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Research article

Photobiomodulation by laser therapy rescued auditory neuropathy induced by ouabain

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HIGHLIGHTS

- Application of ouabain caused spiral ganglion cell damage while sparing the IHCs and OHCs in gerbils.
- Application of PBM using laser partially recovered the functional loss of hearing.
- Photobiomodulation effect on hearing rescue was related with preserved numbers of spiral ganglion cells throughout the cochlear turn, synaptic puncta, and nerve fibers.

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ABSTRACT

Auditory neuropathy is a hearing disorder caused by impaired auditory nerve function. The lack of information about the pathophysiology of this disease limits early diagnosis and further treatment. Laser therapy is a novel approach to enhance nerve growth or induce axonal regeneration. We induced auditory neural degeneration sparing the sensory epithelium with local ouabain application in an animal model and observed the rescue effect of photobiomodulation (PBM), showing recovered auditory function and favorable histologic outcome. Hearing was evaluated using the auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE). Seven days after ouabain application, the animals were sacrificed to evaluate the morphological changes. DPOAE change was not observed in all groups after ouabain application indicating no changes of outer hair cell function. Ouabain application increased the ABR thresholds increase, while the use of ouabain plus laser produced lower threshold compared to the ouabain group. Hematoxylin and Eosin staining of cochlea mid-modiolar sections in animals treated with ouabain showed damaged spiral ganglion cells, neurofilaments, and post synaptic puncta. Ouabain plus laser group showed higher number of spiral ganglion cells, higher density of neurofilaments, and higher number post synaptic puncta counts compared with ouabain application group. Short-term application of ouabain caused spiral ganglion cell damage while sparing the inner and outer hair cells in gerbils. Photobiomodulation alleviated the hearing loss caused by ouabain induced auditory neuropathy. The results indicate the possible role of photobiomodulation therapy for inner ear diseases accompanied by spiral ganglion degeneration.

1. Introduction

Auditory neuropathy (AN), also known as auditory dyssynchrony (AD), is a sensorineural hearing disorder that accounts for 7–10% of all permanent childhood hearing impairment [35]. In recent years, patients diagnosed with auditory neuropathy have been treated at ENT clinics. The hallmark features of AN are normal outer hair cell function and absent/abnormal auditory brainstem

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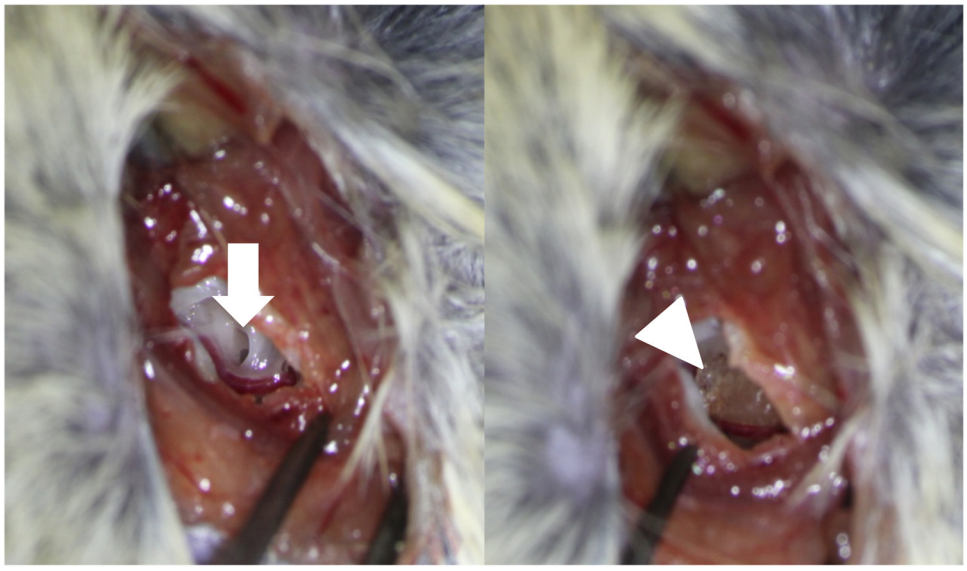

Fig. 1. Ouabain delivery into the inner ear.
Posteroinferior skin was incised in the left retroauricular area and the round window niche was exposed with using an electric drill. Ouabain 3 μL (1 mM) soaked in gelform (arrow head) was applied above the round window membrane (arrow) for 1 h.

responses (ABR) [42]. Unfortunately, there are not any effective therapeutic approaches available for AN, due to its unclear etiology and pathogenesis [34].

