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Sonodynamic induced antitumor effect of radachlorin on solid tumor

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ABSTRACT

In this study, the correlation between sonodynamic antitumor effects on CT26 tumor and the intensity of ultrasound was evaluated and the relation between SDT effects and Radachlorin dose as well. The CT26 tumor was implanted in BALB/c mice and was exposed to 1 MHz ultrasound at the intensity of 1 W/cm^2, 2 W/cm^2 and 3 W/cm^2 for 15 min at 3 h after the Radachlorin injection. The tumor growth was measured at the long and short diameters every day after the treatment. The relationship between SDT antitumor effects and the Radachlorin dose was investigated by using 4 different dose groups: 20 mg/kg, 40 mg/kg, 60 mg/kg of the Radachlorin injection, and the control group. In each group, CT26 tumors were exposed to 1 MHz ultrasound with the intensity of 2 W/cm^2 for 15 min. From these results, we found that both the intensity of ultrasound and the Radachlorin dose play a significant role on increasing antitumor effects using SDT.

1. Introduction

Sonodynamic therapy(SDT) is a promising new approach which uses ultrasound based on photodynamic therapy(PDT) [1]. Ultrasound has an appropriate tissue attenuation coefficient for penetrating tissues and reaching deep-seated tumors with ability to focus the ultrasound energy on a small volume and to locally activate a preloaded sonosensitizer. These attractive features of this modality make it possible for researchers to substitute laser light with ultrasound in PDT for non-invasive treatment of deep-seated tumors. Sonodynamic therapy is based on the local activation of a systemically administered sonosensitizer (sonochemical sensitizer) such as porphyrin and chlorine by ultrasonic exposure. Ultrasound enhances the cytotoxic effects of sonosensitizer and the ultrasonic cavitation phenomenon is characterized by the generation, expansion, and collapse of cytotoxic singlet oxygen in a medium irradiated with ultrasound. The production of the singlet oxygen could be the principle mechanism for tumor destruction in application of SDT and the sonodynamic activation of sonosensitizer attributes to the enhancement of active oxygen generation through acoustic cavitation [2–5].

Chlorin e6 is an efficient photosensitizer since it has a low dark toxicity, fast and sufficiently selective accumulation in target tissue compared to porphyrin and its derivatives [6]. In PDT application, Radachlorin, a chlorophy ll a derivative including mainly sodium chorine e6, is found to efficiently generate singlet oxygen when irradiated with visible light. It has been reported that Radachlorin has very low toxicity in dark, high contrast tumor accumulation, rapid body evacuation, and high cytotoxicity [7]. By using Radachlorin and ultrasonic exposure together, sonodynamic therapy can be an ideal tumor treatment modality since the damage on surrounding normal tissues is minimized by the highly selective tumor accumulation of Radachlorin and the focused ultrasonic exposure. However, there have been only a few studies on the SDT effects of Radachlorin in cancer treatment even though there are several studies on Chlorin e6 which is a major component of Radachlorin [8,9].

In this study, the SDT induced antitumor effects using Radachlorin and ultrasound were investigated on CT26 tumor animal models. The relationship between the antitumor effects and Radachlorin dose, also and the intensity of ultrasound were evaluated to determine the optimum setting for effective sonodynamic therapy.

2. Materials and methods

2.1. Sonosensitizer

The Radachlorin was purchased from the RADA-PHARMA group (RADA-PHARMA Co. Ltd., Moscow, Russia), which was stable in solution at 0 ± 8 °C in the dark.

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2.2. Cell culture and mice

CT26 cells, N-nitroso-N-methyl urethane-induced mouse colon carcinoma cells of BALB/c origin [10], were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in 71% Dulbecco's modified Eagle's medium(DMEM) (Sigma, England) at 37 °C in 5% atmosphere. The male BALB/c mice (7 weeks old) were purchased from Hyochang Biolink(Daegu, Korea), and maintained under pathogen-free conditions. To make a CT26 tumor animal model, the 0.1 ml PBS suspension($1x10^6$ cells/ml) of CT26 cells was injected subcutaneously into the right flank of the mice using a syringe. When the tumor grew to a diameter of about 0.6 cm approximately 12 days after implantation, the treatment study was started.

