing a visually detectable, reproducible ABR response, the values were extrapolated to the nearest 5 dB assuming a log-linear fall-off with intensity. At threshold, the baseline-to-peak amplitude of the largest ABR component was reduced to <10% of that evoked by the 90 dB stimuli.

**Measurement of cochlear blood flow (CBF).** After the ABR recording, a tracheotomy was performed to ensure free breathing. The right femoral artery was cannulated and connected to a pressure transducer (AD Instruments Pty Ltd., Castle Hill, Australia) for arterial blood pressure. Rectal temperature was maintained at 38±1 °C with a servo-regulated heating blanket. The right tympanic bulla was exposed by a ventral approach. It was opened by drilling. After the middle ear mucosa over the bony wall of the cochlea was removed with a cotton pledget, a 1.0 mm needle probe of the laser Doppler blood flow meter (moorLAB™, Moor Instruments Ltd. Devon, UK) was placed on the lateral wall of the basal turn of the cochlea. The CBF output and blood pressure (BP) data were sampled every 20s and analyzed by computer connected with a data acquisition program PowerLab® (AD Instruments Pty Ltd.). The ratio (vascular conductance) of CBF to BP was monitored. And the ratio of normalized CBF in the right ear to that in the left was monitored. The values of CBF, vascular conductance in the left, untreated ear was considered as a baseline for each rat.

To assess the influence of drugs on CBF, PGE₁ (DongAh Pharmacology Co. Korea) which is known to increase the CBF, was applied topically to the round window in vehicle amounts of 10 μl of 1.49x10⁻⁴ μM by a Hamilton syringe after removal of fluid overlying the round window area. The PGE₁ was dissolved into artificial perilymph, as above, before topical application to the round window.

**Statistical analysis.** The data were analyzed using Student’s paired and unpaired t-tests. If the p-value was less than 0.05, the differences were considered to be statistically significant.

Results

**ABR threshold.** The intratympanic LPS inoculation showed ABR threshold elevation. The threshold elevations were larger at higher frequencies. The elevated threshold recovered significantly after intratympanic dexamethasone administration, compared to the PBS treated group (p<0.05) (Figure 1).

**Recovery of CBF.** Intratympanic dexamethasone inoculation to the round window of rats led to a statistically significant increase in CBF compared to the PBS treated group (p<0.05). The recovery rate of vascular conductance compared to the left side was significantly higher in the topical dexamethasone treated group (0.92) than in the PBS treated group (0.75) (p<0.05). The response to round window application of PGE₁ in the dexamethasone treated group was better than in the PBS treated group (Figure 2).

Discussion

Direct intratympanic administration of dexamethasone may result in otopathy and the concentration of dexamethasone reaching the inner ear is difficult to control (19). Dexamethasone acts mainly as a glucocorticoid, not a mineralocorticoid, since it has been observed recently that its action is mediated through the glucocorticoid receptor, especially in the early phase. The presence of mRNAs of glucocorticoid receptors in the cochlea has been reported in rats (20).

An immunological study to determine the presence of glucocorticoid receptors within the lateral wall and the ampullae of the semicircular canals would be of interest since these tissues are believed to be involved with fluid and ionic homeostasis of the inner ear (21). Endotoxin has been shown to up-regulate nitric oxide synthase (NOS) mRNA, with increases in reactive nitrogen intermediates (22). These studies have documented that glucocorticoids inhibit both
the increase of NOS mRNA and the release of reactive nitrogen intermediates. These actions can explain the inhibition of LPS stimulated vestibular disorders (23). According to Sone et al. (10), intratympanic dexamethasone was effective for treatment of cochlear lateral wall damage caused by endotoxin-induced otitis media. Dexamethasone administration following LPS attenuated cochlear lateral wall damage, to some extent, as assessed by transmission electron microscopy and by an increased response of CBF to PGE1. In this present study, the topical application of dexamethasone could attenuate the LPS-induced hearing loss. Our results of the CBF response to PGE1 were similar to those of Sone et al. (10).

Baggett et al. (14) showed that systemic application of dexamethasone before exposure to LPS inhibited the production of middle ear effusion and the proliferation of leukocytes in the middle ear mucosa. These results indicated that dexamethasone can protect cochlear function by inhibiting the production of cytokines by inflammatory cells. In this study, intratympanic dexamethasone treatment protected cochlear function measured by ABR. We did not investigate the cytokine in the middle ear and cochlea. Dexamethasone inhibits the expression of many cytokines and immunomodulatory genes, including TNF-alpha and inducible NOS, at the transcriptional level (24).

The stria vascularis is the main source of the CBF and steroid application to the round window of normal guinea pigs led to a statistically significant increase in CBF (19).

PGE1 has strong vasodilating and platelet anti-aggregating actions, which improves red blood cell flexibility (25). PGE1 is commonly used clinically because of its low cell toxicity compared with other PGs. Systemic administration of PGE1 is associated with problems such as reduced blood pressure and loss of activity when it passes through the lungs. It is important to investigate the effects of topically applied PGE1 on CBF in order to enhance its clinical applications. In rats and chinchillas, topical application of PGE1 to the round window increased CBF (10, 26).

In conclusion, our results suggest that intratympanic dexamethasone treatment was effective in protecting the function of the cochlea against endotoxin-induced otitis media. Further studies are necessary to clarify the role of dexamethasone in the cochlea using molecular studies.

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![Figure 1. Effect of dexamethasone on LPS induced hearing loss. Intratympanic dexamethasone protected the hearing threshold significantly compared to PBS treated group (PBS: phosphate-buffered saline, LPS: lipopolysaccharide, Dexa: 40 μl dexamethasone). *Significant differences.](image-url)
Figure 2. Effect of intratympanic dexamethasone treatment and PGE$_1$ administration on cochlear blood flow (PBS: phosphate-buffered saline, LPS: lipopolysaccharide, PGE$_1$: prostaglandin E$_1$). A) Topical dexamethasone after LPS inoculation. Upper lane: cochlear blood flow; middle lane: systemic blood pressure; lower lane: vascular conductance. B) Topical PBS treatment after LPS inoculation.

References


