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Sonodynamically induced antitumor effect of Photofrin II on colon 26 carcinoma

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Abstract The sonodynamically induced antitumor effect of Photofrin II (PF), was evaluated in mice bearing colon 26 carcinoma. In order to find the optimum timing for ultrasonic exposure after the administration of PF, the PF concentrations in the plasma, skin, muscle, and tumor were measured. The antitumor effect was estimated by measuring the tumor size. Since the highest concentration of PF in the tumor was observed 24 h after administration, an ultrasonic exposure timing of 24 h after the intravenous administration of PF was chosen. When used alone, ultrasound showed a slight antitumor effect, which became increasingly significant as the dose of PF was increased, while use of PF alone showed no significant effect. From these results, it is concluded that PF significantly sensitizes solid tumors to ultrasound, demonstrating a synergistic antitumor effect.

Key words Ultrasound · Photofrin II · Colon 26 · Antitumor effect · Sonodynamic therapy

Abbreviations PF Photofrin II · Hp Hematoporphyrin · HPD Hematoporphyrin derivative

Introduction

There are several major modalities being used clinically for cancer therapy. However, most of these are still far from ideal in terms of tumor-selectivity and side effects. Ultrasound can penetrate deeply into tissues while maintaining its spatial selectivity, typically by being focused. This is a unique advantage when compared to electromagnetic modalities, such as laser beams, in the application to non-invasive treatment of non-superficial tumors. Although the use of ultrasound for tumor treatment has been relatively well-investigated with respect to the thermal effects due to ultrasound absorption (Lele 1982), only a few studies have been reported with respect to non-thermal effects such as the sonochemical effects due to ultrasound cavitation (Kremkau 1979; Kessel et al. 1994; Yumita et al. 1987).

Hematoporphyrin derivative (HPD) is a complex mixture of oligomers of hematoporphyrin (Hp) and is retained preferentially in tumor tissues much longer than in normal tissues. HPD and its more homogeneous form, Photofrin II (PF), are the most widely used sensitizers for photodynamic treatment (PDT) of tumors in combination with laser beam irradiation, both in experimental studies and in clinical trials (Dougherty et al. 1975; Hayata et al. 1982). Photo-induced cytotoxicity of PF occurs through photo-oxidation reactions, initially involving the excitation of a porphyrin molecule by light. The excited photosensitizer may accept or lose an electron leading to free radical reactions (Type I) or may transfer energy to oxygen producing singlet oxygen (Type II).

Recently, we found that photochemically active porphyrins, such as hematoporphyrin (Hp), and a gallium porphyrin complex, 7,12-bis(1-decyloxyethyl)-Ga(III)-3,8,13,17-tetramethyl-porphyrin-2,18-dipropionyl-diaspartic acid (ATX-70), induce significant cell damage when activated with ultrasound (Yumita et al. 1989; Umemura et al. 1993; Yumita et al. 1995). Implanted mouse tumors were treated with ultrasound after administration of such porphyrins and tumor growth was significantly inhibited at an intensity with which ultrasound alone showed only a slight inhibitory effect (Yumita et al. 1990). These results demonstrated that such porphyrins have a potential as a sonochemical sensitizer for tumor treatment with ultrasound. We suggested that this new modality may be referred to as “sonodynamic therapy” (Umemura et al. 1990).
Ultrasonically induced oxidation enhanced by such porphyrins in aqueous solutions was confirmed by detecting nitroxide formation from 2,2,6,6-tetramethyl-4-piperidone by electron spin resonance (ESR) spectroscopy (Yumita et al. 1994). The effect of active oxygen scavengers on the ultrasonically induced cell damage in suspension, and also the effect of deuterium oxide substitution for hydrogen oxide in the suspending medium, were both consistent with a hypothesis that singlet oxygen generation by ultrasonically activated porphyrin molecules was the most important mediator for the porphyrin-enhanced cell damage (Umemura et al. 1990; Umemura et al. 1993). This hypothesis is similar to the above-described Type II mechanism of photo-induced cytotoxicity.

