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Sonodynamic antitumour effect of chloroaluminum phthalocyanine tetrasulfonate on murine solid tumour

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Abstract

The sonodynamically induced antitumour effect of chloroaluminum phthalocyanine tetrasulfonate (AlPcTS) was evaluated on subcutaneously implanted colon 26 carcinoma. A time of 24 h after the administration of AlPcTS was chosen for the ultrasonic exposure, based on the analysis of the AlPcTS concentrations in the tumour, plasma, skin and muscle. The pharmacokinetic analysis showed much faster clearance of AlPcTS than photofrin II from the body, which can be an advantage in view of their potential adverse effects. At an AlPcTS dose not less than 2.5 mg kg^{-1} and at a free-field ultrasonic intensity not less than 3 W cm^{-2}, the synergistic effect between AlPcTS administration and ultrasonic exposure on the tumour growth inhibition was significant. The ultrasonic intensity showed a relatively sharp threshold for the synergistic antitumour effect, which is typical for an ultrasonic effect mediated by acoustic cavitation. These results suggest that AlPcTS is a potential sonosensitizer for sonodynamic treatment of solid tumours.

Introduction

Ultrasound has an appropriate tissue attenuation coefficient for penetrating intervening tissues to reach non-superficial objects while maintaining the ability to focus energy into small volumes. This is a unique advantage when compared to electromagnetic modalities such as laser beams in its application to non-invasive treatment of non-superficial tumours.

Sonodynamic therapy (SDT) is a promising new modality for cancer treatment using ultrasound. SDT is based on the local activation of a systemically administered sonosensitizer by ultrasonic exposure (Yumita et al 1989; Umemura et al 1993). A mechanism for the sonodynamic activation of porphyrins attributed to the enhancement of active oxygen generation through acoustic cavitation has been suggested (Umemura et al 1990). We also have reported that chemical agents such as photofrin II (Porfimer Sodium, PF), the approved sensitizer used for photodynamic therapy, induced significant antitumour effect when activated with ultrasound (Tachibana et al 1997; Yumita et al 2000a). These results demonstrated that PF also has potential as a sonosensitizer, a sonochemical sensitizer, for tumour treatment with ultrasound (Yumita et al 2000b). However, the patients have to stay in a relatively dark environment for a few weeks after the injection of PF to avoid skin photosensitization because of its long retention time (Bellnier et al 1989; Peng et al 1991; Dougherty 1993).

Recently, certain sulfonated phthalocyanines have been developed as the second-generation photosensitizers for photodynamic therapy (Rosenthal 1991). They are eliminated more quickly from the body and cause less significant side effects than PF (Bellnier et al 1989). Among these phthalocyanines, chloroaluminum phthalocyanine tetrasulfonate (AlPcTS) showed the longest lifetime in the reactive triplet state when activated by photons, which can be a great advantage in the efficient generation of reactive oxygen species (Spikes 1986). Furthermore, AlPcTS maintains the characteristic of being more preferentially retained by tumours than normal tissues (Darwent et al 1982). Significant tumour tissue destruction was demonstrated using AlPcTS in combination with laser exposure. These results suggest that AlPcTS has a great potential

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as a photosensitizer for photodynamic therapy (Evensen & Moan 1987; Berg et al 1989a; Chan et al 1990; Peng et al 1990a; Peng & Moan 1995; Allen et al 2002; Vrouenraets et al 2002).

Assuming that the reactive state of AlPcTS also has a long lifetime when ultrasonically activated, it may be of interest to know whether AlPcTS has a potential as a sonosensitizer. In this study, the in-vivo effect of the combination of AlPcTS and ultrasonic exposure on a subcutaneously implanted solid tumour was investigated using ultrasound at 2 MHz in a standing wave mode. Colon 26 carcinoma, which is not responsive to many of the antitumour drugs including adriamycin, taxol, etc., was chosen for the experimental tumour because of its well-established malignancy.

