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Novel After-loading Interstitial Photodynamic Therapy of Canine Transmissible Sarcoma with Photofrin II and Excimer Dye Laser

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Novel after-loading interstitial photodynamic therapy was performed in a canine transmissible sarcoma (CTS) model, utilizing photofrin II and an excimer dye laser. First, photofrin II was injected intravenously at a dose of 5 mg/kg, then 48 h later, laser-proof plastic tubing was inserted into the CTS, followed by photoradiation of the tumor from the inside. The mean diameter of tumor necrosis rapidly increased in parallel with increase in total irradiation energy below 240 J/cm; the mean diameter of tumor necrosis was 20.7 mm at an energy of 120 J/cm, and 24.5 mm at 240 J/cm. Beyond 240 J/cm, the diameter gradually increased to 26 mm at 960 J/cm. As a side effect, cutaneous tissue showed a deep open ulcer at 240 J/cm, a shallow open ulcer at 180 J/cm, and a scar healing at 120 J/cm. The thermal effect of laser light is considered negligible below 480 J/cm.

Key words: Canine transmissible sarcoma — Photofrin II — Excimer dye laser — Interstitial photodynamic therapy — Laser-proof plastic tube

Photodynamic therapy (PDT) is a cancer treatment, based on the synergistic photochemical reaction between tumor cells containing accumulated photosensitizer and laser light.[1] The mechanisms of killing of malignant cells were studied by several researchers.[2–5] Photosensitizers accumulate 3 to 9 times more in malignant cells than in normal cells,[6] and this concentration difference contributes to the selective killing of the malignant cells. In general, the transmission capacity of light in tissue seems to be low,[7–9] and a high light energy delivered for a long period, causes thermal change in the tissue.[10] Therefore, provided the malignant tumor is treated with a sufficiently low light energy to avoid thermal effects, the use of photosensitizers and highly transmissive laser light should be favorable. Long-wavelength pulsed laser light is considered to be the best regimen in PDT,[11] and an excimer pumped dye laser seems to give better light transmission than a continuous-wave argon-pumped dye laser.[11] Furthermore, an effective light delivery system must be established. A cylindrical diffuser fitted to the fiber tip has been used to treat subcutaneous tumors[12] and obstructing malignant tumors in the gastro-intestinal and tracheo-bronchial tract under endoscopic observation.[13] Photofrin II (DHE) has been officially adopted for PDT in Japan,[14] and in recent clinical trials, superficial cancers of the stomach, bronchus, esophagus and cervix were treated with PDT with a cure rate of more than 80%.[14] This success encouraged us to try the application of PDT to non-superficial cancer. In order to test the efficacy of our novel after-loading interstitial PDT, we employed a canine transmissible sarcoma (CTS) model.[15] First, laser-proof plastic tubing was inserted into the CTS, followed by the insertion of an optic fiber and photo-radiation of the tumor from the inside.

The aim of this study was to investigate the effects and side-effects of this new method on the malignant tumor and normal tissues, utilizing CTS-bearing dogs treated with photofrin II (DHE) and an excimer dye laser.

MATERIALS AND METHODS

Photosensitizer Photofrin II was supplied by Quadralogics Technologies, Inc., Vancouver, Canada.[16] Photofrin II contains 86.5% porfimer sodium and 13.5% related compounds. It was dissolved in 5% glucose solution at a concentration of 2.5 mg/ml and administered intravenously in volumes ranging from 8 to 14 ml. The pharmaceutical data in dogs have already been reported.[17]

Animal and tumor models Five female beagle dogs, 12 to 16 weeks old, bearing CTS,[11] were supplied by the Department of Veterinary Surgery, Faculty of Agriculture, University of Tottori, Tottori, Japan. CTS is of mesodermal origin, and it was maintained as subcutaneous tumors in the inguinal region by injection of 10^7 cells of the CTS tumor.[15] The mean body weight of the five dogs was 6.2 ± 0.6 kg, and they were anesthetized with an intramuscular administration of ketamine at a dose of 20 mg/kg during the experiment. The experiments were started when the diameter of the tumor reached about 35 mm, at about 4 weeks after transplantation, since a

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tumor of more than 35 mm in diameter frequently exhibits central necrosis. The same dogs were used in experiments designed to investigate normal skin damage following PDT.

