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A Comparison between Argon-dye and Excimer-dye Laser for Photodynamic Effect in Transplanted Mouse Tumor

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Photodynamic therapy (PDT) utilizing a hematoporphyrin derivative (HpD) as a sensitizer has become a viable option for the local treatment of neoplastic disease. The argon-dye laser system is commonly used as a light source in this treatment modality. The excimer-dye laser, on the other hand, delivers high-energy red light in a pulsatile fashion. In this investigation, we treated BALB/c mice bearing mouse kidney sarcoma cell tumors with PDT using HpD at the dose of 5 mg/kg body weight as a photosensitizer and either a standard argon-dye laser or the pulsatile excimer-dye laser as the light source. At equal light energy doses (50 J/cm^2), necrotic changes at depths averaging 4 mm from the tumor surface were obtained with the argon-dye laser (200 mW power output) while tumor necrosis at depths exceeding 15 mm from the tumor surface was obtained using the excimer-dye laser (6 mJ/pulse, 5 Hz). To determine the best conditions for photoirradiation with the excimer-dye laser, tumor-bearing mice were treated with different total light doses (10, 30 and 50 J/cm^2), dose rates (1, 3 and 6 mJ/cm^2), and frequencies (5, 15 and 50 Hz) of light exposure. Our results indicate that the optimal effects obtained with the excimer-dye laser are related to the total light dose used and the dose rate, but not to the frequency of light exposure.

Key words: Photodynamic therapy — Excimer-dye laser — Transplanted mouse tumor

The effectiveness of PDT[4] in the treatment of human tumors was first reported by Dougherty *et al.*[1] and since then increasing attention has been paid to this new treatment modality. Over 3000 patients with a wide variety of malignancies have been treated by this method.[2] Despite many advances in this rapidly developing field, improvements in the technique, photosensitizer agents, and instrumentation employed in PDT are needed to achieve greater efficacy in therapy.

The development of new photosensitizers having a greater selectivity for tumor tissue remains an active area of research, as does developments in laser technology.[3–5] As a light source, the argon-dye laser is used to provide the red beam necessary for photoirradiation of tumor tissue sensitized by dye.[6] However, there are limitations, the most important of which remains the limited tissue penetrability achieved with current light sources.

Since 1982, we have developed a new diagnostic and therapeutic endoscopic system which utilizes an excimer-dye laser capable of emitting a high-energy pulsed laser beam.[7] Medical research concenrning the applicability of the excimer laser is ongoing,[8–10] and its role in cancer therapy is yet to be established. The excimer-dye laser has advantages over other delivery systems in PDT. Pulsed dye-laser systems can provide more high-energy photons in bursts as short as 10 nanoseconds that are capable of exciting HpD deep in tumor tissue.[11] These short high-energy pulses also insure against rapid heating and vaporization of tissues.[12]

In the present study we compared the excimer- and argon-dye laser systems with respect to their therapeutic effectiveness in the PDT of mice with actively growing m-KSA tumors. We also examined the effects of various levels of total energy, pulse power and frequency of the excimer-dye laser in an attempt define optimal treatment parameters for this laser system.

MATERIALS AND METHODS

HpD preparation for injection HpD (Porphyrin Products, Logan, UT) was dissolved in 30 ml of sterile PBS (5 mg/ml), pH 7.2, and the solution was stirred for 1 h at room temperature, adjusted to a total volume of 75 ml, and stored at −20°C in the dark. HpD was administered intravenously to mice via the dorsal tail vein at a dose of 5 mg/kg body weight.

Laser light delivery system Two fundamentally different laser systems were compared in this investigation. The argon-dye laser system (Spectra Physics Co. Mountain View, CA) consists of an argon laser (Model 171-08, 15 W, 457.9 to 514.9 nm wavelength emission) coupled to a

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[4] The abbreviations used are: PDT, photodynamic therapy; HpD, hematoporphyrin derivative; m-KSA, mouse kidney sarcoma; PBS, phosphate-buffered saline.