Prior to the study described above (Yumita et al 2000a), it was confirmed that the optimum timing required to maximize the sonosensitizer concentration in the tumour rather than in the plasma is the ultrasonic exposure timing for sonodynamic tumour treatment, which is basically the same as for photodynamic treatment (Dougherty 1993). In order to determine the optimum timing for the ultrasonic exposure of the tumour, the time course of AlPcTS concentrations in the plasma, tumour, muscle and skin were measured. The tumour was exposed to ultrasound at the time when the AlPcTS concentration in the tumour was at its maximum.

Materials and Methods

Materials

AlPcTS was purchased from Porphyrin Products (St Louis, MO). All the other reagents were commercial products of analytical grade.

Tumour cells and animals

Colon 26 carcinoma was supplied by the Cancer Institute (Tokyo, Japan). The cell lines were passed weekly through male BALB/c mice (5 weeks old). Transplanted tumours were initiated by subcutaneous trocar injection of approximately 1 mm^3 pieces of fresh tumour into the left dorsal scapula region of male 5-week-old CDF_1 mice. When the tumours grew to a diameter of about 10 mm, approximately 14 days after implantation, the pharmacokinetic and treatment studies were started. The experimental animals were treated according to the guideline proposed by the Science Council of Japan.

Determination of AlPcTS concentration in plasma and tissue

AlPcTS was dissolved in a sterilized saline solution and administered to the tumour-bearing CDF_1 mice at a dose of 5 mg kg^{-1} by intravenous injection in the caudal vein. Under pentobarbital anaesthesia, the blood samples were obtained by a heart puncture 1, 5, 10, 30 min and 1, 2, 6, 12, 24, 48 and 72 h after injection. Immediately after sampling, the blood was placed in a heparin-coated test-tube and centrifuged at 2500 $\times g$ for 10 min to separate the plasma. The tumour, muscle and skin were taken immediately after the sacrifice of animals 6, 24, 48 and 72 h after injection. The tissues were excised, blotted dry and weighed. The samples were stored at $-20\,°C$ until used. Plasma and tissue samples were taken from the same animals. These samples were digested with 0.1 M NaOH (10 mL per 0.1 g wet weight tissue). After centrifuging at 3000 $\times g$ for 10 min, the clear supernatant was aspirated and the fluorescence intensity of the extracts was measured using a fluorescence spectrophotometer (model 650-10L, Hitachi, Tokyo, Japan; excitation 403 nm, emission 628 nm). A standard curve was obtained by adding known concentrations of AlPcTS to the corresponding tissue digests prepared from untreated animals.

Pharmacokinetic analysis

Pharmacokinetic analysis of the plasma disappearance of AlPcTS was performed based on a two-compartment open model. The plasma concentration of AlPcTS (C(t)) is described by equation (1). The observed plasma concentrations were fitted to this equation and pharmacokinetic parameters, A, α, B and β were determined by means of a non-linear least-squares method:

$$C(t) = A\ \exp(-\alpha t) + B\ \exp(-\beta t) \quad (1)$$

The area under the plasma concentration curve (AUC) from time zero to infinity, the plasma total body clearance (Cltot) and the distribution volume at the steady state (Vdss) were then calculated using the following equations:

$$AUC = A/\alpha + B/\beta \quad (2)$$

$$Cltot = dose/AUC \quad (3)$$

$$Vdss = dose(A\beta^2 + B\alpha^2)/(B\alpha + A\beta)^2 \quad (4)$$

Ultrasonic exposure system

The ultrasonic exposure set-up is shown in Figure 1. A piezoelectric ceramic disk transducer, 12 mm in diameter, was tightly bonded onto an aluminum matching layer,

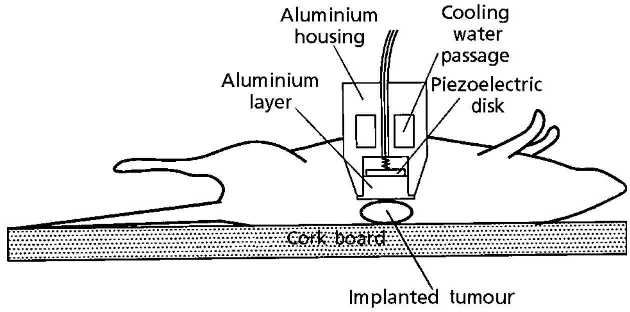

Figure 1 Schematic diagram of the ultrasonic exposure set-up. A cross-section of the ultrasonic transducer is shown.