***In vivo* light source and light delivery system** An excimer pumped dye laser (Hamamatsu Photonics K.K., Hamamatsu), was used in all *in vivo* PDT experiments.[18] The laser was tuned to 630 nm for photofrin II-mediated PDT. The wavelength of emitted light was recorded with a spectroscope and the laser output was measured with a power meter in every experiment. The peak energy of each pulse was 500 kW and its duration was 10 ns. Pulses were delivered at a rate of 80 Hz. A single quartz fiber interfaced to the dye laser was used to deliver light. In order to radiate light from the inside of the tumor, laser-proof polypropylene-based plastic tubing (external and internal diameters, 1.5 mm and 1.2 mm respectively; thickness 0.15 mm) (Yokohama Electronics Engineering Laboratory, Yokohama) (Fig. 1) was inserted into the tumor,[19] and an optic fiber was passed into it. The characteristics of the plastic tubing were as follows: specific gravity, 1.41 at 23°C; maximum tension, 620 kgf/cm^2 at 23°C; modulus of bending elasticity, 26400 kgf/cm^2 at 23°C; melting point, 135°C; specific heat, 0.35 kcal•m/kg•°C at 20°C. The external and core diameters of the optic fiber were 1.0 mm and 0.6 mm, respectively. The distal tip of the optic fiber was cut at an angle of 45°, so that the laser beam was delivered perpendicular to the optical axis of the optical fiber (Fig. 2). As the plastic tubing was fabricated from scattering materials, the laser beam was emitted with scatter in a conical shape with large divergence (conical angle: 33°) (Fig. 3). The fiber tip was rotated at a speed of 12 rpm and performed a reciprocal motion from one end to the other at a speed of 18 mm per min. The radiation dose was expressed as the integrated energy emitted from 1 cm of tubing during one reciprocal motion of the fiber tip.

Tumor temperature measurements A separate group of dogs was used to investigate tumor temperature profiles as a function of shifting of the fiber tip, that is, the light source. An 18-gauge thermocouple needle (Technoseven Inc., Tokyo) was inserted into the tumor, 3 mm from the plastic tubing and perpendicular to its axis. Intratumor temperatures were expressed as a function of shifting of the fiber tip.

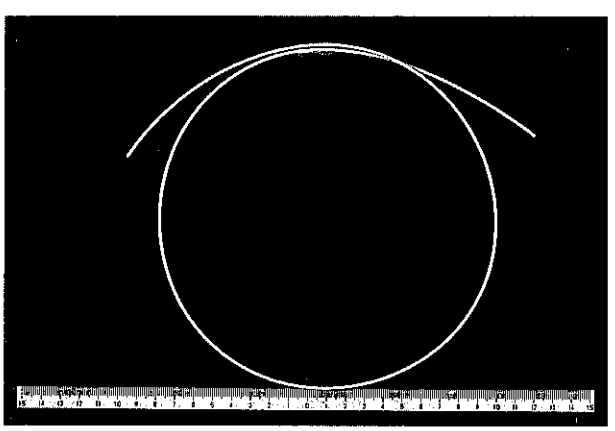

Fig. 1. Laser-proof plastic tubing. The color is milky white, specific gravity is 1.4 at 23°C, melting point is 135°C, maximum tension is 620 kgf/cm^2 at 23°C and the modulus of bending elasticity is 26400 kgf/cm^2 at 23°C. The external diameter varies from 0.7 mm to 1.5 mm.