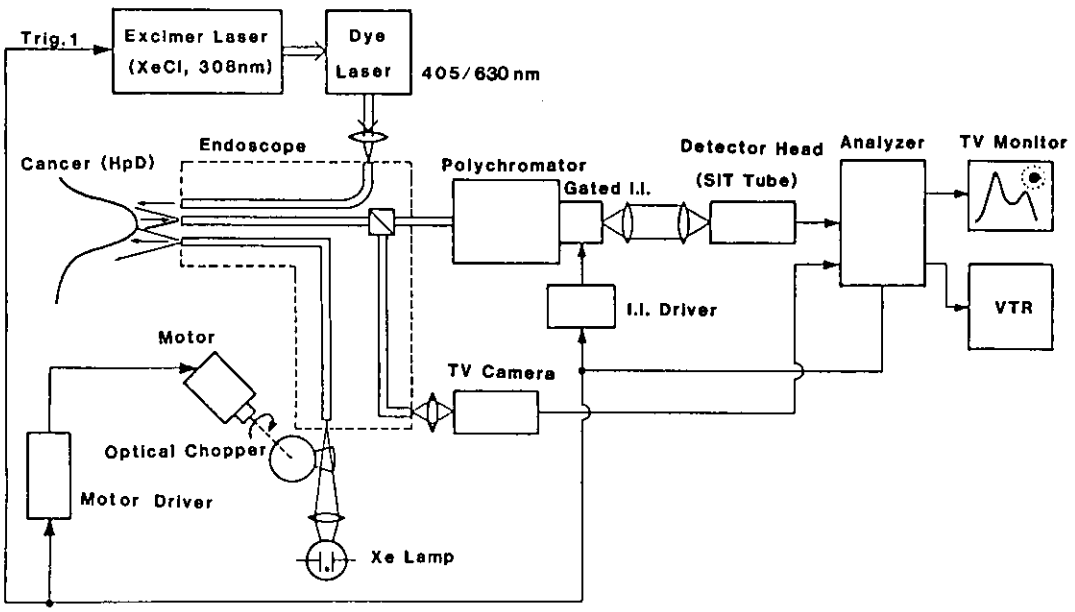

Fig. 1. The endoscope, delineated by dotted lines, is equipped with 3 channels which contain the fiber optics for field visualization (Xe lamp), the excimer-dye laser, and the fluorescence detector which can function simultaneously in this system. Note that the wavelength of the excimer laser is varied by coupling to a dye laser circulating either Rhodamine B in ethanol (630 nm for treatment) or diphenyl sulphan in dioxane (405 nm for diagnostic purposes). The chopper functions in alternating the delivery of white light and laser light. The input from the fluorescence detector is fed through a polychromator which functions by recording the intensity of light at each individual wavelength along its emission spectrum. The resulting spectrum is plotted by the analyzer and displayed by the monitor. Light from the detector is also processed by a separate path in order to generate the endoscopic field which is displayed alongside the emission spectrum. The final outputs are simulataneously recorded for further review.

dye laser using Rhodamine B dye (Model 375–03, 630 to 640 nm emission wavelength). The newer system consists of an excimer laser which emits a pulse laser beam coupled to a dye laser (Hamamatsu Photonics Co., Shizuoka). A block diagram of this system is shown in Fig. 1. The excimer laser uses a gas mixture containing 0.9% Xe, 0.1% HCl and 99% helium at 2 atm pressure in order to generate its characteristic high-energy beam. The optimal performance of this laser is obtained at 30 mJ/pulse to one-half peak power $\times$ 10.9 nanoseconds at 308 nm. The XeCl excimer laser (308 nm) can be coupled to a pump system containing saturated diphenyl sulphan dye in dioxane, which converts the beam to 405 nm, used for diagnostic purposes. To generate the 630 nm high-energy beam used in PDT, the excimer laser is coupled to a system containing 2 *M* Rhodamine B dye in ethanol. The radiation from both the excimer- and argon-dye lasers is focused onto 400-μm fused silica fibers (Fuji Photo Optical Co. Tokyo), the tips of which are fitted with microlenses for improved homogeneity of light distribution throughout the treatment field. The final circular area of illumination was 2 cm^2.

