

Cochlea Hair Cell Rescue after a Noise-Induced Hearing Loss using a Low Level Laser Therapy (LLLT)

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ABSTRACT

Aim: To see the effect of LLLT on noise-induced hearing loss. **Methods:** Eleven rats were exposed to noise (120 dB, 16 kHz, 6 h) and left ears were irradiated at 60J/cm², 830 nm laser for 12 days. Right ears were control. Hearing levels were measured at frequencies of 4, 8, 12, 16, 32 kHz before noise exposure and after 12th irradiations. **Results:** The initial hearing levels were 26.5±4.7, 24.5±5.0, 24.0±5.2, 24.0±3.2, 24.5±5.5 dB SPL. After noise exposure, thresholds were 63.5±15.1, 64±16.8, 71.5±11.3, 73.5±15.6, 67.5±14.4 dB SPL in 4, 8, 12, 16, 32 kHz. After 12th irradiation, thresholds of treated ears recovered significantly 21±4.2, 20±3.5, 24±11.9, 24±12.9, 21±2.2 dB SPL and that of the untreated right ears measured 36.3±22.9, 45±15.8, 66.3±22.9, 50±16.8, 43.8±21.4 dB SPL. **Conclusion:** LLLT may promote recovery of hearing after noise-induced hearing loss.

Introduction

One of the most common factors that cause hearing disorders is noise trauma. Noise is an increasing hazard and it is pervasive, which makes it difficult to take precaution and prevent noise-induced hearing losses (NIHL) beforehand. A number of studies have been carried out regarding the effects of noise on the cochlea and have reported that intense noise exposure initiates a cascade of events which ultimately induces cochlear damage¹. It has been revealed that exposure to noise promotes cochlear microcirculatory changes, such as hypoperfusion and ischemia. The oxidative stress that result from these changes produces a variety of reactive oxygen species (ROS), including hydrogen peroxide (H₂O₂), the superoxide anion (O₂⁻) and the hydroxyl radical (OH)²⁻⁵. Production of these ROS alters the homeostasis in cells and the imbalance brings forth conditions such as vascular insufficiency, ultimately resulting in necrosis^{6,7}.

The human inner ear is mature at birth and the hair cells are fully differentiated and functional. Because such crucial role is played by the hair cells, damages done to the inner ear are immediately displayed as reduced hearing abilities and unlike most of the other cells, the hair cells normally do not regenerate when lost. Much research have been carried out to find ways to restore the hearing level and although numerous cases have been reported in which certain modality seemed to be effective, no definite treatment to mediate the issue has been established yet.

Already applied in numerous areas, the laser-induced phototherapy is widely accepted as a universal treatment and the dependency on the laser is still growing. The interests in the LLLT have been growing especially for the past decade and the number of studies and clinical trials performed has increased markedly.

The LLLT has been discovered to be effective in wound healing, chronic pain, musculoskeletal complications, and has been found to enhance various biological processes⁸⁻¹². Some studies have carried out nerve conducting models and reported that the low level laser has biostimulatory effect on traumatic nerve injury¹³⁻¹⁷. Although the exact mechanism of the laser therapy is not fully clarified, it has been documented that once absorbed, the light can modulate cell biochemical reactions and stimulates mitochondrial respiration, enhancing the production of molecular oxygen, ATP synthesis, and collagen deposition¹⁸⁻²⁰. In certain studies, release of growth factors has also been reported(Kipshidze²¹). Overall, the low level laser irradiation increases cell proliferation, thus positively modulating repair processes^{22,23}.

Despite the vastly growing interests in low level laser and its variety of utilized fields, the effects of the laser on the prevention of hair cell loss and post-regeneration have not been thoroughly investigated. In the present study, the ability to recover hearing thresholds after a noise-induced hearing loss has been assessed using 830 nm diode laser.