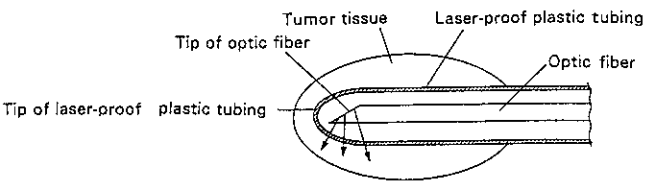

Fig. 2. Schematic model of interstitial PDT. Laser-proof plastic tubing was implanted in the tumor. An optic fiber, whose tip was cut at an angle of 45°, was inserted into it. Laser light is delivered in a direction perpendicular to the axis of the optic fiber. The fiber tip is rotated at 12 rpm, and performs a reciprocal motion at 18 mm/min. The external and internal diameters of the plastic tubing were 1.5 mm and 1.2 mm, respectively.

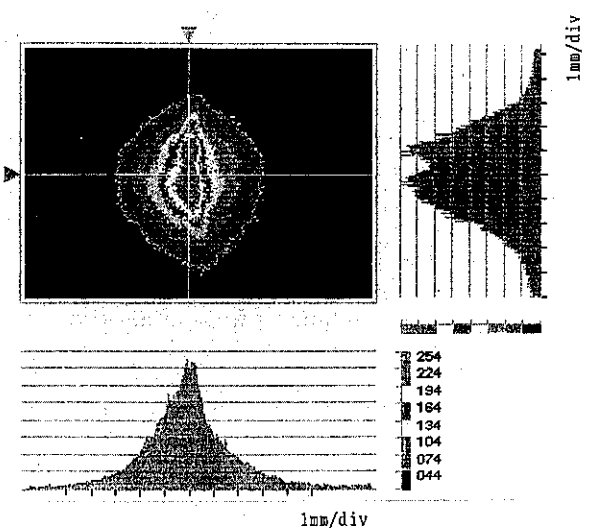

Fig. 3. Distribution of light intensity. A light source was positioned 1 cm from the CCD camera. An optic fiber with a 45° cleaved tip, which was covered with plastic tubing having scattering properties, provides a conical light beam directed perpendicular to the optical axis with a large divergence (conical angle 33°). The distribution of light intensity was almost circular.

PDT tumor treatments Macro- and microscopic examinations of a cross section of the tumor were used to evaluate photofrin II-induced PDT responses. Prior to tumor treatment, photofrin II was administered intravenously at a concentration of 5 mg/kg. At 48 h thereafter, PDT was performed. A 400 mJ/s light dose (5.0 mJ/pulse, 80 Hz) was adopted for all PDT experiments, and the total light doses ranged from zero to 1200 J/cm. The tumors were excised 6 days later and fixed in 20% formalin solution. The macro- and microscopic findings in the tumor cross-section were examined. The tumor was cut every 5 mm along the axis of optic fiber, and the degree of tumor necrosis was calculated as the average diameter of round-shaped tumor necrosis in each cross-section. The diameter was measured eight times with the tube hole as the center by shifting by an angle of 22.5° and then calculating the mean diameter. The largest diameter was chosen to represent the tumor necrosis.

PDT-mediated normal skin response Normal skin response to PDT was evaluated in five dogs administered with photofrin II. As CTS was transplanted in the subcutaneous tissue, the normal skin tissue near the tube was, as a matter of course, irradiated simultaneously during PDT. The cutaneous damage was classified into five groups. Group I: scar healing, group IIa: shallow open ulcer, group IIb: deep open ulcer, group III: fistula, group IV: drainage of necrotic tissue.