which was cooled by circulating water to keep the transducer and bearing temperature below a certain level. The overall resonant frequency of the transducer was 1.92 MHz. Sine waves were generated by a wave generator (model MG442A, Anritsu, Tokyo) and amplified by an RF amplifier (model 210L, ENI, Rochester, New York). The sinusoidal drive signal of the transducer was monitored with an oscilloscope during the exposure. A standing wave exposure mode was chosen for the relatively easy generation of reproducible cavitation. However, the output acoustic power from the transducer was calibrated in a free field (progressive wave mode) to avoid difficulty in acoustic power estimation. The output acoustic pressure was measured in degassed water 30 mm from the transducer surface using a 1-mm-diameter polyvinylidene difluoride needle-type hydrophone (Medicoteknisk Institut, Denmark). Spatial average intensity was calculated by scanning the probe for 4 mm axially and laterally to eliminate the effect of ripples in the field due to Fresnel diffraction. The measured intensity was approximately proportional to the square of the peak-to-peak driving signal voltage of the transducer in the voltage range used for the exposure. In the in-vivo ultrasonic exposure experiments, the transducer was driven at a voltage corresponding to a certain free-field intensity, which is used to specify the intensity of ultrasonic exposure in this paper.

Treatment protocol

The tumour-bearing mice were divided into four groups of four mice: (1) the control group, those treated with (2) AlPcTS alone, (3) ultrasound alone, and (4) AlPcTS + ultrasound. For the treatments with AlPcTS, this was administered to a mouse via the caudal vein. For the treatments with ultrasound, a mouse was anaesthetized with sodium pentobarbital (40 mg kg^{-1}, i. p.). The hair over the tumour was shaved and ultrasound gel was applied to the naked skin. The mouse was fixed on a cork board with the tumour upwards. The thermistor probe (Anritsu) was inserted into the tumour to monitor the temperature. The transducer was placed tightly on the tumour, which was exposed to ultrasound for 15 min. The transducer was cooled by circulating water at 25 °C during the exposure to keep the temperature of the tumour below 35 °C, which is much lower than the hyperthermia level. For the combined treatment, the tumour was exposed to ultrasound 24 h after AlPcTS administration.

Evaluation of antitumour effect

The long and short diameters (a and b in mm) of the tumour were measured with a slide calliper every day after transplantation. The tumour size was calculated as $(a + b)/2$. The mean and standard deviation (s.d.) were calculated for each group.

Results

The concentrations of AlPcTS in the plasma after its intravenous administration are shown in Figure 2. The observed data were best fit by the bi-exponential equation (equation 1); the calculated pharmacokinetic parameters are listed in Table 1. The elimination half-life at the terminal phase ($t_{1/2\beta}$) was 3.16 h. The time courses of AlPcTS concentration in the tumour, skin and muscle are shown in Figure 3. The highest concentration of AlPcTS in the tumour was

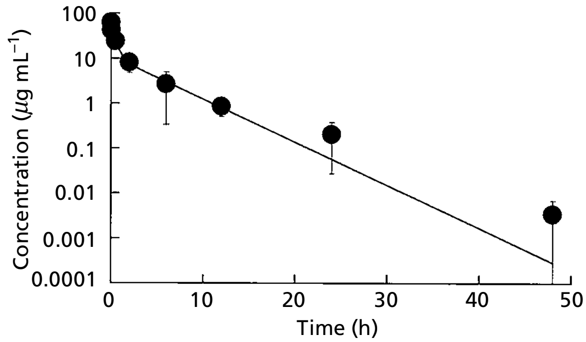

Figure 2 Time course of AlPcTS concentration in plasma after intravenous administration. Each point and vertical bar represents the mean ± s.d. of four mice. The data are fitted with a bi-exponential curve.