MATERIALS AND METHODS

Animals

Adult male SD rats weighing approximately 200 g (Narabio, Korea) were divided into noise only and combination of noise and laser groups. For anesthetics, zoletil (Virbac Laboratories, France) and rumpun (Bayer Korea, Korea) were mixed in a 4:1 ratio, respectively. The animals were anesthetized before each auditory brainstem response recordings or irradiation. Approximately 0.1 ml of the anesthetics was administered for every 100 g. The animals were sacrificed after the 12 days of treatment for scanning electron microscope (SEM) studies

Noise Exposure

An acrylic noise box was designed with a Beyma Loudspeaker CP800Ti (Beyma, Spain) attached on top. A dual channel real-time frequency analyzer (Bruel and Kjaer, Naerum, Denmark) was used to calibrate the noise generator and confirm that the amplifier projected the exact settings of the generator. The rats were placed in small, separate cages to prevent defensive behaviors such as blockage of the ears and were set inside the noise box. The subjects were given a one-time exposure to a narrow band noise of 120 dB SPL centered at the frequency of 16 kHz for 6 h. The hearing thresholds of the animals in both groups were recorded once before the exposure and also after 1st, 5th, 10th and 12th irradiations to observe the recovery of hearing thresholds.

Laser Irradiation

An 830 nm diode laser (Hi-tech optoelectronics, Daejeon, Korea) was used to irradiate the ears. From the following day after the noise exposure, the rats were irradiated at their left ears for 60 min at an energy density of 165 mW/cm² for 12 days in a row. The laser fiber was delivered through a hollow tube into the external auditory canal so that the distance from the tip of the fiber to the surface of the tympanic membrane was approximately 1 mm.

Auditory Brainstem Response Recordings

The auditory brainstem responses (ABR) were recorded using a signal-processing system (System III, Tucker Davis Technologies) with tone-burst, frequency specific stimulus-generation modules. The subjects were placed in a soundproof booth and three electrodes were inserted subcutaneously, one at the vertex and the other two ventrolateral to each ear, beneath the pinna. The tone-burst auditory stimuli were delivered through a tube inserted into the ear canal of the rat and the measurements were taken at each frequency of 4, 8, 12, 16 and 32 kHz to observe the changes in the hearing thresholds of the test subjects. The ABRs were measured before the exposure to noise for control values, immediately after the noise exposure and on the 1st, 5th, 10th and 12th day of irradiation.

SEM studies

After the 12 consecutive irradiations, ABRs of the rats were recorded and the subjects were sacrificed under general anesthesia. Intracardiac perfusions were performed with 20 minutes of 0.1 M phosphate-buffered saline (PBS) and 20 minutes of 4% paraformaldehyde (PFA). The animals were then decapitated and the cochleae were removed by locating the bulla. Microperfusions were performed once more on the

harvested cochleae and afterwards, a diamond burr dissecting drill (Saeshin Precision Co., LTD., Daegu, Korea) was used to remove the bone and the lateral wall (spiral ligament and stria vascularis) under a dissection microscope (Olympus Corporation, Tokyo, Japan). The separated cochleae were fixed in 2% glutaraldehyde overnight and then were rinsed with 0.1 M PBS. The samples were then postfixed with a 1% osmium tetroxide for 3 to 5 min and were gently rinsed again with 0.1 M PBS. After being dehydrated in graded series of ethanol, the critical-point dryer (Hitachi, Tokyo, Japan) was used to fully dehydrate the specimens. The prepared cochlea samples were then attached to aluminum stubs and were sputter-coated with platinum-palladium using E-1030 PT-PD target assembly (Hitachi, Tokyo, Japan). The surfaces of the basilar membrane with hair cells were examined using an S-4300 scanning electron microscopy (Hitachi, Tokyo, Japan).

Other than morphological observations, the cochleae were also quantitatively analyzed by recording the number of remaining hair cells. The cochleae were divided into apical, middle and basal turns at approximately $0^{\circ}\sim 270^{\circ}$, $270^{\circ}\sim 540^{\circ}$ and $540^{\circ}\sim 810^{\circ}$, respectively, and the hair cells were counted at three different sites of each turns at x600 magnification. A hair cell was considered as absent if the bundle of stereocilia was missing.

Statistical Analysis

All data were analyzed statistically by analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS, Chicago, USA) software. Data were expressed as mean \pm SD and differences were considered statistically significant when $p<0.05$.

Results

Threshold Shifts

The hearing abilities of the rats were observed through the ABR recordings at each frequency of 4, 8, 12, 16 and 32 kHz. ABRs were monitored before the noise exposure to confirm that they are functional at a normal range and also after 1st, 5th, 10th and 12th irradiations to keep up with the changes in the hearing thresholds.

