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Efficient treatment of pigmented B16 melanoma using photosensitized long-circulating magnetofullerenosomes

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Abstract

Magnetic targeting was used for melanoma treatment with magnetofullerenosomes. After their intravenous administration, a permanent magnet was attached to the surface of B16 tumors in C57 mice for 24 h, followed by irradiation with an infrared laser pulse and subsequent illumination with a 776 nm diode laser for conventional photodynamic treatment. Tumor response was substantially better than that obtained with either treatment alone.

Keywords: Magnetic targeting; Melanoma B16; Magnetofullerenosomes; Laser pulses; Photodynamic therapy; Fullerenosomes; C57 mice; Tumor treatment

1. Introduction

The most widely used methods of treating malignant tumors include surgery, chemotherapy, radiation therapy, hyperthermia, and immunotherapy. Essentially only the last modality in some extent offers the possibility of selective destruction of tumor without injury to normal tissue. Specific drug delivery methods potentially serve to minimize toxic side effects, lower the required dosage amounts, and decrease costs for the patient [1]. One of the most promising carriers are liposomes, especially sterically stabilized long-circulating liposomes characterized by polyethylene glycol coating display prolonged bioavailability, but decreased cellular uptake, as compared with conventional liposomes [2].

Another method used to fight cancer, photodynamic therapy (PDT), is a treatment that is based upon the differential uptake of photosensitizing agents by cancerous cells, followed by irradiation of the cells to cause a photochemical reaction generating chemically disruptive species, such as singlet oxygen [3]. These disruptive species in turn injure the cells through reaction with cell parts e.g. cellular and nuclear membranes. However, certain types of cancers, such as the very

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virulent pigmented melanoma, are known to be poorly responsive to PDT with commonly used photosensitizing agents whose lowest energy absorption band lies in the 600–700 nm spectral range. This lack of response is generally ascribed to optical filtering of the incident light by the melanin granules, which are expressed with a particularly high frequency in this tumor type. To overcome these shortcomings a new method was proposed [4–6] comprising the steps: (1) subjecting the cancer tissue to relatively high power light which is absorbed by the pigment so as to substantially reduce its amount in the pigmented cancerous cells; (2) treating the tumor with photosensitizing agent; followed by (3) subjecting the tumor tissue to low power light absorbed by the photosensitizer so that the photosensitizer is excited to a more reactive state and induces the disruption of the integrity of the tumor cells.

Many of the pharmacological properties of conventional "free" drugs can be improved through the use of drug delivery systems. Recently we have developed fullerenosomes, a novel nanosystem prepared by incorporation of C_{60} fullerenes into lipid bilayers of phosphatidylcholine liposomes. As we have shown [7–9], single nanosecond near-infrared laser pulse energy absorbed by C_{60} leads to explosive release of encapsulated material. Our aim in this study is to show that sterically stabilized long-circulating photosensitized magnetofullerenosomes (fullerenosomes with coencapsulated dextran magnetite) may substantially improve photodynamic therapy of pigmented B16 melanoma using the above-mentioned method of laser mediated tumor bleaching combined with magnetic targeting, which has been shown very useful in many areas, e.g. in cancer radiotherapy [10].

2. Material and methods

2.1. Magnetofullerenosome preparation

Thirty-five milligrams of dipalmitoyl-phosphatidylcholine and 2 mol% of distearylphosphatidylethanolamine-PEG-2000 (Sigma, St. Louis, USA) were dissolved in 10 ml of a diethyl ether and chloroform mixture (1:1, v/v) in rounded bottom flask. To this mixture were added required amounts of fullerene C_{60} (kindly supplied by Dr. H. Michnik). Finally bis(di-isobutyloctadecylsiloxy)-2,3-naphthalocyanato silicon (isoBO-SiNc) prepared by chemical synthesis [11] and dextran magnetite were added to the flask and emulsified in a Labsonic 2000 sonicator bath (Branson Ultrasonics, Danbury, UK) at 100 W for 5 min at 45 °C. The emulsion was then evaporated at 45 °C under a reduced pressure (30 mmHg) in a rotary evaporator [12] until an opaque suspension of magnetofullerenosomes with coencapsulated isoBO-SiNc and dextran magnetite had formed. Magnetofullerenosomes were separated from non-encapsulated C_{60}, dextran magnetite, and isoBO-SiNc by centrifugation (at 10,000g) and resuspended in phosphate buffered saline (PBS) at pH 7.4.