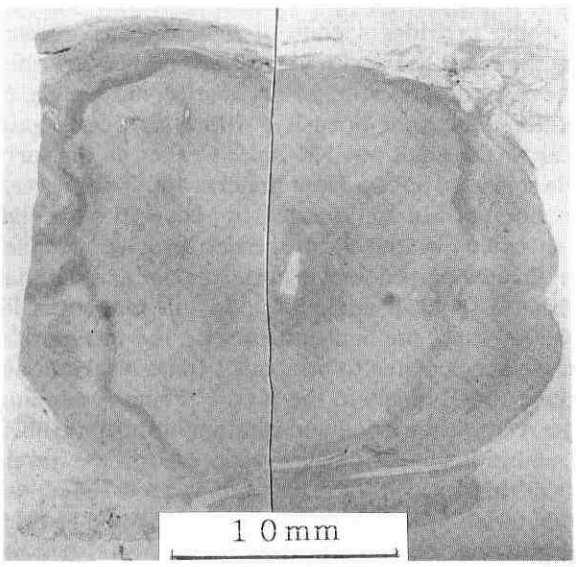

Fig. 4. Histology of a cross section of an irradiated tumor. The irradiated tumor was cut at intervals of 5 mm along the axis of the plastic tubing. The mean diameter was 24 mm with 5.0 mJ/pulse at 80 Hz. The total energy of radiation was 720 J/cm.

RESULTS

The irradiated tumor showed a round-shaped necrosis in the cross-section of CTS (Fig. 4). Inflammatory cells and hemorrhages were found in the marginal region of the necrosis (Fig. 4). In that region, collagen fibers and necrotic tissues may disturb light transmission and reduce the tumoricidal effect. In the case of photo-radiation without administration of photosensitizer, the tumor necrosis also displayed a round shape, whose mean diameters and standard deviations were 1.5 ± 0.8 mm at 240 J/cm, 3.2 ± 0.8 mm at 720 J/cm, and 6.4 ± 1.6 mm at 1200 J/cm. The thermal effect of photo-radiation is minimal up to 240 J/cm. The malignant tissue surrounding the necrosis seemed to be edematous. As shown in Fig. 5, the photo-radiation dose is correlated to the mean diameter of tumor necrosis after photosensitizer administration. The mean values and standard deviations of diameters of tumor necrosis were 20.7 ± 3.1 mm at 120 J/cm

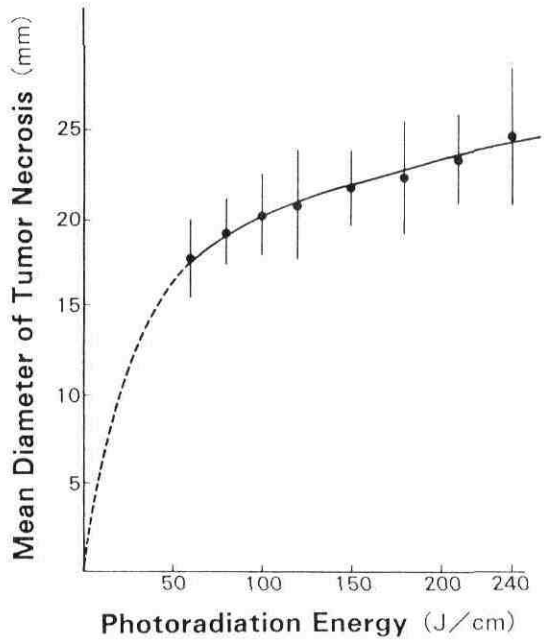

Fig. 5. Relationship between mean diameter of tumor necrosis and photo-radiation energy. After PDT with 5.0 mJ/pulse at 80 Hz, tumors were cross-sectioned at intervals of 5 mm along the axis of the plastic tubing. The mean diameter of the largest cross-sectional area of tumor necrosis was adopted as an index of PDT efficacy. The mean values and standard deviations of tumor necrosis are presented at various levels of radiation energy. The diameter of tumor necrosis was 20.7 mm at 120 J/cm, and 24.5 mm at 240 J/cm. The side effects of PDT on cutaneous tissues were as follows: scar healing at 120 J/cm, a shallow ulcer at 180 J/cm, and a deep ulcer at 240 J/cm.