2.3. In vitro and in vivo sonodynamic therapy

A piezoelectric disk transducer, 5 cm^2 in size and made of high-quality titanium, was the sonodynamic therapy unit SONOSTAT 135 (gbo Medizintechnik AG, Germany). *In vitro* insonation, an acoustically transparent gel(Pharmaaceutical Innovation inc., Newark, NJ) was placed between the top of ultrasound probe and the film base of the 24-well plate. Ultrasound irradiation was done using a 1.0 MHz device at intensity of 1 W/cm^2, 2 W/cm^2 and 3 W/cm^2 for 5 min. The CT26 cells were exposed to ultrasound at 2 h after the incubation of Radachlorin(2 μg/ml). The MTT assay is a quantitative colorimetric method to determine cell viability. The 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) assay was used to monitor the cytotoxicity of Radachlorin-mediated SDT on CT26 cells. Immediately after exposure, cells in 100 μl were added to 96 well culture plates, and viability was determined by adding 10 μl MTT solution(5 mg/ml in PBS) to each well, and the mixture was incubated for 4 h at 37 °C in CO_2 incubator. The formazan crystals were dissolved in 100 μl 10% SDS, 0.01 M HCI solution, and the absorbance at 540 nm was recorded using a multiscanner autoreader(Flurostar optima, BMG labtech, Germany) against the reference value at 620 nm. The cell survival of treated samples was then obtained by comparing the results of the incubated to the non-exposed control samples. *In vivo* insonation, thhe tumor-bearing mice were divided into 4 groups of 4 mice each; the control group, and those treated with Radachlorin alone, ultrasound alone and Radachlorin+ultrasound. Radachlorin was administered via the caudal vein. For the ultrasound treatment, mice were anesthetized with ketamin (100 mg/kg, i.p.). The hair over the tumor was shaved and ultrasound gel was applied to the naked skin. The mouse was fixed on a cork board and transducer was placed tightly on the tumor, which was exposed to ultrasound for 15 min(1.0 MHz). The tumor was exposed to ultrasound 3 h after Radachlorin administration.

2.4. Fluorescence system and fluorescence detection

The fluorescence measurements are performed using an FS-003V spectrometers(CLUSTER Ltd., Russia) with a working range of 410∼1000 nm, a spectral resolution of 3 nm (with fiber-optic probe), and an inverse linear dispersion of 0.5 nm/pixel. The excitation light source was 532 nm line of diode-pumped solid-state laser with 10 mW of power, which is low enough to avoid thermal effect in the target tissue. A Y-shaped ring fiber-optic probe(seven fibers with a diameter of 100 μm and a numerical aperture of 0.22) is used to deliver the excitation laser radiation to the tissue and to transmit the fluorescence radiation to the photodetector. The fluorescence light was filtered with a standard optical filter placed at the spectrometer entrance, allowing clear observation of the peaks without the influence of the original wavelength excitation fluorescence.

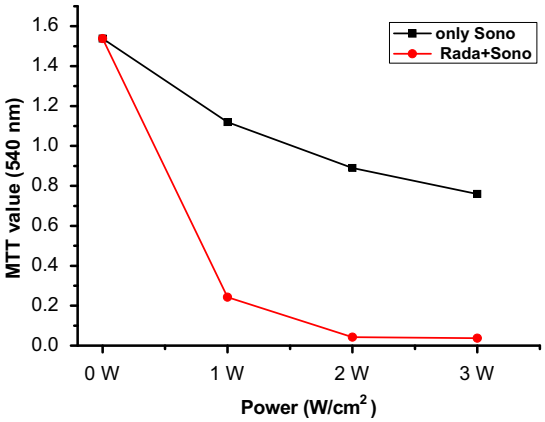

Fig. 1. *In vitro* effect of Radachlorin and/or ultrasound on isolated CT26 cells.

2.5. Evaluation of antitumor effect

The long and short diameters(a and b in mm) of the tumor were measured with a slide caliper every day after treatment. The tumor size was then calculated as (a+b)/2. The mean and standard deviation were calculated for each group.

2.6. Light microscopy examination

Mice solid tumor was harvested as described above, and fixed in 10% neutrally buffered formalin. Prepared tissue blocks of mice solid tumor were longitudinally sectioned at 10 μm thickness. Sections were stained with hematoxylin and eosin(H&E) and observed under a light microscope(LM).

3. Results and discussion

Several research groups have been focused on the studies of SDT using sonosensitizer to overcome the difficulties in PDT for the treatment of deep-seated malignant tumors. Ideal sonosensitizer should have certain characteristics: very low toxicity in the absence of ultrasound, high selectiveness of tumor accumulation, rapid body evacuation, water solubility, and finally high cytotoxicity. The studies for effects of porphyrins, chlorins, phthalocyanines, purpurins, and other chemical compounds have been reported in order to search for the most suitable sonosensitizer [2]. The sensitizer Chlorin e6, a Chlorin compound, has a good solubility in both polar and non-polar solution because it is hydrophilic. This property of Chlorin e6 results in high contrast of tumor accumulation and rapid body evacuation [11].

Two different sonodynamic mechanisms were proposed in the literatures. One mechanism proposed is that the augmentation of the cellular membrane disruption by sonosensitizers near the cavitation bubbles is the origin of the mechanism for SDT [12]. The other proposal is that reactive oxygen species(ROS) play an important role in antitumor effects in SDT [13]. In the generation of reactive oxygen species, molecules of the sonosensitizer are irradiated by ultrasound and the molecules become excited triplet state oxygen. When the triplet oxygen gets more ultrasonic energy, it will be excited to singlet oxygen. This singlet state oxygen is cytotoxic and initiates an oxidizing agent to kill tumor cells. The other point of view on ROS generation is that ultrasound activates sonosensitizer and then generates cavitation bubbles. The bubbles