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Antitumor effect of sonodynamically activated pyrrolidine tris-acid fullerene

音響化学的活性化されたピロリジントリス酸フラーレンによる抗腫瘍効果

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1. Introduction

Ultrasound has a tissue attenuation coefficient appropriate for penetrating intervening tissues to reach non-superficial objects while maintaining the ability to focus energy into small volumes. Sonodynamic therapy is a new and promising strategy for cancer treatment based on the generation of reactive oxygen species during irradiation by ultrasound in the presence of sonosensitizers selectively accumulated in the malignant target tissue [1-4].

Medical applications of nanoparticles are being extensively studied for the diagnosis and treatment, of disease because nanoparticles have interesting characteristics, such as small size and high reactivity. Fullerene, a nanoparticle about 1 nm in diameter, is smaller than the most nanoparticles (1-100 nm diameter) and holds promise for improving drug delivery. The absorption of visible light by fullerene allows it to transfer energy to oxygen molecules and very efficiently, generate reactive oxygen species. Since reactive oxygen promotes cell damage, we anticipated that fullerene might induce an antitumor effect [5-6].

Functionalized fullerenes, such as pyrrolidine tris-acid fullerene (PTF), have attracted particular attention due to their water solubility and potential application in tumor imaging and therapy as carbon nanomaterials. We here studied the sono-dynamically induced antitumor effect of PTF in combination with ultrasound using isolated sarcoma 180 cells and solid tumors isolated from mice injected with colon 26 carcinoma cells.

2. Materials and Methods

Ultrasound Exposure Apparatus

The ultrasound transducer used a piezoelectric ceramic disk 12 mm or 24 mm in diameter. A frequency of 2 MHz was used to maximize tumor absorption and penetration.

Evaluation of in vitro effect.

Sarcoma 180 cells (4×10^6 cells/ml) were prepared and transferred to a piezoelectric ceramic disk for exposure to ultrasound for up to 3 min in the presence or absence of PTF. The unstained fraction of cells was determined by counting the number of trypan blue-unstained cells.

Measurement of Singlet Oxygen.

ESR and spin trapping agents were used to measure singlet oxygen. Ultrasound induces nitroxide production; nitroxide produced in the presence or absence of PTF was reacted with an aqueous solution of 50 mM 2,2,6,6-tetramethyl-4-piperidone in the presence or absence of oxygen scavengers. The reaction product, 4-oxo-2,2,6,6-tetramethyl-4-piperidin-1-oxyl (4-Oxo-TEMPO), was measured by ESR spectroscopy.

Evaluation of antitumor effect.

Colon 26 carcinoma cells were transplanted into male 5-week-old CDF1 mice. The treatment study was started once the tumor grew to a diameter of about 10 mm (approximately 14 days after implantation). The tumor-bearing mice were divided into four groups of four mice: the control group, mice treated with PTF, and mice treated with ultrasound alone or PTF and ultrasound for 15 min. The tumor was irradiated in standing wave mode at a free-field intensity. The long and short diameters of each tumor were measured daily with a slide caliper following transplantation. Fourteen days after the treatment, the mice were sacrificed and the tumors were weighed, and stored in fixative solution.

3. Results and Discussion

PHF significantly enhanced ultrasonically

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induced *in vitro* damage, of isolated sarcoma 180 cells (Fig. 1), comparable to hematoporphyrin at the same concentration. In addition, the combination of PTF and ultrasound enhanced nitroxide generation. Fig. 2 shows the amounts of 4-Oxo-TEMPO ultrasonically generated in air-saturated aqueous solutions of 50 mM 2,2,6,6-tetramethyl-4-piperidone with and without PTF.