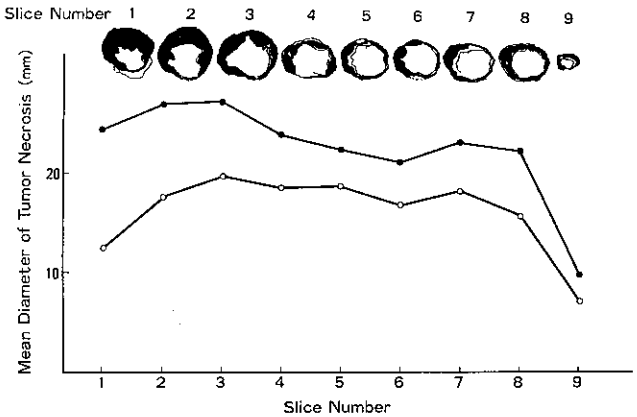

Fig. 6. Serial cross section of tumor necrosis in an irradiated tumor. The irradiated tumor was cut at intervals of 5 mm along the axis of the plastic tubing after photoradiation at 720 J/cm. The mean diameter of tumor necrosis is not constant for a single stroke of the optical fiber. The total light energy seems to be more integrated in the central part of the tumor. The closed circles (●) represent the mean diameters of the tumor cross section. The open circles (○) represent the mean diameters of tumor necrosis.

and 24.5±3.8 mm at 240 J/cm. The cutaneous tissues near the tubing after PDT showed scar healing at 120 J/cm, a shallow ulcer at 180 J/cm, a deep ulcer at 240 J/cm and a fistula above 960 J/cm. In every dog, the tumor temperature was measured continuously during photo-radiation up to 720 J/cm; there was a transient rise of temperature (4°C), when the laser light passed near the detector at 3 mm from the tubing. After photoradiation, no rise in temperature was observed.

As shown in Fig. 6, the mean diameter of tumor necrosis was not constant for a single stroke of the optical fiber. The total light energy seemed to be more concentrated in the central part of the tumor. Dosimetry in the tissue may be required for uniform photoradiation.

DISCUSSION

The usefulness and effectiveness of PDT on superficial cancer is generally accepted.[14] The present procedure of PDT is as follows. Forty-eight hours after systemic administration of photofrin II, malignant tumors are irradiated with an excimer dye laser at a wavelength of 630 nm. The total radiation dose is 96 J when a tumor is irradiated at 160 mJ/s/cm^2 (4.0 mJ/pulse, 40 Hz) for 10 min. Under these conditions, the extent of necrosis from the surface of a malignant tumor is about 10 mm. Photoradiation at more than 160 mJ/s/cm^2 causes a thermal change in the irradiated tissue.[20] Such success of PDT on superficial cancers led us to try to extend the applicability of PDT to larger solid tumors deeper in the body. In the preliminary experiment on CTS, no tumor necrosis was found with 200 J photoradiation from a point 10 mm away from and directly above the CTS, but 8 or 9 mm depth of tumor necrosis was found with 200 J photoradiation when an optic fiber, installed in plastic tubing, was directly applied to the skin. In our experience, a directly inserted optic fiber is the most efficient way to transfer light energy to an organ deep in the body. Another way to transfer light energy is to use a vessel as a guide tube. However, this procedure induces tissue hypoxia owing to interruption of blood flow.[3] Accordingly, for the present purpose, we decided to use thin plastic tubing (external diameter: 0.7–1.5 mm) as a fiber guide. An optic fiber with a bulb tip and a cylindrical diffuser have already been used for interstitial PDT.[13, 21, 22] In general, the side effects of PDT arise from: (1) a thermal effect, and (2) damage to normal cells induced by photochemical synergistic interaction between intracellular photosensitizer and the light. The light intensity at an optic fiber tip is expected to be extremely high, and, consequently, thermal change and subsequent tissue necrosis will occur in the tissue around a directly inserted fiber tip.[10] Heat generation from PDT has to be balanced with the cooling capacity of blood flow.