Table 1 Pharmacokinetic parameters of AlPcTS after intravenous administration.

Parameters[a]	
A	40.3 μg mL^{-1}
B	11.1 μg mL^{-1}
α	2.16 h^{-1}
β	0.219 h^{-1}
Cltot	0.145 mL h^{-1} kg^{-1}
Vdss	249 mL kg^{-1}

[a]Calculated from the mean plasma concentrations of four mice.

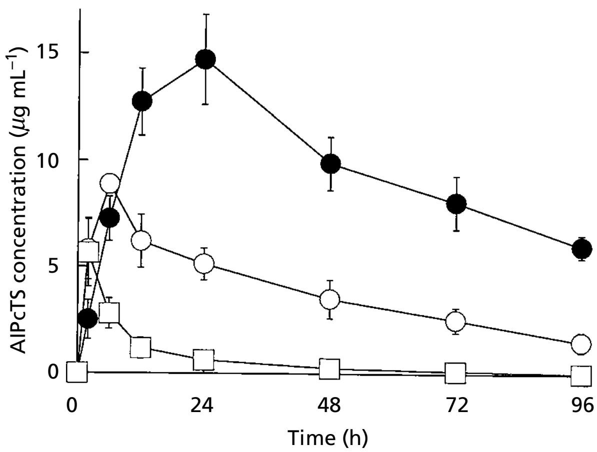

Figure 3 Time course of AlPcTS concentration in tumour, skin and muscle after intravenous administration. ●, Tumour; □, muscle; ○, skin. Each point and vertical bar represents the mean ± s.d. of four mice.

observed 24 h after administration. Twenty four hours or longer after the administration of AlPcTS, its concentration in the tumour exceeded those in the plasma and the muscle by an order of magnitude and was approximately three times higher than that in the skin.

The effect of each treatment on the growth of colon 26 carcinoma is compared in Figure 4 by plotting the tumour size for two weeks after the day of the treatment. AlPcTS alone at a dose of 2.5 mg kg^{-1} had no inhibitory effect. Ultrasound alone at a free-field intensity of 3 W cm^{-2} showed a slight inhibitory effect. AlPcTS + ultrasound showed such a significant antitumour effect that the tumour size decreased to smaller than half three days after the treatment. The tumour started growing again after that point, but the ratio of the treated tumour size to the untreated was kept constant at approximately a third.

The effect of ultrasonic intensity on the tumour growth at an AlPcTS dose of 2.5 mg kg^{-1} is shown in Figure 5. The five curves correspond to free-field ultrasonic intensities of 0, 1, 2, 3 and 5 W cm^{-2}, respectively. The ultrasound intensity threshold for the synergistic antitumour effect is clearly seen between the free-field intensities of 2 and 3 W cm^{-2}.

The effect of AlPcTS dose on the tumour growth at a free-field ultrasonic intensity of 3 W cm^{-2} is shown in Figure 6. The five curves correspond to AlPcTS doses of 0, 0.5, 1.0, 2.5 and 5.0 mg kg^{-1}, respectively. The synergistic antitumour effect became more and more significant as the AlPcTS dose increased.

Discussion

The adverse effect of sonodynamic as well as photodynamic treatment can be minimized by choosing the exposure timing when the tumour-to-plasma and tumour-to-normal-tissue ratios of the sensitizer concentration are significantly high (Dougherty 1993). In order to determine the optimum timing for ultrasonic exposure, the concentration of AlPcTS in the plasma, tumour, muscle and skin was measured and analysed. The AlPcTS concentration in the plasma was well explained by the two-compartment open model, resulting in the distribution volume (Vdss) of 249 mL kg^{-1} and the plasma total body clearance (Cltot) of 0.145 mL h^{-1} kg^{-1}. This small value suggests that AlPcTS does not markedly distribute in normal tissues. Twenty four hours after the administration, the AlPcTS concentration in the tumour reached its maximum and was at least a few times higher than those in normal tissues such as plasma, skin and muscle. These results agree well with previous

