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The effect of topically administered latanoprost on the cochlear blood flow and hearing

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ABSTRACT

Objective: The application of intratympanic latanoprost ($PGF_{2\alpha}$ analog) has been recently used to alleviate vertigo, disequilibrium and to improve hearing in Meniere's disease patients. However, there is no known report on the effect of topically applied latanoprost on hearing and cochlear hemodynamic parameters including cochlear blood flow (CBF) and vascular conductance. Our goal was to assess the influence of topically applied latanoprost on cochlear blood flow (CBF) and hearing.
Materials and methods: Twenty male Sprague-Dawley rats were randomly divided into the group A, 50 μl of latanoprost (1 ml containing 50 μg, $n = 10$) and group B, 100 μl (1 ml containing 50 μg, $n = 10$). Topical application of latanoprost was performed at the right side, and the left side was applied with phosphate buffered saline (PBS) as a negative control. Five rats at each group were used to measure cochlear blood flow (CBF). And the others at each group were used for hearing test by auditory brainstem response (ABR). After physiological examination, bullas were extracted. The changes of cochlear hair cells were observed by performing the field emission-scanning electron microscopy (FE-SEM).
Results: The CBF of both groups was found to be decreased compared to the PBS applied left side. Significant decrement of CBF was observed in group B compared to the group A. Significant elevation of hearing threshold at high frequencies was observed in both groups compared to the PBS applied group. However, inner and outer hair cells were intact.
Conclusion: Topically administered latanoprost decreased the CBF and impaired hearing. Based on our findings, additional studies are required to evaluate the side effects of intratympanic latanoprost before its use in clinical practice.

1. Introduction

Meniere's disease results from endolymphatic hydrops, an abnormal swelling of certain structures of the inner ear. However, the exact cause of this swelling is not entirely clear. Although Meniere's disease is often seen in the adult population, children can also be affected by this debilitating inner ear disorder [1,2].

The prostaglandins (PGs) are autacoids that exert effects through the seven specific transmembrane G-protein coupled metabolic type cell membrane receptors [3]. It has previously been documented that prostaglandins can be produced by cochlear tissue of several animal species [4–6]. In addition, the PGs may have many other functions in the inner ear. For instance, some PGs have been shown to increase the cochlear blood flow [7–9]. Among the PGs, $PGF_{2\alpha}$ is abundantly distributed in many structures of physiological importance for normal hearing system in both of guinea pig and humans.

Latanoprost is a $PGF_{2\alpha}$ analog (13, 14-dihydro-17-phenyl-18, 19, 20-trinor-$PGF_{2\alpha}$-isopropyl ester). Recently, application of intratympanic latanoprost has been used to alleviate vertigo, disequilibrium and to improve hearing in patients with Meniere's disease [10]. The compound has been shown to reduce intraocular pressure by increasing uveoscleral outflow. Previous studies reported no change of ocular blood flow increment one-week or two-weeks after the latanoprost treatment [11,12]. The mechanism by which $PGF_{2\alpha}$ exerts its vascular effects has not been precisely defined, but latanoprost may interact with a novel receptor on the vascular bed of the stria vascularis or on vascular smooth muscle cells similar to, but distinct from, the thromboxane receptor [13]. The concentration of $PGF_{2\alpha}$ in biological fluids has

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been considered as the most reliable biochemical index of lipid peroxidation and oxidative stress. Intense noise induces formation of vasoactive lipid peroxidation products in the cochlea by increasing the level of $PGF_{2\alpha}$ [14].

No report has been made on the effects of cochlear blood flow onto the round window membrane (RWM) by the topical application of latanoprost. The present study was designed to evaluate whether topically application of latanoprost to the RWM would decrease cochlear blood flow or change the hearing threshold.

2. Materials and methods

2.1. Animals

Experiments were performed by using 20 male Sprague-Dawley rats weighing 250 g (Samtaco Bio Korea, Osan, Korea) with normal tympanic membranes and Preyer's reflexes. To assess the influence of topically applied latanoprost on cochlear blood flow (CBF) and hearing, twenty male Sprague-Dawley rats were randomly divided into the two groups. The animals were housed in a room at a constant temperature of 22 °C, humidity of 50% and an ambient noise level less than 40 dB. All animal experiments were approved by the local ethical committee at the Research Center for Resistant Cells, Chosun University. For the A group, 50 μl of latanoprost (1 ml containing 50 μg) was applied onto the 10 rats, and 100 μl was applied onto the 10 rats at the B group. Five rats at each group were used for cochlear blood flow (CBF) measurement. The others at each group were used for hearing test (ABR). After physiological examination, bullas were extracted. The changes of cochlear hair cells were observed by performing the field emission-scanning electron microscopy (FE-SEM). The animals were anesthetized by the intraperitoneal injection of Zolletil® (a 1:1 combination of tiletamine and zolazepam, Virbac, Carros, France) and xylazine hydrochloride. Ear canals and the tympanic membranes were examined under an operating microscope. Ear infection was excluded by the examination of the external auditory canal and the tympanic membrane.