Tumor system and animal preparation Three-week-old BALB/c female mice weighing 18–22 g were used. The tumor system used was the m-KSA cell-line obtained as an *in vitro* culture from the National Institute of Health, Tokyo. Tumor cells were cultured in RPMI 1640 media (Flow Laboratory, London, UK) containing 10% newborn calf serum (Flow Laboratory). Tumor growth was initiated by intradermal injection of cells harvested from cultures in exponential growth (1×10^7 cells in 0.1 ml of sterile PBS) into the dorsum of mice. Tumors generally reached 18 to 22 mm in diameter and 10 to 15 mm in thickness and, at these sizes, were homogeneously white, with little or no necrosis evident on pathological examination of selected specimens. Furthermore, in a preliminary experiment, the incidence of spontaneous necrosis was less than 1% (data not shown). Tumor-bearing mice with shaven dorsa were picked at random and were fixed prone without anesthesia on plastic boards. These mice were treated 24 h after the intravenous administration of HpD in all experiments.

Comparison of the excimer- and argon-dye lasers in PDT Animals were divided into 3 groups of 10 (Table I).

Groups I and II were treated with the argon-dye laser (ADL) at total light doses of 50 and 200 J/cm^2, respectively, at a power density of 200 mW. The excimer-dye laser (EDL) was used to treat animals in group III at 50 J/cm^2, a pulse power of 6 mJ and a frequency of 30 Hz.

Control studies consisted of 5 animals given HpD without PDT from either laser, 5 animals not given HpD but treated with 200 J/cm^2 (200 mW/cm^2 power output) of argon-dye irradiation, and 5 animals not given HpD but treated with 50 J/cm^2 (6 mJ/pulse, 30 Hz) of excimer-dye irradiation.

Determination of optimal parameters for PDT with the excimer-dye laser Nine groups of 10 tumor-bearing mice per group were treated with the excimer-dye laser 24 h after HpD injection under conditions in which the total power per area, frequency and pulse power of laser light were varied in order to difine the optimal treatment parameters for photoirradiation with this laser in this tumor model (Table II).

Histology Tumors were removed from animals 48 h after PDT, fixed in buffered formalin, sectioned to 5 μm thickness, and stained with hematoxylin-eosin. The average depth of necrosis at the mid-sagittal section of a tumor was assessed, beginning at the sub-dermal margin in all specimens. Statistical comparisons were made using Student's t test for independent variables.

RESULTS

In Table I, the quantified photodynamic effects of the argon- and excimer-dye lasers are summarized. Mice treated with the argon-dye laser exhibited tumor necrosis at mean depths of 4.1 and 9.4 mm from subdermal margins at 50 and 200 J/cm^2 total light dose, respectively. On the other hand, mice treated with the excimer-dye laser at a total energy dose of 50 J/cm^2 (6 mJ/pulse, 30 Hz) demonstrated a depth of effect averaging 14 mm, which was significantly different from the results obtained in group I ($P<0.001$) and group II ($P<0.002$). In Fig. 2, typical m-KSA specimens from each group of mice treated are shown. Note the well delineated areas of massive hemorrhagic tumor necrosis with vacuolization. Viable tumor was evident below the areas of necrosis and appears most dark.

Table II indicates the photodynamic results obtained with the excimer-dye laser under various treatment conditions. In groups I to IV, the depth of necrosis increased from 1.2 mm to 11.9 mm as the total dose was varied from 1 to 50 J/cm^2. In groups IV to VII, only the frequency was varied. The resulting mean depths of necrosis were not significantly different from each other. In groups IV, VIII and IX, only the pulse power was varied. Depth of necrosis increased significantly with increasing pulse power from 8.7 mm at 1 mJ/pulse to 11.9 mm at 3 mJ/pulse ($P<0.025$) and to 14.3 mm at 6 mJ/pulse ($P<0.01$). Groups of control mice showed no sign of necrosis.

DISCUSSION

Photodynamic therapy, a relatively new modality used in the treatment of cancer, has gained considerable acceptance in the past decade. At our institution, PDT has been used in the treatment of more than 300 cancer patients since 1980 with quite favorable results, including the only known patient with lung cancer who has survived disease-free for more than 10 years after treatment with PDT alone.[13, 14] There are, however, restrictions imposed by the current light sources commonly used in PDT, which include limited tissue penetrability. In the past, the gold-vapor laser, which also emits a pulse beam, was developed with the expectation of greater tissue penetrability. The major disadvantage of this system, however, is its complicated and costly maintenance.[15] The excimer-dye laser was developed for use in PDT in order to address these issues.