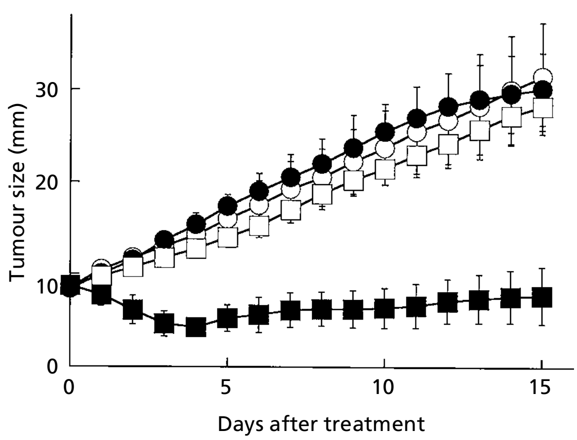

Figure 4 Effect of AlPcTS and/or ultrasound on growth of colon 26 carcinoma. ○, Control; ●, AlPcTS alone; □, ultrasound alone; ■, AlPcTS + ultrasound. AlPcTS was administered 24 h before the treatment at a dose of 2.5 mg kg^{-1} and a free-field ultrasonic intensity of 3 W cm^{-2} was used. Each point and vertical bar represents the mean ± s.d. of four mice.

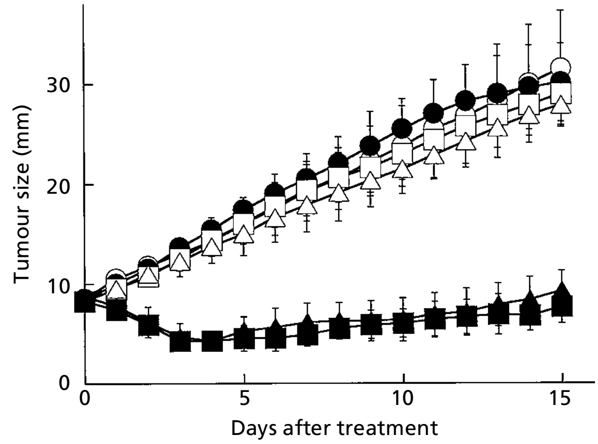

Figure 5 Effect of ultrasonic intensity on tumour. AlPcTS was administered 24 h before the treatment at a dose of 2.5 mg kg^{-1} except for the control mice. ○, Control; ●, free-field ultrasonic intensity of 0; □, 1 W cm^{-2}; △, 2 W cm^{-2}; ▲, 3 W cm^{-2}; ■, 5 W cm^{-2}. Each point and vertical bar represents the mean ± s.d. of four mice.

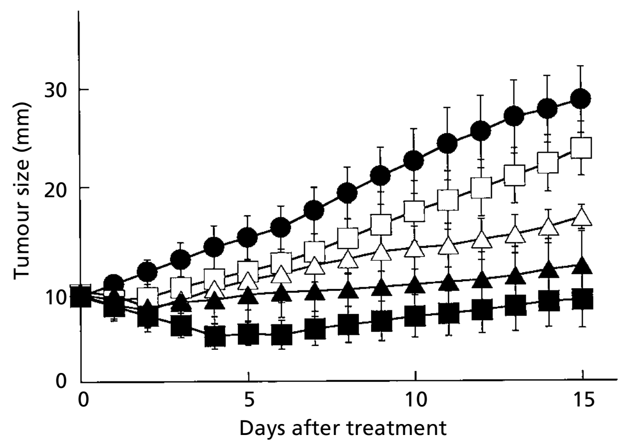

Figure 6 Effect of AlPcTS dose on tumour growth. AlPcTS was administered 24 h before the treatment, and the tumour was exposed to ultrasound at the free-field intensity of 3 W cm^{-2}. ●, AlPcTS dose of 0; □, 0.5 mg kg^{-1}; △, 1.0 mg kg^{-1}; ▲, 2.5 mg kg^{-1}; ■, 5.0 mg kg^{-1}. Each point and vertical bar represents the mean ± s.d. of four mice.

studies indicating that the uptake of AlPcTS in tumour tissues was higher than in normal tissues (Evensen & Moan 1987; Berg et al 1989b; Peng et al 1990a; Peng & Moan 1995). We chose the ultrasonic exposure timing of 24 h after the intravenous administration of AlPcTS based on these results.