2.2. Measurement of cochlear blood flow and systemic blood pressure

The CBF was measured in each animal by following the method that was previously reported by Jang et al. [9]. In brief, the right femoral artery was canulated and connected to a pressure transducer (AD Instruments, Castle Hill, Australia) for arterial blood pressure. The right tympanic bulla was exposed by following a ventral approach. The right tympanic bulla 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 a laser Doppler blood flow meter (moorLAB, Moor Instruments, Axminster, Devon, UK) was placed on the lateral wall of the basal turn of the cochlea. The CBF output and systemic blood pressure (SBP) data were sampled every 20 seconds and were analyzed by a computer equipped with a data acquisition program (PowerLab, AD Instruments).

To assess the influence of drugs on CBF, 50 μl ($n = 5$, group A) and 100 μl ($n = 5$, group B) of latanoprost (Xalatan Eye Drop 50 μg/ml, Pfizer, Seoul, Korea) were soaked in a small piece of gelfoam by a Hamilton syringe after the removal of fluid overlying the RWM. For the prevention of mucosal resorption of latanoprost, lateral side of the gelfoam was covered by fibrin glue (Greenplast, Greencross, Yongin, South Korea).

Latanoprost was placed on the RWM of rats for 1 h. The baseline of CBF and SBP were measured before gelfoam placement. After the removal of gelfoam, CBF, SBP and vascular conductance (CBF/SBP) measurements at 10 min individual time segments were averaged for each experimental group. Vascular conductance (VC) was calculated from the ratio of CBF to SBP, it was used to reflect CBF changes not directly related to SBP changes. By using the data acquisition program (PowerLAB, Labchart system), automatic VC channel was created. CBF and SBP were recorded over 60 min following latanoprost soaked gelfoam placement on the RWM. Since there was some variance in the actual values of CBF and SBP among the animals, the experimental values of the CBF and SBP were presented as a percentage change from the initially measured value (100%).

To assess the influence of drugs on hearing threshold, auditory brainstem response (ABR) was recorded using an evoked potential system (Tucker-Davis Technologies, Alachua, FL, USA) and a Samsung computer. Stimuli were digitally synthesized by using the Siggen© software and were presented through an ER-2 insert earphone (Etymotic Research, Elk Grove Village, IL, USA). Acoustic stimuli consisting of click (low frequencies less than 4 kHz) and 4, 8, 16, and 32 kHz tone bursts were produced. The tone bursts are the 3 ms envelope that are consisted with 1 ms ramp onset, 1 ms plateau and 1 ms decay. ABR was recorded through Grass® stainless steel needle electrodes placed subcutaneously at the vertex (active), right cheek (inverting) and at the left cheek (common). The resulting signal was band-pass filtered (100–3000 Hz), amplified (10,000×), and digitized by using a TDT Bioamp (Tucker Davis Technologies). Responses were collected and averaged at 30 presentations per second for up to 512 times. The stimulus was presented at 90 dB SPL and progressed downward in 10 dB steps until no response was identifiable. ABRs were assessed 1 h after placing the latanoprost soaked gelfoam by transbullar approach at the right side. And the PBS soaked gelfoam placed at the left side as a negative control. The opening area of the bullar wall was covered using compressed dry gelfoam with cyanoacrylate adhesive. Click and tone burst-evoked ABR threshold testing took between 40 and 50 min to complete in one side. After measurement of the left side, right side was performed.

Data were analyzed by using the Mann–Whitney *U* test and Kruskal Wallis ANOVA (Analysis of variance). The statistical significance was considered if *P* value is less than 0.05.

2.3. Scanning electronmicroscopic observation

After the final ABR recordings, the anesthetized rats were perfused intracardially with 4% paraformaldehyde while they were under general anesthesia. The temporal bones were isolated and the perilymphatic spaces of the cochlear were gently perfused with 2.5% glutaraldehyde in 0.1 M phosphate-buffered solution (PBS) by performing cochleostomies at the round and oval windows. The bony capsules were removed to expose the organ of Corti, after which the specimens were post-fixed in 2.5% glutaraldehyde overnight at 4 °C. The specimens were washed three times in PBS, and then post-fixed in 1% osmium tetroxide for 1 h at 4 °C. The Organ of Corti specimens were then dehydrated through a graded series of ethanol solutions and then they were critical-point dried using liquid carbon dioxide. The critical-point dried specimens of the Corti organs were attached to aluminum SEM stubs with aluminum paint and then sputter-coated with platinum. The surface of each sample was examined by using a S-4700 field emission scanning electron microscope (Hitachi, Tokyo, Japan).

3. Results

Fig. 1A–C shows the CBF, blood pressure and vascular conductance response (mean ± standard deviation) for the two different concentrations of latanoprost in rats, respectively. The CBF values were expressed relative to the baseline value, which was set at 100% for each condition. In group A, CBF began to fall within 10 min