There were highly significant differences in the photodynamic effects obtained between the argon- and excimer-dye lasers as measured by the depth of necrosis in m-KSA tumor specimens. A greater than 3-fold increase

Table I. Comparison of Photodynamic Effects between the Argon-dye Laser and the Excimer-dye Laser Systems

Group	Laser[a)]	Conditions of irradiation	Total power (J/cm^2)	Number of mice	Depth of effect means±SD (mm)
I	ADL	200 mW	50	10	4.1±0.78
II	ADL	200 mW	200	10	9.4±1.2
III	EDL	6 mJ/pulse×30 Hz	50	10	14.0±1.55

a) ADL, argon-dye laser, EDL, excimer-dye laser.
Ten tumor-bearing mice in each of three groups given HpD were treated with either the argon-dye laser (total dose of 50 or 200 J/cm^2) or the excimer-dye laser (50 J/cm^2). The average depth of necrosis at the cross-section of a tumor center was assessed.

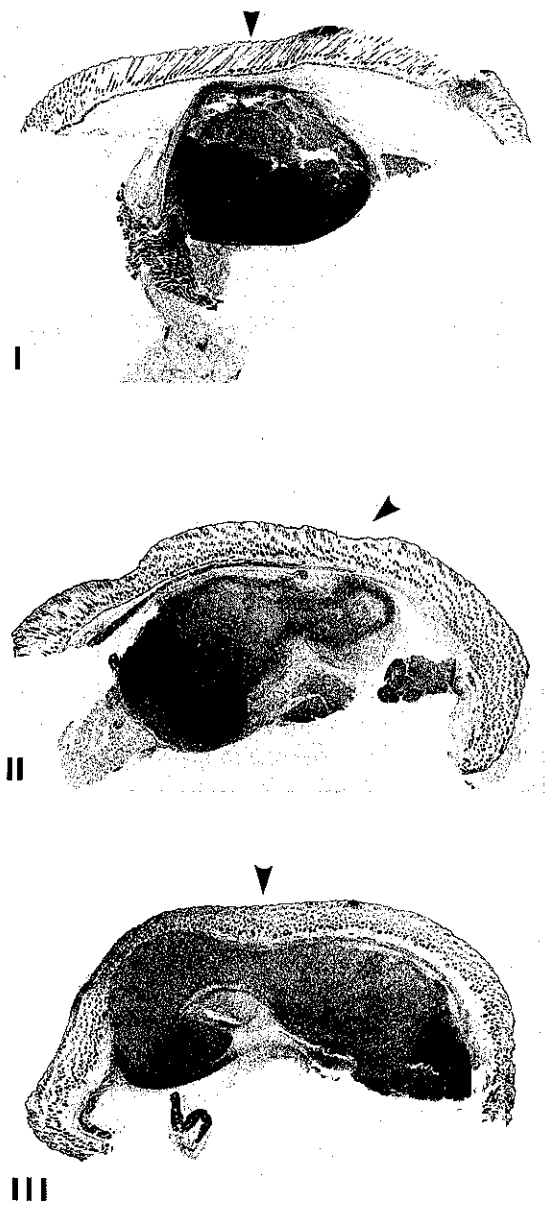

Fig. 2. The m-KSA tumor specimens from animals treated by PDT with differing laser light sources. In group I (50 J/cm^2) and group II (200 J/cm^2 total light dose) the continuous-wave argon-dye laser was used, while group III animals were treated with the pulse-wave excimer-dye laser (50 J/cm^2 total light dose). The arrows represent the paths of the incident laser beams. Lighter regions beginning at the sub-dermal margins represent areas of tumor necrosis while viable tumor remains mostly dark. Note the well-delineated margins between necrotic and viable tumor in these specimens. Observe the areas of massive hemorrhagic necrosis with extravasation of blood, which appear white and are more pronounced in group II, and most abundant in group III animals. Viable islands of tumor cells in areas of necrosis are evident, perhaps reflecting the uneven distribution of HpD within tumor tissue (H&E, $\times 15$).

in depth of necrosis was evident with the use of the excimer-dye pulse laser as compared to the argon-dye system. Gomer *et al.* have demonstrated that the photodynamic effects obtained with the argon-dye laser are not related to power density.[16] Using the excimer-dye laser, however, we have shown that a positive correlation exists between pulse power and the photodynamic effects obtained as measured by depth of necrosis (Table II). No thermal changes were apparent within the tissue treated with the excimer-dye laser under the conditions used in this experiment.[12] This indicates that the tumor cell necrosis observed with the excimer-dye laser in this study is a result of photodynamic effects.

