Indocyanine green-mediated photodynamic therapy on glioblastoma cells in vitro

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Abstract: Photodynamic therapy (PDT) is an alternative therapy which is administered with non-toxic drugs, called photosensitizers (PSs), along with irradiation at a specific wavelength of light to damage tumor cells. Different wavelengths of light sources and photosensitizers have been investigated in treatment of many cancer types. In this study, we investigated whether photodynamic therapy using indocyanine green (ICG), also a cyanine dye used in medical diagnostics, can be used to inhibit cell proliferation of glioblastoma. Glioblastoma cells were irradiated with a diode laser (λ = 809 nm, 100 J cm⁻²) in continuous wave operation mode. Cell proliferation was measured by XTT assay at 24 h after light irradiation. While only ICG application (10 µg/ml and 50 µg/ml concentrations) had no effects on glioblastoma cell proliferation, ICG in combination with laser irradiation (ICG-PDT) caused a significant decrease of cell proliferation. In conclusion, ICG-PDT may be valuable therapeutic approach as potential treatment for cancer such as glioblastoma. The results could be used as primary data for efficacy of ICG-PDT on malignant tumors.

Keywords: photodynamic therapy, indocyanine green, glioblastoma, cell proliferation.

1. Introduction
Photodynamic therapy (PDT) involves the combination of tumor-localizing photosensitizer (PS) with the light of a specific wavelength (commonly from a laser source). The irradiated light is absorbed by the PS which in the presence of oxygen leads to the generation of reactive oxygen species that can destroy tumor cell [1]. PDT has advantages such as selective targeting, minimal invasiveness and reduced toxicity that allows for repeated treatment [2]. PDT is dependent on the delivered light energy and the concentration of the photosensitizer [3]. Recent studies focus on new photosensitizer delivery systems and light delivery devices to improve the efficacy and safety of PDT. The optimal concentrations and types of PS are also being investigated [4]. Indocyanine green (ICG) is also a promising photosensitizer agent for PDT. It has been widely used as a diagnostic aid for blood volume determination, cardiac output, liver function and visualization of retinal and choroidal vasculature [5]. ICG has rapid excretion and strong absorption at 805 nm which can be used in PDT [6]. The use of PDT is new strategy for cancer therapy because of its easy application, high specificity and selectivity [7]. Glioblastoma is the most common and aggressive type of brain cancer; and it is resistant to all conventional therapies [8],[9]. New therapeutic approaches are needed for inhibition of cell proliferation in cancer. Recently, several photosensitizer agents (photofrin, 5-aminolevulinic acid and haematoporphyrin derivative) have been tested in treatment of glioblastoma [3]. In this study, the anti-proliferative effects of ICG-PDT were investigated in glioblastoma cell line.

2. Material and methods
2.1. Cell line
C6 rat glioblastoma cell lines were grown in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), and 1% penicillin–streptomycin. DMEM, FBS and penicillin–streptomycin were purchased from Gibco BRL-Invitrogen. Cells were kept at 37 °C in a humidified incubator with 5% CO₂.

2.2. Laser set-up
A commercial 809 nm diode laser module (Spectra-Physics Lasers Inc., CA, USA) was used to develop a computer controlled laser system in-house (10 W, CW). This system uses a RS232 interface for communication, and it is controlled by a software user interface developed in C. The laser output was coupled to a 400 µm silica optical fiber (Spindler-Hoyer, Göttingen, Germany). The fiber was fixed at 50 cm above a well plate to ensure homogeneous illumination of 3x3 wells. (Only the center of the Gaussian distribution profile was used). Blackout foil (Thorlabs Inc., NJ, USA) was utilized to cover and protect the remaining wells from illumination (Figure 1). Using this optical setup, the diode laser system was operated at maximum power (10 W) resulting in a power density of 140 mW cm⁻² throughout the target area, which was measured using an optical power meter (1918-R, Newport Corp., CA, USA). The wells were irradiated for 12 minutes to ensure the delivery of the designated dose (100 J cm⁻²).
2.3. Cell proliferation assay

Glioblastoma cells were seeded in a 96-well plate (TPP, Switzerland) and incubated for 24 h. Plates were divided into 4 main groups as Control, ICG, Laser and PDT with ICG (ICG-PDT). ICG and ICG-PDT groups were incubated in 10 μg/ml and 50 μg/ml ICG (dissolved in medium) for 24 h. Laser and ICG-PDT groups were treated under 809 nm diode laser for 12 min with a power density of 140 mW cm². Cell proliferation was determined by XTT assay (Biological Industries). The experiments were repeated three times. XTT assay was done according to the manufacturer’s instructions. The cells were incubated for 4 h at 37 °C. The cells were directly exposed to XTT right after treatment. The optical density was measured at 450 nm with a microplate reader (Bio-Rad iMark Absorbance Reader). The number of cell counts was compared using one-way analysis of variance (ANOVA) and subsequently separated using Tukey’s Honestly Significant Difference (HSD). SPSS software program (version 18.0 for Windows, SPSS Science, Chicago, IL) was used for data analysis. Results were considered statistically significant when P < 0.05.

3. Results

PDT using ICG was able to decrease cell proliferation in a dose-dependent manner in C6 cell line. We found that PDT with 10 μg/ml, 50 μg/ml ICG and only laser irradiation significantly inhibit cell proliferation when compared with control and 10 μg/ml and 50 μg/ml ICG (F: 20.078; df: 5,12; P: 0.000). We also determined that 50 μg/ml ICG was suitable for ICG-PDT application rather than 10 μg/ml ICG for anti-proliferative activity in C6 glioblastoma cell line (Figure 2).

4. Conclusions

Photodynamic therapy has been applied to many cancer types with several photosensitizers. Tumor cells that contain a photosensitizer are destroyed with the use of lasers at a specific wavelength. ICG is shown to have anti-proliferative effect as a photosensitizer in pancreas cancer [10], [11], colon cancer [12], metastatic breast cancer [6], over cancer [13], cervical cancer [14]. Our results have showed that ICG-PDT application also has anti-proliferative activity in glioblastoma cell lines. Anti-proliferative activity is higher with 50 μg/ml ICG-PDT application and light energy (100 J cm⁻²). Our research shows promising results for treatment of glioblastoma cell line. Our results contain primary data of ICG-PDT anti-proliferative effects on C6 glioblastoma cell line. New treatment approaches such as ICG-PDT are needed to be studied thoroughly to develop cancer therapy. Future researches should focus on the effect of temperature increase by laser exposure.

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References


