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Comparison between sonodynamic and photodynamic effect on MDA-MB-231 cells

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ABSTRACT

Photodynamic therapy (PDT) and sonodynamic therapy (SDT) are therapeutic modalities for tumors. In this study we investigated the combined cytotoxic effect of 0.36 W/cm^2 and 0.72 W/cm^2 ultrasound with various Ce6 concentrations (1, 2, 5, 10 μg/ml), and that of 1 μg/ml Ce6 with different laser light dose (650 nm; 10.4 mW/cm^2; 0.3, 0.6, 1.2 and 2.5 J/cm^2) on MDA-MB-231 cells. Both high reactive oxygen species (ROS) production and a decline in mitochondrial membrane potential (MMP) were detected with high Ce6 concentrations (5 and 10 μg/ml) combined with 0.72 W/cm^2 ultrasound and 1.2, 2.5 J/cm^2 laser light with 1 μg/ml Ce6. In addition, cell membrane integrity was evaluated by using propidium iodide (PI), revealing membrane damage was aggravated with the increasing ultrasound intensity, but no significant difference on cell membrane integrity could be observed after PDT treatment. These results suggest ROS may play an important role both in SDT and PDT. Besides, mitochondria may be an initial target in PDT while SDT can cause multi-site damages in MDA-MB-231 cells.

1. Introduction

Breast cancer has been one of the most fatal cancers threatening females' health in the world for decades [1]. Traditional therapies such as radiotherapy and chemotherapy are not the best way to treat breast cancer because of poor prognosis and serious side effects [2]. Although surgical treatment is a relatively effective method for the sufferer, but it has higher recurrence rate and may cause great injury for the physical body and beauty [3]. Therefore, searching for novel therapeutic strategies in tumor treatment, especially noninvasive approaches, is urgently required.

Photodynamic therapy (PDT) has been increasingly used for treatment of various tumors [4]. The basis of this therapy is the photosensitizer preferentially accumulates in malignant tissue and is subsequently activated by light of an appropriate wavelength [5,6]. Following absorption appropriate light wavelengths, the photosensitizer is excited into a high-energy state, from which it is returned, accompanied by the transfer of an electron to adjacent molecules, referred to as a type I photochemical reaction, or energy to ground state of molecular oxygen, type II photochemical reaction [7,8]. The reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide radical anion ($O_2^{\cdot-}$), hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2), produced in this process are harmful to cells [9]. As demonstrated by previous studies, PDT has been successfully applied in some clinical cases [4]. However, lasers are unable to penetrate and reach deep tissues to activate the photosensitizer, so the application of PDT is considered to be limited in the future development [6].

Sonodynamic therapy (SDT), another new promising tumor treatment method, which is derived from PDT, applies ultrasound to activate sensitizers and could eventually kill cancer cells [10]. Ultrasound has an appropriate tissue attenuation ability, allowing it to penetrate into tissues and reach non-superficial objects while maintaining the ability to focus energy into small volumes and activate sensitizers [11]. Among noninvasive treatments, this advantage is unique compared to PDT. Recently, in vitro and in vivo experiments have demonstrated the significant anti-tumor effects of SDT, but the mechanisms have not been understood clearly. Hiraoka et al. [12] reported that the mechanism of ultrasound is primarily due to certain mechanical stress, such as physical disruption of cellular membrane, and the synergistic effect of ultrasound and sensitizers is suggested owing to the photo-excitation by the sonoluminescence produced in collapsing cavitation [13]. Studies have suggested that SDT-induced caviation may be responsible for the sonochemically generated radical production and a quantitative phenomenon [14,15]. Li et al. investigated the combined anti-cancer effect of PDT and SDT which was called Sono-Photodynamic therapy (SPDT) [16], however, the involved mechanisms remain unclear.

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In this study, we applied a sensitizer chlorin e6 (Ce6), which has been reported to preferentially accumulate in tumor tissues [17]. By evaluating the antitumor effect of Ce6 mediated PDT and SDT on human breast cancer MDA-MB-231 cells, we attempt to compare the distinct mechanisms between the two anti-cancer therapies.

2. Materials and methods

2.1. Cell culture

Human breast cancer MDA-MB-231 cells were obtained from the cell bank of Chinese Academy of Science, Shanghai, China. The cell line was cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO, Life Technologies, Inc., USA) supplemented with 10% fetal bovine serum (FBS, HYCLONE, USA), 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Cells in the exponential phase of growth were used in each experiment.

2.2. Chemical

Ce6 was purchased from Sigma chemical (St. Louis, MO, USA) and the purity was greater than 95%. Ce6 was dissolved in sterilized PBS (0.01 M, pH 7.4) at a stock concentration of 2.5 mg/ml, aliquoted and stored in the dark at −20 °C. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide tetrazolium (MTT), N-acetylcysteine (NAC), propidium iodide (PI) and rhodamine 123 (Rho123), were also supplied by Sigma. 2′,7′-Dichlorodihydrofluorescein-diacetate (DCFH-DA) was supplied by Molecular Probes Inc. (Invitrogen, CA, USA). All other reagents were commercial products of analytical grade.