The explanation for the increased tissue penetrability of pulse lasers remains both unclear and the subject of much speculation. While the overall amount of quantum energy delivered to a given area by a continuous wave argon-dye laser and a pulsed excimer laser might be the same, the amount of peak energy obtained by the latter is greater, which might result in deeper penetration of this light and hence more extensive stimulation of the photosensitizer. It is speculated that because the relaxation time of excited HpD is much greater than the pulse time of the excimer laser, unabsorbed photons at the tissue surface are free to pass to deeper tissue planes as re-excitation of surface dye does not occur. Although the maximum power output of the argon dye laser is in the region of 1000 mW, the excimer-dye laser provides a 10 nanosecond pulse of 5 mJ, the equivalent of 5×10^5 W, delivered extremely rapidly. This extremely short quantum pulse and the greater mass of tissue which can absorb each pulse of energy may explain why little or no tissue heating is observed with current methods of detection.[11, 12]

The percent decrease in tumor area in a mid-saggital section of a tumor after PDT is often used to quantify photodynamic effects.[3, 17] We used the depth of tumor necrosis 48 h after treatment as our main parameter because of the difficulty involved in producing transplanted tumors of fairly uniform size, which we feel are necessary for an accurate comparison of tumoricidal effects. Histologically, no differences in specimens treated with either laser in this study were noted except for the depth of necrosis. A grading system quantifying the effects of laser phototherapy should be developed, however, to facilitate standardization and communication among investigators. It is important to note that in some of our tumor specimens, very small islands of viable tumor cells were found within areas of necrosis (Fig. 2). This is consistent with the fact that the distribution of HpD in any given tumor may be uneven.[11] Areas of low grade necrosis were also observed adjacent to margins of viable tumor. Studying the speciments 48 h after therapy may have contributed to these observations.

Table II. Photodynamic Effects with the Excimer-dye Laser

Group	Pulse power (mJ/pulse)	Frequency (Hz)	Total power (J/cm^2)	Number of mice	Depth of effects means±SD (mm)
I	3	30	1	10	1.2±0.33
II	3	30	10	10	3.5±0.48
III	3	30	30	10	8.6±0.92
IV	3	30	50	10	11.9±0.89
V	3	5	50	10	12.2±1.02
VI	3	15	50	10	12.0±1.25
VII	3	60	50	10	11.5±0.85
VIII	1	30	50	10	8.7±0.89
IX	6	30	50	10	14.3±1.15

Nice groups of 10 tumor-bearing mice per group given HpD injection were treated with the excimer-dye laser under conditions in which the total power per area, frequency and pulse energy of laser light were varied.

The excimer-dye laser system is also designed for diagnostic purposes (Fig. 1).[7] In photodynamic diagnosis (PDD), the excimer laser is coupled to a pump unit containing diphenyl sulphan in dioxane and the resulting pulsed beam (405 nm) can maximally excite HpD, while fluorescence detectors in a neighboring part of the endoscope discriminate between tumor fluorescence and that of adjacent normal tissue. During the time intervals between laser pulses, white light illuminates the endoscopic field, permitting visualization. The endoscopic environment as well as the spectral pattern of tumor fluorescence can both be viewed and recorded on a video monitor.

The excimer-dye laser system has been in use at our institution for both the diagnosis and treatment of cancer patients for several years now. A phase III study for Photofrin II is being performed in Japan and an excimer dye laser was officially permitted to be used in this study. Seventeen patients with lung cancer including 7 CIS cases have already treated by PDT with the excimer dye laser.[18] Some advantages of the excimer dye laser, such as deeper necrosis of the tumor for larger tumors and reduction of treatment time, were proven clinically. It is hoped that further investigations will confirm the promise of this new laser system and contribute to its improvement.

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