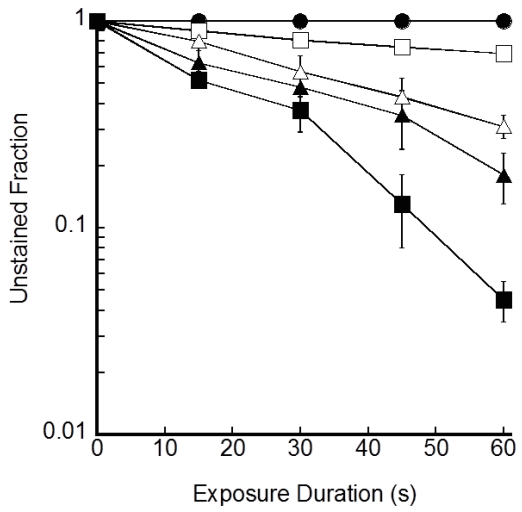

Fig. 1. *In vitro* effect of ultrasound with and without PTF on isolated sarcoma 180 cells. ●, 80 μM PTF alone; □, ultrasound alone; △, ultrasound + 20 μM PTF; ▲, ultrasound + 40 μM PTF; ■, ultrasound + 80 μM PTF. Data are presented as the mean ± SD of four experiments.

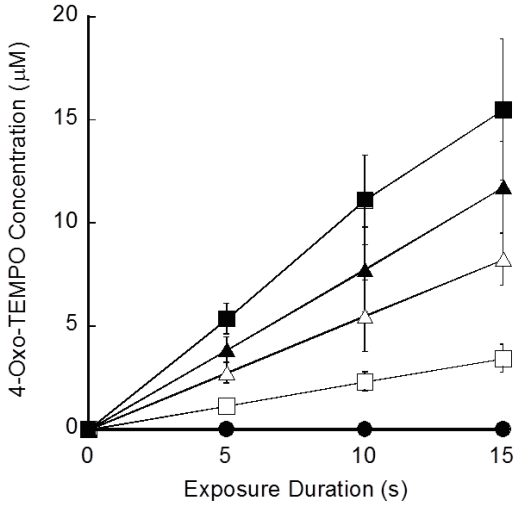

Fig. 2. 4-Oxo-TEMPO generation in an air-saturated solution of 50 mM 2,2,6,6-tetramethyl-4-piperidol-N-oxyl during exposure to ultrasound in the presence and absence of PTF. ●, 80 μM PTF alone; □, ultrasound alone; △, ultrasound + 20 μM PTF; ▲, ultrasound + 40 μM PTF; ■, ultrasound + 80 μM PTF. Data are presented as the mean ± SD of four experiments.

The amount of ultrasonically generated 4-Oxo-TEMPO increased linearly as the insonation time increased. PTF at a concentration of 80 μM enhanced the rate approximately three-fold. 4-Oxo-TEMPO generation was not observed with PHF alone.

Histologic sections of the mouse tumors are compared in Fig. 3. No significant histological change was observed in the tumors treated either with PTF alone or ultrasound alone (Fig. 3b and c). In contrast, combination treatment with PTF and ultrasound produced massive necrosis in the tumor region (Fig. 3d).

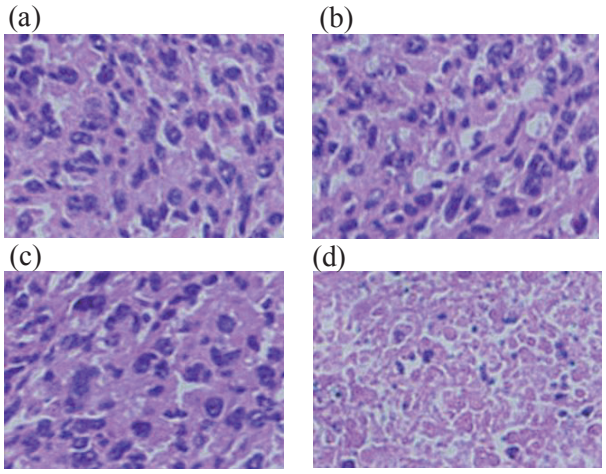

Fig. 3. Histologic sections (×400) of tumors are compared for (a) control, (b) PTF alone, (c) ultrasound alone, and (d) PTF + ultrasound.

These *in vitro* and *in vivo* results suggest that PTF is a potential sensitizer for sonodynamic tumor treatment. The results reported here are preliminary, but significantly support the possibility of the clinical sonodynamic treatment of tumors using water-soluble functionalized fullerenes, such as PTF.

4. References

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