Effect of 850 nm photobiomodulation on the adenosine diphosphate/adenosine triphosphate measure of apoptosis in geniculate ganglion neuronal cells in vitro

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ABSTRACT

Introduction

This study was designed to investigate the influence of photobiomodulation(PBM) on geniculate ganglion cell by measuring cell viability using MTT(Tetrazolium salt) assay, ELISA, and ADP/ATP ration assay.

Methods

This in vitro study examined the effects of PBM on geniculate ganglion cells. Geniculate ganglion neuronal cells (GGNs) were cultured and exposed to different parameters of PBM. The cell viability, proliferation, and adenosine diphosphate/adenosine triphosphate (ADP/ATP) levels were assessed.

Results

PBMT at 10 J increased the cell viability three and seven days after PBMT via an MTT assay. The ADP/ATP assay results showed a significant difference in the ADP/ATP ratio of PBM-treated cells to the control group the days after PBM treatment. PBMT at doses of 1 and 5J showed a significant increase in the ADP/ATP ratio compared with the control group, while the 10 J group exhibited a marked difference relative to other groups.

Conclusions

This study supports potential effect of PBM therapy by acceleration of oxygenation and ATP production, which can promote the regeneration of damaged geniculate ganglion neuronal cells.

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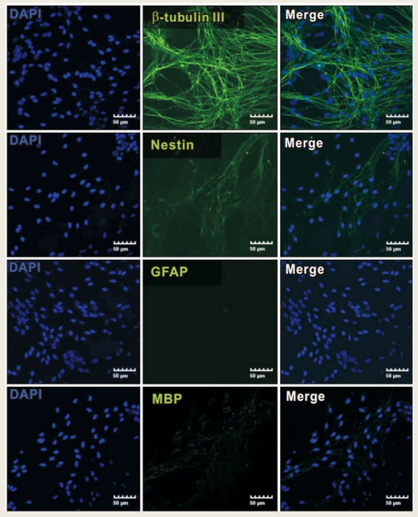
Background

- Photobiomodulation therapy (PBMT) is a non-invasive treatment that uses low-level light therapy to stimulate the cellular function and promote tissue healing.
- The geniculate ganglion(GGN) is a complex network of nerve fibers that plays a vital role in the sensory processing of the head and face, including taste perception.
- A dysfunction of geniculate ganglion cells can result in taste disorders and other conditions, such as facial nerve palsy and tinnitus.
- Herein, we investigated the effects of 850 nm PBMT on the primary culture of GGNs with a particular role in taste function. The goal is to determine the potential protective or therapeutic effect of PBMT on sensory neurons such as GGNs regarding cell viability, proliferation, and ATP production.

METHODS AND MATERIALS



Cell isolation, culture, and characterization

- GGNs isolated from neonatal rats (P1; Orientbio Corp.), allowed to grow for three days
- Immunofluorescence staining characterized isolated GGNs with neuronal and geniculate ganglion cell-specific markers.

Fig. 1. Geniculate ganglion neuronal cells maintained in culture for 3 days consist of mature neurons expressing beta III tubulin, minimal neural progenitor cells (Nestin), with no detection of astrocyte (GFAP) and oligodendrite marker (MBP) positive cells.

PBMT

- GGNs seeded in 24-well culture plates coated with PDL and exposed to PBMT
- 850 nm light emitting diode (LED) panel with a power setting of 9.70mW
- Irradiation in an incubator set at 37°C and 5% CO2
- Treatment groups were divided into four:
  control (no treatment), PBMT of 1J(103 seconds), 5J(515 seconds), and 10J (1,031 seconds)
- The PBMT was performed once on the third day after cell seeding

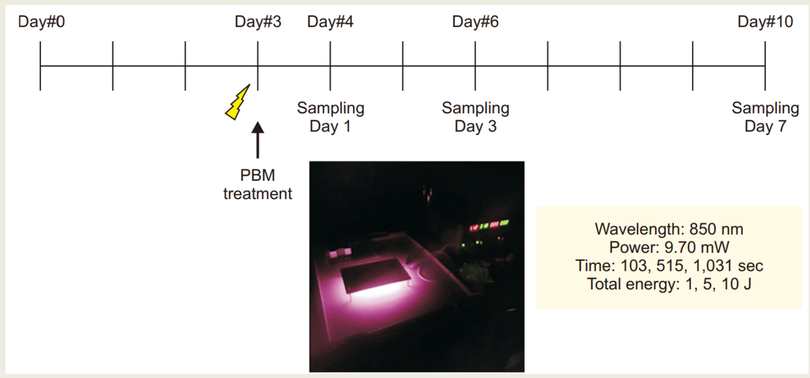


Fig. 2. Photobiomodulation (PBM) treatment scheme.
PBM therapy performed using 850 nm light emitting diode device panel with 9.70mW power output at varying durations equivalent to 1, 5, and 10J energy treatments

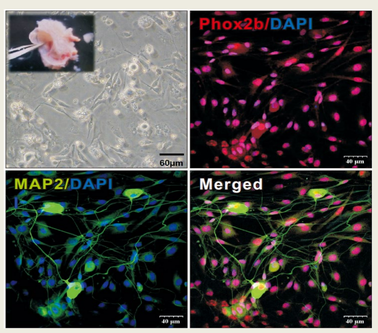
Cell viability assay

MTT assay was performed to assess the effects of PBMT on cell viability. This measures the activity of mitochondrial enzymes, which reflects the metabolic activity of cells.

