Special issue of the International Conference on Computational and Experimental Science and Engineering (ICCESEN 2014)

Cytotoxic Effects of Different ICG Concentrations and Laser Parameters on Neuroblastoma

A. Ak[a,*], Ö. Kaya[b], D.T. Coşan[c] and M. Gülsoy[b]

[a]Erzincan University, Biomedical Engineering Department, Erzincan, Turkey

[b]Boğaziçi University, Institute of Biomedical Engineering, İstanbul, Turkey

[c]Eskişehir Osmangazi University, Medical Biology, Eskişehir, Turkey

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 groups as control, only ICG concentrations (25 and 50 μg/ml), only laser treatment I (50 J/cm^2), only laser treatment II (100 J/cm^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 (λ = 809 nm, 70 mW/cm^2, 50 & 100 J/cm^2) in continuous wave operation 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^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 inhibite 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_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^2),
- only laser treatment II (100 J/cm^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.

*corresponding author; e-mail: ayseak@erzincan.edu.tr

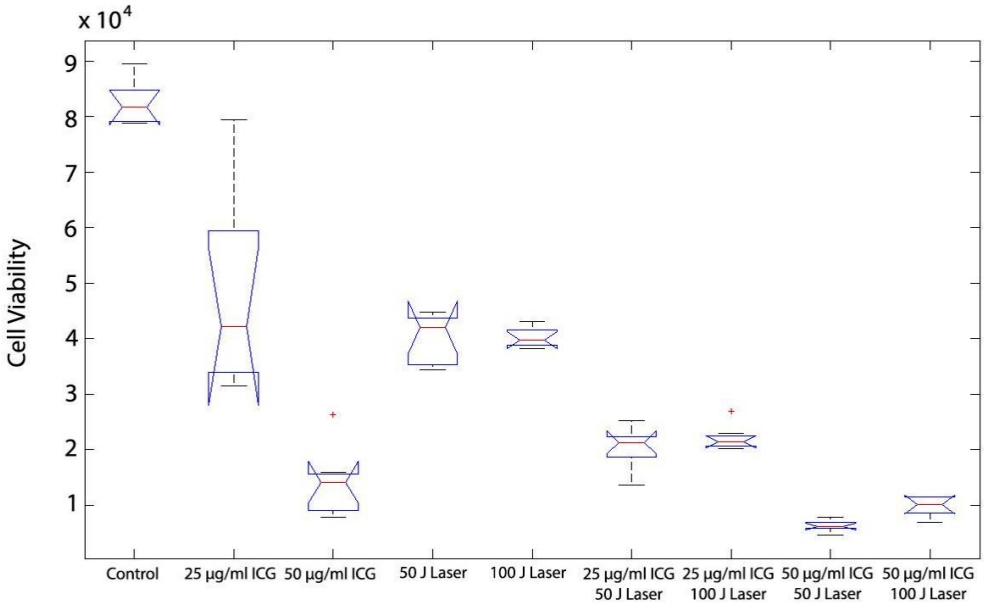

Fig. 3. PDT treatment in neuroblastoma cells.

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 ($\lambda = 809$ nm, 70 mW/cm^2, 50 & 100 J/cm^2) 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/cm^2). 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

[1] M.-C. Tetard, M. Vermandel, S. Mordon J.-P. Lejeune, N. Reyns, *Photodiagnosis Photodyn. Ther.* **11**, 319 (2014).

[2] T.J. Dougherty, C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, *J. Natl. Cancer I.* **90**, 889 (1998).

[3] K. Morimoto, T. Ozawa, K. Awazu, N. Ito, N. Honda, S. Matsumoto, D. Tsuruta, *PLOSone* **9**, e105173 (2014).

[4] Y. Morita, T. Sakaguchi, N. Unno, Y. Shibasaki, A. Suzuki, K. Fukumoto, K. Inaba, S. Baba, Y. Takehara, S. Suzuki, H. Konno, *Int. J. Clin. Oncol.* **18**, 232 (2013).

[5] K. Urbańska, B. Romanowska-Dixon, Z. Matuszak, J. Oszajca, P. Nowak-Śliwinska, G. Stochel, *Acta Biochim. Pol.* **49**, 387 (2002).

[6] R. Radzi, T. Osaki, T. Tsuka, T. Imagawa, S. Minami, Y. Nakayama, Y. Okamoto, *J. Vet. Med. Sci.* **74**, 545 (2012).

[7] Y. Li, A. Nakagawara, *Cells* **2**, 432 (2013).

[8] D. Bechet, S.R. Mordon, F. Guillemin, M.A. Barberi-Heyob, *Cancer Treat. Rev.* **40**, 229 (2014).

[9] K. Berg, P.K. Selbo, A. Weyergang, A. Dietze, L. Prasmickaite, A. Bonsted, B.O. Engesaeter, E. Angell-Petersen, T. Warloe, N. Frandsen, A. Hogset, *J. Microsc.* **218**, 133 (2005).

[10] M.E. Wieder, D.C. Hone, M.J. Cook, M.M. Handsley, J. Gavrilovic, D.A. Russell, *Photochem. Photobiol. Sci.* **5**, 727 (2006).

[11] W. Bäumler, C. Abels, S. Karrer, T. Wei, H. Messmann, M. Landthaler, R.-M. Szeimies, *Br. J. Cancer* **80**, 360 (1999).

[12] W.R. Chen, R.L. Adams, A.K. Higgins, K.E. Bartels, R.E. Nordquist, *Cancer Lett.* **98**, 169 (1996).

[13] W.W. Tseng, R.E. Saxton, A. Deganutti, C.D. Liu, *Pancreas* **27**, 42 (2003).