The thresholds of the subjects were recorded to be 26.5, 24.5, 24.0, 24.0, and 24.5 dB SPL before the noise exposure. After the NIHL, the recordings increased markedly to 58.3, 62.8, 76.7, 60.6 and 60.0 dB SPL. Signs of change were observed after three to five days of irradiation and after 12th irradiation, the thresholds of the treated ears had recovered significantly (26.4, 25.9 \pm , 27.7, 27.7 \pm 10.3, and 26.4 dB SPL, $p<0.05$). That of the untreated ears measured 46.7, 51.7, 63.3, 50.6, and 48.9 dB SPL after 12th irradiation. All ABR recordings were arranged in order, corresponding to that of the five frequencies, 4, 8, 12, 16 and 32 kHz, respectively, and for accurate measurements, 11 ears were averaged each treated and control groups.

Morphological Changes and Hair cell Survival

The cochlea was divided into apical, middle and basal turns and the hair cells were counted at three different sites for each turn and were averaged for more precise data. The number of hair cells over 200 μ m of the basilar membrane was averaged for each group for comparison. Total of 6 ears for control group and 16 ears for laser group were included in this study. In the cochlea of the rats that have received neither noise nor the laser, the hair cell count was recorded to be 176.5, 146, and 154.8 cells and hair cells of the noise only group counted to be 122.5, 113.5, and 133.3 for apical, middle and basal turns, respectively. The cochlea of the treated (left) ears displayed promising results with hair cell counts of 167.4, 140.4 and 147 counts, respectively. Although the differences in the morphologies of the hair cells were not as distinctive as ototoxicities induced by pharmaceutical agents, the cochleae of the noise only group displayed some distortion or deformities in their stereocilia.

DISCUSSION

The prevailing view of NIHL was that it was caused by mechanical destruction of the membranes of hair cells and supporting structures of the organ of Corti until it was discovered that there was more at work²⁴⁻³¹. Recently reviewed evidence made clear that noise exposure can cause mechanical or metabolic impairment as well as neuronal disturbances. The noise trauma may be justified as abrupt damages done to the inner ear structures by disproportionate kinetic sound energy which overwhelms the physical resistance

capacity of the inner ear tissues³²⁾. The damages projected upon the inner ear drive mitochondrial activity and free radical productions, reduce cochlear blood flow, cause excitotoxic neural swelling, and induce both necrotic and apoptotic cell deaths in the organ of Corti^{33,34}. Excess ROS generated by elevated hair cell metabolic activity during intense noise exposure could overwhelm the antioxidant buffering capacity of the cell, leading to permanent loss or injury of hair cells³⁵. These free radicals degrade lipids and damage membrane-bound organelles such as mitochondria and nuclei. Attenuation of ROS production by neutrophils may play a role in the effects of LLLT in the treatment of inflammatory tissues³⁵. The LLLT may attenuate production of ROS in the hair cells and may increase the antioxidant buffering capacity of the hair cells, avoiding permanent loss or injury of hair cells. The hydroxyl (OH) radicals also initiates lipid peroxidation, which leads to oxidative lipid deterioration and damages to proteins embedded in cell membranes. It has been reported that the stria vascularis (lateral wall), outer hair cells and inner hair cells of the cochlea were targeted heavily³.

In previously done studies, prevented NIHLs have been reported on treatments with variety of antioxidants, glutamate antagonists and nitric oxide synthase (NOS) inhibitors³⁶. Pharmacological effects of NIHL prevention were found on chemical compounds such as N-acetylcysteine, D-methionine and ebselen, and also on natural products like flavonoid baicalein, ginsenoside Rb1 and Korean red ginseng³⁷⁻⁴⁰. No definite modality has been established, however, especially for post-exposure treatments which will be more applicable in clinical therapies.

Although exact mechanisms of laser therapy at work have not been clarified, the present LLLT on the left ears of the rats with NIHL improved the left ear hearing loss significantly compared to the control right ear hearing loss. The LLLT into the left ear ultimately appeared to cause positively modulated repair processes. The LLLT was observed to be effective as well in treating gentamicin-induced ototoxicity^{41,42}, but its positive results on noise-induced hearing losses will prove to be more significant in clinical practices. Further studies are encouraged, however, to observe the efficacy of delayed treatments after the noise damage and for an accurate understanding of its mechanisms.

CONCLUSION

The present study yielded positive results on restoring the hearing levels in animals after a noise-induced hearing loss. After careful observations, it may be suggested that the low level laser promotes hair cell survival and recovery of hearing thresholds. This potential modality for treating noise damages poses more possibilities of application in clinical practices than other procedures established for drug-induced hearing losses.

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