Adenosine diphosphate/adenosine triphosphate (ADP/ATP) assay

The adenosine diphosphate/adenosine triphosphate (ADP/ATP) ratio assay measures the relative levels of ATP and ADP in cells, reflecting the energy status of cells.

RESULTS



Successful cell culture & characterization

The cultured GGNs exhibited characteristics of mature neuronal cells (MAP2 and Phox2b), with suggested the purity of the neuronal population (absence of astrocyte marker GFAP and oligodendrocyte marker MBP).

Fig. 3. Isolated and cultured geniculate ganglion neuronal cells express MAP2 neuronal marker and Phox2b marker associated with taste function.

Cell viability assay

- The cell viability in the PBM-treated cells was not significantly different from the control group on day 1 (Fig. 4).
- PBMT at a dose of 10 J significantly increased cell viability compared to other treatment groups on day 3 and day 7 ($p < 0.05$).
- PBMT at doses of 1 and 5 J did not show a significant increase in cell viability compared to the control group, suggesting that a certain energy level is required to enhance cell viability with PBMT.

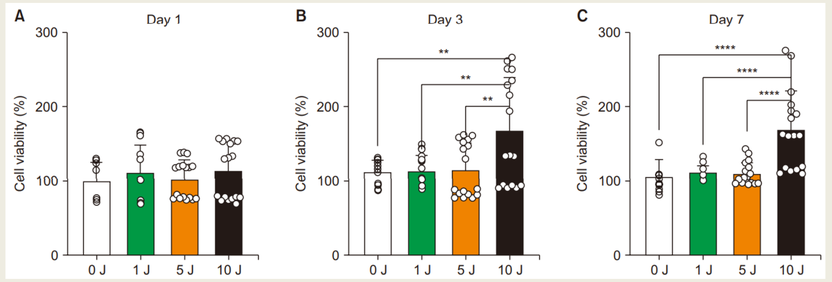


Fig. 4. Cell viability via MTT assay after photobiomodulation treatment using 850 nm light-emitting diode device panel with 9.70 mW power output at varying durations equivalent to 1, 5, and 10 J energy treatments. **$p < 0.01$ and ****$p < 0.0001$.

ADP/ATP assay

- There is a significant difference in the ADP/ATP ratio between the PBM-treated cells and the control group on the third day after PBM treatment (Fig. 5).
- PBMT at doses of 1 and 5 J showed a significant increase in the ADP/ATP ratio compared with the control group, while the 10 J group exhibited a marked difference relative to other groups.
- The effects of PBMT become evident three days after treatment, suggesting enhanced metabolic properties and improved cell proliferation.
- Therefore, PBMT in these types of cells does not present immediately but enhances the cells' metabolic properties as proliferation improves in the latter days.

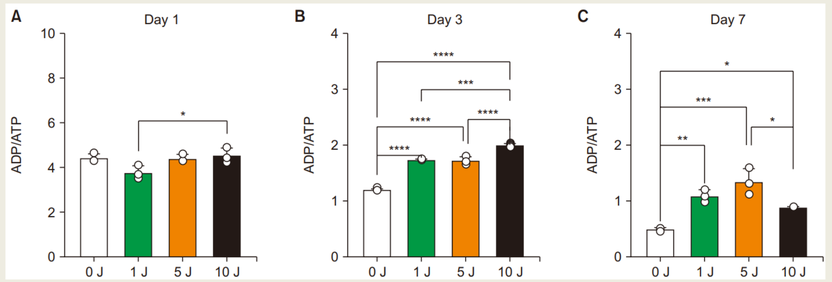


Fig. 5. ADP/ATP ratio apoptosis analysis after photobiomodulation treatment using 850 nm light-emitting diode device panel with 9.70 mW power output at varying durations equivalent to 1, 5, and 10 J energy treatments. *$p < 0.5$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

DISCUSSION

- PBM may be a safe and non-invasive approach for treating the geniculate ganglion, potentially for addressing taste dysfunction.
- PBM treatment of 10 J significantly improved the cell viability in the GGNs and prevented its apoptosis.
- Further studies are needed to optimize the parameters(wavelength, power density, and treatment duration) and investigate the underlying mechanisms and potential side effects of PBM therapy in the GGNs as well as the long-term effects of PBMT.
- Future studies need to address the potential side effects of PBM therapy, such as oxidative stress and DNA damage, which may occur at high doses or prolonged exposure to PBMT.

CONCLUSIONS

- The PBM treatment of 10 J improved the cell viability significantly in the GGNs and prevented apoptosis. Therefore, PBMT may be a safe and non-invasive approach for treating the geniculate ganglion, potentially addressing taste dysfunction.
- The study provides valuable insights into the potential therapeutic applications of PBMT at an appropriate energy level for taste or neurological diseases involving geniculate ganglion tissue.

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