As described in the results, the thermal effect in the present experiments was minimal or negligible below 240 J/cm. However, the photochemical synergistic reaction on normal tissue is considerable. When using PDT for superficial cancer, we have used an optic fiber with a flat tip, which provides a cone of light directed forwards along the optical axis with narrow divergence determined by the numerical aperture of the fiber (conical angle: 6°). Therefore, the light intensity, with which early gastric cancer was treated, was 196 times weaker on the surface of the stomach, 3.7 cm from the fiber tip.[14] However, an optic fiber with a 45° cleaved tip, covered with plastic tubing having scattering properties, provides a cone of light directed perpendicular to the optical axis with large divergence (conical angle 33°). In this interstitial light delivery system, the light intensity on the surface of the tubing was calculated to be about 3 times weaker than at the fiber tip. Damage to the plastic tubing is minimized by the reciprocal motion and rotation of the optic fiber in the tubing. Recently, we have succeeded in the development of thin laser-proof plastic tubing (ϕ 0.7 mm) using new material. Translucent plastic tubing would be preferable, however.

The photochemical synergistic effect of PDT on normal tissue close to the tubing is critical. Gastric cancer in its early stages can be treated by PDT with 10 min photo-radiation at 160 mJ/s/cm^2.[14, 23] When normal gastric mucosa was treated in a similar fashion, edema

was observed at the irradiated site 48 h later with complete cure after three weeks.[24] It is also important whether the normal tissue surrounding a malignant tumor is sensitive or insensitive to the side effects of PDT. As CTS is a subcutaneous tumor, the tubing penetrates cutaneous tissue and the skin close to the tubing may suffer side effects such as ulcers and fistulas. We found an ulcer at 240 J/cm, but only a scar which healed at 120 J/cm. The intracellular concentration of photofrin II, which frequently causes skin photosensitivity,[17] in the cutaneous tissue is 50% of that in the malignant cells. In a preliminary experiment on dogs, the side effects of PDT on tissues in the stomach, intestine, pancreas and liver were negligible at 120 J/cm. The macro- and microscopic findings in the irradiated area showed inflammatory changes. The levels of enzymes such as transaminases and amylase, were moderately increased. As shown in Fig. 3, the mean diameter of tumor necrosis at 120 J/cm was 20.7 mm with negligible cutaneous side effects, compared to 24.5 mm at 240 J/cm. In view of these results, we recommend that the radiation dose for interstitial PDT be from 100 J/cm to 200 J/cm. In order to attenuate damage to normal tissues when using interstitial PDT, the following measures can be considered: (1) to decrease the total radiation energy, (2) to move the light source more rapidly, (3) to decrease the number of pulses or the energy of each pulse, (4) to use a more highly selective photosensitizer.

It has been suggested that the destructive effect on tumor tissue in the clinical setting is mediated by stromal damage rather than by direct tumor cell damage.[25] However, our observation of round-shaped tumor necrosis shows that light transmission is almost the same in all directions, suggesting that the tumor necrosis is mediated by direct cell damage rather than by stromal damage.

In addition to progress in laser technology, development of longer-wavelength photosensitizers, such as chlorins[26,27] and benzoporphyrin derivatives,[28] may afford superior results. The present results suggest that after-loading interstitial PDT may be applicable to a wider range of malignancies with efficacy and safety. However, it is debatable whether it is desirable to treat large solid tumors with PDT, because such tumors are often associated with metastatic disease. Nevertheless, as with interstitial brachytherapy,[29] PDT is likely to find a role in the treatment of solid tumor without remote metastasis and of directly infiltrating tumor cells around the main tumor. Photodynamic therapy does have potential advantages over ionizing radiation because the total dose of ionizing radiation has an upper limit. Therefore, the present novel interstitial PDT is a breakthrough in local therapy against cancer.

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