Cytotoxic Effects of Different ICG Concentrations and Laser Parameters on Neuroblastoma

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Photodynamic therapy (PDT) is a minimally invasive treatment for cancer therapy. It can be administered in combination with other treatments such as chemotherapy, radiotherapy, and surgical excision. PDT involves a photosensitizing agent that is activated by exposure to a specific wavelength of light. PDT is a cold photochemical process, there is no tissue heating. In our study, we investigated whether different laser parameters with different concentrations of indocyanine green (ICG) have cytotoxic and anti-proliferative effects on neuroblastoma. Plates were divided into groups as control, only ICG concentrations (25 and 50 µg/ml), only laser treatment I (50 J/cm\textsuperscript{2}), only laser treatment II (100 J/cm\textsuperscript{2}), 25 µg/ml ICG + laser treatment I and 25 µg/ml ICG + laser treatment II, 50 µg/ml ICG + laser treatment I and 50 µg/ml ICG + laser treatment II. Neuroblastoma cell lines were irradiated with an in-house developed diode laser system ($\lambda = 809$ nm, 70 mW/cm\textsuperscript{2}, 50 & 100 J/cm\textsuperscript{2}) in continuous wave mode after ICG application. Cell proliferation was measured by XTT assay after light irradiation. Cell proliferation was decreased in a dose-dependent manner in 25 and 50 µg/ml ICG concentrations when compared with control. The applied ICG concentrations (especially 50 µg/ml) had cytotoxic effects for neuroblastoma cell lines, SH-SY5Y. There was no difference between laser treatment groups (L 50 & 100 J/cm\textsuperscript{2}). However, PDT groups (laser exposure with ICG) showed significant inhibition of cell viability ($p < 0.05$). Additionally, laser exposure did not increase the well temperature above the incubation parameter. In conclusion, PDT has cytotoxic effects in neuroblastoma cell lines. Appropriate ICG dose — laser parameter combinations must be determined for each cell type. Different energy densities may cause different effects of PDT on inhibition of cell viability.

DOI: 10.12693/APhysPolA.128.B-381
PACS: 42.62.–B, 87.19.XJ

1. Introduction

Photodynamic therapy (PDT) is showing great promise as a minimal invasive strategy in the treatment of various cancers. PDT requires a chemical agent that is called as photosensitizer and activation of the agent by light of a specific wavelength to produce oxygen-dependent cytotoxic reaction [1–3].

Indocyanine green (ICG), a photosensitizer with a molecular weight of 775 Da, has been used as a diagnostic agent to determine cardiac output, hepatic function and blood flow [4, 5]. ICG has low toxicity and has been approved by Food and Drug Administration (FDA) [6].

Neuroblastoma (NB) is one of the most common malignant solid tumors arising from neural crest cells [7]. The first aim is to inhibit cell proliferation in cancer treatment [8]. For this purpose we investigated whether different laser parameters with different concentrations of indocyanine green (ICG) have cytotoxic and anti-proliferative effects on neuroblastoma.

2. Material and methods

Neuroblastoma cell lines (SHSY-5Y) were grown in the Dulbecco modified eagle medium (DMEM), supplemented with 10% fetal bovine serum (FBS), and 1% penicillin–streptomycin. Cells were kept at 37°C in a humidified incubator with 5% CO\textsubscript{2}. Plates were divided into 9 main groups as

- control,
- only 25 µg/ml ICG concentrations,
- only 50 µg/ml ICG concentrations,
- only laser treatment I (50 J/cm\textsuperscript{2}),
- only laser treatment II (100 J/cm\textsuperscript{2}),
- 25 µg/ml ICG + laser treatment I,
- 25 µg/ml ICG + laser treatment II,
- 50 µg/ml ICG + laser treatment I,
- 50 µg/ml ICG + laser treatment II.

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25 µg/ml and 50 µg/ml ICG concentrations were applied and left for incubation for period of 24 hours. Neuroblastoma cell lines were irradiated with an in-house developed diode laser system (λ = 809 nm, 70 mW/cm², 50 & 100 J/cm²) in continuous wave operation mode after ICG application. Cell proliferation was measured by XTT assay after light irradiation. The optical density was measured at 450 nm with a microplate reader (Bio-Rad iMark Absorbance Reader). The results of the cell viability test were analysed using One-Way ANOVA technique and graphed as a boxplot in MATLAB. Multiple comparison technique was utilised to analyse these results revealing the groups that have a statistically significant difference.

3. Results

Cell proliferation was decreased in a dose-dependent manner in 25 and 50 µg/ml ICG concentrations when compared with control. The applied ICG concentrations (especially 50 µg/ml) had cytotoxic effects for neuroblastoma cell lines.

There was no difference between laser treatment groups (L 50 & 100 J/cm²). However, PDT I and PDT II groups (laser exposure with ICG) showed significant inhibition of cell viability (p < 0.05) (Fig. 1).

4. Conclusion

Photodynamic therapy has been used with several photosensitizers in cancer diagnosis and treatment [9, 10]. Photoactivated ICG is shown to have anti-proliferative effects in colon cancer, breast cancer, pancreatic cancer [11–13]. Our results have showed that all doses of ICG may be effective. When ICG is applied with laser, PDT I and PDT II cause cytotoxic effects in neuroblastoma cell lines. Appropriate ICG dose — laser parameter combinations must be determined for each cell type. Different energy densities may cause different effects of pdt on inhibition of cell viability. Results contain primary data of ICG-PDT anti-proliferative effects on neuroblastoma cell line. New treatment approaches such as ICG-PDT are needed to be studied thoroughly to find cancer treatment.

References