The accumulation of phthalocyanine compounds in tumours has been reported in a variety of tumours in experimental animals and human beings (Evensen & Moan 1987). Recent in-vitro and in-vivo studies suggest the involvement of the low-density lipoprotein (LDL) receptor pathway as the mechanism of the accumulation of porphyrin and phthalocyanine compounds (Kessel 1986; Peng et al 1990b). The elimination half-life of 3.16 h was about half the reported value of PF, and the total body clearance of 0.145 $mL\,h^{-1}\,kg^{-1}$ was about an order of magnitude larger than PF (Yumita 2000b). This could be an advantage of AlPcTS over PF regarding their potential adverse effects.

When both the AlPcTS dose and ultrasonic exposure intensity were higher than certain levels, a significant antitumour effect was observed. At an AlPcTS dose not less than 2.5 $mg\,kg^{-1}$ and at a free-field ultrasonic intensity not less than 3 $W\,cm^{-2}$, the synergistic effect between AlPcTS administration and ultrasonic exposure on the tumour growth inhibition was marked.

The ultrasonic intensity showed a relatively sharp threshold. This is typical for an ultrasonic effect mediated by acoustic cavitation, which is known to consist of two stages: (1) nucleation and growth of microbubbles under acoustic pressure and (2) their sudden collapse. Sonochemical effects such as active oxygen generation are induced at the second stage, while the first stage requires ultrasonic intensity higher than a certain level, termed the 'cavitation threshold', which is much higher than the intensity required for the second stage.

The AlPcTS dose showed a broader threshold and the antitumour effect was gradually intensified as the dose increased. The observed effective dose of AlPcTS is one or two orders of magnitude lower than its lethal dose ($LD_{50} = 150\,mg\,kg^{-1}$, i.v., for a mouse) (Evensen & Moan 1987). Thus, as a potential adverse effect in the sonodynamic treatment with AlPcTS, the toxicity of AlPcTS alone may be much less important than the potential photosensitive dermatitis. From this point of view, the considerable accumulation of AlPcTS in the tumour can be an advantage for the sonodynamic treatment using AlPcTS as a sensitizer.

Assuming that AlPcTS concentration in the tumour increases steadily as the dose increases in the range of dose in this study, the observed synergistic antitumour effect can be regarded as being highly dependent on the AlPcTS concentration in the tumour. Therefore, based on the presented results and the previously reported in-vitro experimental results (Evensen & Moan 1987), we think that the observed in-vivo cytotoxic effect may also be attributed to sonochemical activation of AlPcTS.

Because of the synergistic antitumour effect between AlPcTS and ultrasound at their proper doses, the average tumour size continued to decrease for three days after the treatment. It then started growing gradually again, but the ratio of the treated to untreated tumour size remained approximately constant at a third or less. The present series of experiments was carried out in accordance with the protocol under which one course consists of a single treatment for simplicity although it is expected that further repeated treatment may yield results with a higher clinical impact.

In summary, the presented pharmacokinetic properties of AlPcTS in the tumour and normal tissues in combination with the presented ultrasonically induced inhibitory effect on the tumour growth suggest that AlPcTS is a potential sonosensitizer for tumour treatment. The results reported in this paper are experimental, but they significantly support the possibility of sonodynamic treatment using AlPcTS. In future studies, experiments with animals of a size similar to humans, using focused ultrasound rather than plane waves, need to be performed. In this way, the synergy between the molecular selectivity of the sonosensitizer and the geometric selectivity of focused ultrasound will be achieved so as to suppress the adverse effects that may otherwise take place outside of the region to be treated.

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