Although HPD and PF are most widely used for PDT, ultrasonically induced antitumor effects of PF have only briefly been reported (Tachibana et al. 1993; Tachibana et al. 1994). Tachibana et al. (1993, 1997) observed an enhancement of ultrasonically induced in vitro cell killing and in vivo liver tissue damage in the presence of PF, but the effects on tumors have not yet been reported. The use of PF in combination with ultrasonic exposure may also be effective for tumor treatment as with the porphyrins described above.

In this study, in vivo effects of the combination of PF and ultrasound on the solid tumor of colon 26 carcinoma were investigated using ultrasound at 2 MHz in standing wave modes. In order to establish the optimum timing for the ultrasonic exposure of the tumor, the time course of PF concentrations in the plasma, tumor, muscle, and skin were measured and analyzed. The tumor was exposed to ultrasound at the time when PF concentrations in the tumor were at their maximum.

Materials and methods

Materials

PF was supplied by Nihon Ledari (Tokyo, Japan). All the other reagents were commercial products of analytical grade.

Tumor 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 tumors were initiated by subcutaneous trocar-injection of approximately 1 mm³ pieces of fresh tumor into the left dorsal scapula region of male 5-week-old CDF₁ mice. When the tumor grew to a diameter of approximately 10 mm approximately 14 days after implantation, the pharmacokinetic or treatment study was started. The experimental animals were treated according to the guidelines proposed by the Science Council of Japan.

Determination of PF concentration in plasma and tissue

PF was dissolved in a sterilized saline solution and administered to tumor-bearing CDF₁ mice at a dose of 5 mg/kg by intravenous injection from the caudal vein. Under pentobarbital anesthesia the blood samples were obtained by heart puncture 1, 2, 6, 12, 24, 48, and 72 h after injection. Immediately after the sampling, the blood was placed in a heparin-coated test tube and centrifuged at 2500 g for 10 min to separate plasma. The tumor, the muscle, and the skin were taken immediately after the animals were killed 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 (0.2 ml) was mixed with 2.5 ml of 10 mM ethylenediaminetetraacetic acid (EDTA) and 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 7.4). A portion of tissue (0.25 g) was homogenized in 2.5 ml of the same buffer. Then the sample was shaken with 6 ml of chloroform:methanol mixture (1:1, v/v) for extraction. After centrifuging at 3000 × g for 10 min, the chloroform layer was removed and the aqueous layer was shaken with 3 ml of chloroform for the second extraction. Both first and second chloroform layers were combined and evaporated to dryness in a water bath at 30°C. The residue was dissolved in 0.1–1.0 ml of methanol. The concentration of PF was determined by measuring the fluorescence intensity using a fluorescence spectrophotometer (model 650–10L, Hitachi, Tokyo, Japan, excitation 403 nm, emission 628 nm).

Pharmacokinetic analysis

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

\[
C(t) = A \exp(-\alpha t) + B \exp(-\beta t)
\]  

(1)

The area under the plasma concentration curve (AUC) from time zero to infinity, the plasma total body clearance (Cl_tot), and the distribution volume at the steady state (V_dss) are given by the following equations:

\[
AUC = \frac{A}{\alpha} + \frac{B}{\beta}
\]

(2)

\[
Cl_{tot} = \frac{Dose}{AUC}
\]

(3)

\[
V_{dss} = \frac{Dose(\alpha + \beta^2 + B \times \alpha)}{(B \times \alpha + A \times \beta)^2}
\]

(4)

Ultrasound exposure system

The ultrasonic exposure set-up was shown in Fig. 1. A piezoelectric ceramic disk transducer, 24 mm in diameter, was tightly bonded onto an aluminum matching layer, which was cooled by circulating water to keep the transducer and tumor 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 MG442 A, Anritsu, Tokyo) and amplified by an RF amplifier (model 210L, ENI, Rochester, N.Y., USA). The sinusoidal drive signal of the transducer was monitored by an oscilloscope during the ultrasonic 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 (Medicotechnik Institut, Denmark). Spatial average intensity was calculated by scanning the probe, for 4 mm axially and 10 mm laterally, to eliminate the effects of rippling and 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 as “the free-field intensity” in this paper.