Ouabain is a cardiac glycoside that selectively inhibits Na^+/K^+-ATPase activity. Ouabain has been used for many years to investigate the role of Na^+/K^+-ATPase in maintaining ion homeostasis in the inner ear and to determine how Na^+/K^+-ATPase inhibition alters various cochlear potentials [37,43]. The application of ouabain to a round window membrane (RWM) of cochleae in gerbils and mice results in a rapid loss of type I spiral ganglion neurons with minimal damage to the sensory hair cells, which mimics the clinical presentation of auditory neuropathy [6,24].

Photobiomodulation (PBM), has been used in various fields of medicine. Recently, the United States Food and Drug Administration (FDA) approved PBM for the treatment of several diseases including carpal tunnel syndrome [9–12] and alopecia [8]. PBM improves wound healing [26] and inflammation [1], provides pain relief [17], promotes nerve regeneration [4], and improves hearing loss and tinnitus [7,16,31,41]. Electrochemical potential and ion channels are influenced by laser treatment and neural functions are reported to be significantly improved after PBM [8] suggesting the possibility of neural recovery or protection of the peripheral nervous structures of cochleae after neural degeneration.

We investigated the effect of the treatment with PBM on ouabain-induced auditory neuronal degeneration in Mongolian gerbils and determined the rescue effect of PBM, showing increased auditory function and favorable histologic outcome.

2. Material and methods

2.1. Animals

Female Mongolian gerbils (N = 30) aged 4–8 months with healthy external ears were used. The animals were provided by the Animal Center of the Ministry of Food and Drug Safety in Korea. All animals were treated in accordance with the Guide for Care and Use of Laboratory Animals (7th edition, 1996), as formulated by the Institute of Laboratory Animal Resources of the Commission on Life Sciences. This study was approved by Institutional Animal Care and Use Committees (IACUC) of Dankook University (DKU-14-023). The animals were divided into three groups: control group (N = 11 ears), ouabain only group (N = 18 ears) and ouabain plus laser group (N = 18 ears).

2.2. Surgery for ouabain application

Unilateral auditory nerve denervation was accomplished by application of an ouabain solution to the round window niche, as described previously [23]. Zolazepam (Zoletil; Virbac, Carros Cedex, France) and Xylazine (Rompun; Bayer, Leverkusen, Germany) were mixed in a 1:4 ratio (0.1 mL/100 g, i.m) for anesthesia. A posteroinferior skin incision was made in the retroauricular area of the unilateral ear. The underlying muscles and facial nerve were identified and dissected using an iris scissor to expose the lateral wall of the bulla, and the round window niche was exposed after drilling the bone. Gelform soaked with 3 μL of 1 mM ouabain using a 10 μL micropipette was applied to the round window niche for 1 h (Fig. 1). The bulla was covered with muscle and fascia, the incision was closed with non-absorbable 5-0 suture.

2.3. PBM (low level laser therapy)

An 808-nm diode laser (WONTECH Co., Ltd, Korea) was used to irradiate the ear. Optic fiber (core size: 62.5 mm) was delivered through a hollow tube into the external acoustic canal (Fig. 2). Laser was irradiated to the tympanic membrane, which is lateral to the cochlea. Laser energy was expected to be delivered to the cochlea, specifically spiral ganglion (Rosenthal canal) which is located within the cochlea. Energy was measured using the cadaveric animal model, and was 52.7 mW (26% of the initial output) (Supplementary Fig. 1). One day after ouabain treatment, gerbils in the ouabain plus laser group were irradiated for 1 h at an energy power of 200 mW for 7 consecutive days. The power of the laser was measured at the distal end of the optic fiber before irradiation with a Field MaxII-power meter (Coherent, USA) and a PM3 broadband sensor (Coherent) detector head. Description of the laser setup is shown in Table 1.