2.2. Treatment protocols

Female C57 mice (18–20 g body weight) were used in an experimental model. B16 pigmented melanoma was subcutaneously implanted into the upper flank by injecting 10^6 cells of a sterile suspension in PBS. All of our experiments were performed one week after injection of B16 melanoma cells, when the tumor volume was $\sim$0.04 cm^3. IsoBO-SiNc incorporated into magnetofullerenosomes was slowly injected in 0.15 ml at a dose of 1 mg/kg body weight into the caudal vein of C57 mice bearing the B16 pigmented melanoma. Four groups of mice (five mice per each time point) were used for treatments: unirradiated control mice; mice with injected isoBO-SiNc irradiated with 776 nm diode laser alone (representing conventional PDT) mice irradiated with the Nd:YAG laser (Quantel, Continuum Corp., St. Clara, USA; wavelength 1064 nm, 8 ns pulse with 500 mJ energy), with 776 nm diode laser immediately after the 1064 nm pulse; and finally mice irradiated with pulse as well as 776 nm light, but with small neodymium magnet (with surface magnetic induction 0.32 T) attached 24 h to tumor surface to increase accumulation of magnetofullerenosomes in the tumor. All these treatments were done 24 h after their intravenous administration of magnetofullerenosomes. During the

treatments animals were anesthetized with i.p. injected Ketamine (Pfizer) 5 mg/100 g. For each group the effectiveness of the treatment was evaluated by measuring the rate of tumor growth daily with a calliper. Assuming a hemiellipsoidal structure for the tumor nodule and measuring the two perpendicular axes D_1 and D_2 and the height D_3, individual tumor volumes were calculated according to the formula $V = \pi/6(D_1 \times D_2 \times D_3)$.

3. Results and discussion

Tumor growth kinetics for mice injected with magnetofullerenosomes with isoBO-SiNc (1 mg/kg) and after 24 h irradiated with 500 mJ/pulse of 1064 nm followed by 400 J/cm^2 of 776 nm light with and without the magnetic targeting, as well as conventional PDT alone, compared with the rate of tumor growth in control untreated mice are shown in Fig. 1.

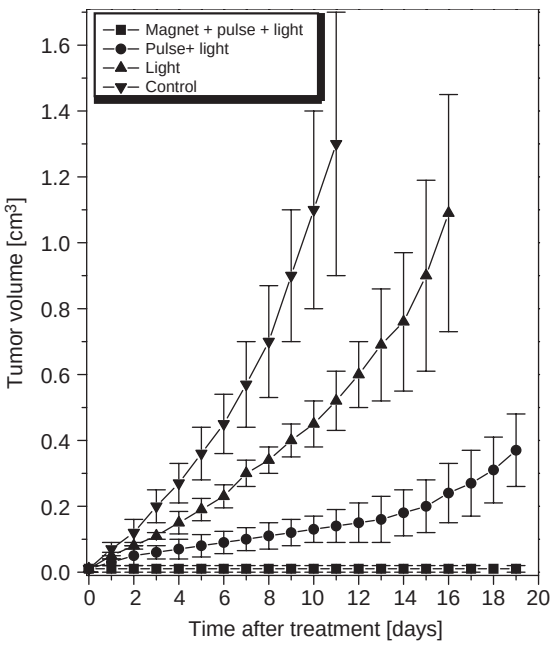

Fig. 1. Melanoma growth kinetics after various modes of treatment with magnetofullerenosomes (each time point represents an average result of experiments on five mice ± standard deviation).

Irradiation of B16 pigmented melanoma subcutaneously transplanted in C57 mice with a single 500 mJ pulse (8 ns) of 1064 nm light caused visible instantaneous bleaching of the pigmented tissue (via destruction of melanin granules [13]) and in such a way enhance tumor susceptibility to conventional photodynamic therapy.

Let us compare the tumor growth to the volume of ~0.4 cm^3 (which is about 10 times larger than the tumor volume at the beginning of the treatment). This volume was attained after 6, 10, and 20, days for control, PDT, and PDT after infrared pulse, respectively. Therefore, treatment of the B16 pigmented melanoma by photodynamic therapy from a 776 nm diode laser immediately after the 1064 nm irradiation results in ~2 weeks delay of tumor regrowth. If the magnetic field was applied in 80% cases (four out of five mice) complete tumor eradication has been achieved, which is a remarkable result not easily obtained for this kind of skin cancer by other common methods. It should be stressed that infrared pulse itself may have beneficial effects for tumor destruction, due to the laser mediated melanocytes destruction and also by capabilities of C_{60} to produce cytotoxic singlet oxygen upon irradiation, via multiphoton infrared light absorption [9].

In conclusion, the present investigations involving irradiation with 8 ns near-infrared laser pulse followed by conventional PDT using 776 nm light with isoBO-SiNc delivered via sterically stabilized magnetically targeted magnetofullerenosomes accumulating preferentially in tumors, demonstrated the occurrence of a significant tumor response which is markedly larger than that obtained with either treatment alone. Proposed method therefore provides a new way to multifunctional photodynamic therapy of highly virulent pigmented melanomas, and after further elaboration, may have potential clinical applications.

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