2.3. Photodynamic and ultrasound treatment

The laser light (excitation wavelength: 650 nm; manufacturer: Institute of Photonics & Photon Technology, Department of Physics, Northwest University, Shaanxi, China) were used as a source for evocation of the photodynamic effect. Irradiance was measured by the radiometer system (Institute of photonics & photon-technology, Department of physics, Northwest university). For the laser light, the power output: 1.3 mW; power intensity: 10.4 mW/cm^2; irradiation time: 30–240 s; emitting aperture area: 0.125 cm^2; area of the beam: 9.62 cm^2; The power intensity (10.4 mW/cm^2) = the output power (1.3 mW)/the detector area (0.125 cm^2) and the energy intensity was calculated using the following equation: energy intensity (J/cm^2) = power intensity (W/cm^2) × irradiation time (seconds), so the final light dose was varied from 0 (dark control) to 2.5 J/cm^2 in this experiment. When cells reached around 70% of confluence in a 35 mm diameter culture dish, cells were divided randomly into four groups: (1) control, (2) Ce6 alone, (3) laser light alone, (4) Ce6 plus laser light (PDT). For Ce6 alone and PDT groups, cells were incubated in the serum-free DMEM with 1 μg/ml Ce6 for 4 h, allowing the sensitizer uptake of the cells to reach a maximum level. Instead of Ce6, an equivalent quantity of serum-free DMEM was used in the control and laser light alone groups. The cells in PDT group were irradiated with light from 0.3 J/cm^2 to 2.5 J/cm^2.

A 35 mm diameter planar transducer (Institution of Applied Acoustics, Shaanxi Normal University) was submerged in a circular water bath filled with degassed water. Continuous-wave ultrasound was generated by the amplifier (T&C Power Conversion, Inc., Rochester, NY). For SDT, cells in 35 mm culture dish were placed in the water bath and 1 cm above the top of the transducer in a horizontal position. Cells were sonicated at intensities of 0.36, 0.72 W/cm^2 with the frequency of 1.0 MHz, for each intensity the concentration of Ce6 varied form 1 μg/ml to 10 μg/ml. After the treatment procedure, cells were re-suspended in fresh medium and cultured for an additional time as specified in the text and then subjected to different analysis.

Experiments were carried out in low-level light to minimize any influence of photo-activation.

For inhibitory experiment, 5, 7.5, 10 mM N-acetylcysteine (NAC), the ROS scavenger, was added to serum-free DMEM 1 h before loading Ce6. The inhibitor at the used concentration did not yield any significant cell damage to cultured cells.

2.4. Cytotoxicity

The cytotoxicity of PDT and SDT on MDA-MB-231 cells was analyzed using the MTT assay. Briefly, cells after different treatment were added to 96 well culture plates, and viability was determined by adding 20 μl MTT solution (2.5 mg/ml in PBS) to each well and the mixture was incubated for additional 4 h at 37 °C in a CO_2 incubator. After incubating, the mixture was removed and 150 μl pure DMSO was added per well. After shaking for 15 min at room temperature, the absorbance at 570 nm was recorded using a microplate reader (Bio-Tek ELX800, USA) against the reference value at 630 nm. Cytotoxicity was calculated using the following equation:

$$\text{Cytotoxicity}(\%) = (\text{OD}_{\text{control group}} - \text{OD}_{\text{treatment group}})/\text{OD}_{\text{control group}} \times 100\%$$

2.5. Determination of intracellular ROS

Intracellular ROS production was studied by measuring the fluorescence intensity of dichlorofluorescein (DCF) as described in our previous papers [3]. 2,7-DCF-diacetate(DCFH-DA), a non-fluorescent cell-permeant compound, is cleaved by endogenous esterases within the cell and the de-esterified product can be converted into the fluorescent compound DCF upon oxidation by intracellular ROS.

At 1 h after different treatment, cells were washed with PBS and incubated with serum-free DMEM containing 4 μM DCHF-DA at 37 °C in an incubator for 20 min with gentle shaking on a small shaking table. After incubation, samples were washed by PBS and harvested by trypsinization, then immediately detected by flow cytometry (Guava easyCyte 8HT, Millipore, USA). Histograms were analyzed using FCS Express V3 software.

2.6. Determination of mitochondrial membrane potential

Rhodamine 123 (Rho 123) was used to evaluate perturbation in mitochondria membrance potential (MMP) as previously described [18]. At 1 h after different treatment, cells were harvested and washed with PBS then incubated at 37 °C with 1 μg/ml Rho 123 in serum-free DMEM for 20 min followed by washing with PBS. Then samples were immediately detected by flow cytometry.

2.7. Cell membrane integrity

To monitor membrane permeability after SDT and PDT, PI was added. PI can stain the nuclei by intercalating between the stacked bases of nucleic acid. PI can enter cells only if the cell membrane becomes permeable, so it is widely used to measure the integrity of the plasma membrane. Briefly, after PDT or SDT cells were harvested in the indicated times and washed three times with PBS then re-suspensed in PBS containing 5 μg/ml PI for 5 min in the dark. The samples were then immediately detected by flow cytometry. Histograms were analyzed using FCS Express V3 software.