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Temperature Rise During Laser Photodynamic Therapy in a Mouse Tumor Model

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=Abstract=

Radiation-induced fibrosarcoma tumors were grown on the flanks of C3H mice. The mice were divided into two groups. One group was injected with Photofrin Ⅱ, intravenously (2.5mg/kg body weight). The other group received no Photofrin Ⅱ. Mice from both groups were irradiated for approximately 15 minutes at 100,300, or 500 mW/cm^2 with the argon (488nm/514.5 nm), dye(628nm) and gold vapor (pulsed 628 nm) laser light. A photosensitizer behaved as an added absorber. Under our experimental conditions, the presence of Photofrin Ⅱ increased surface temperature by at least 40% and the temperature rise due to 300 mW/cm^2 irradiation exceeded values for hyperthermia. Light and temperature distributions with depth were estimated by a computer model. The model demonstrated the influence of wavelength on the thermal process and proved to be a valuable tool to investigate internal temperature rise.

1. INTRODUCTION

In photodynamic therapy (PDT), a photosensitizer (light-activated drug) such as hematoporphyrin derivative is injected intravenously; after a portion of the dye is retained by the tumor, the area is illuminated by light to produce a photochemical reaction. Singlet oxygen generated in the process is believed to cause oxidative damage to biological components leading to tumor destruction. Typically, a sufficient radiant dose is given to activate the drug during a treatment of 5-30 minutes. However, excessive irradiance can cause hyperthermia or even thermal damage. Light dosimetry for PDT depends on many variables such as laser power, exposure time, wavelength, beam profile, drug concentration and tumor size. Dosages may be more than several hundred joules with irradiances up to several hundred mW/cm^2 [1,2].

A certain degree of temperature rise is inevitable at these levels of irradiance. An increase of temperature above about 42℃ will produce a hyperthermia effect in addition to photodynamic action. Since a synergistic interaction between PDT and hyperthermia has been reported [3,4,5] examining temperature can provide valuable information for the study of PDT. In this report, surface temperature on mouse tumors were measured using a thermal camera for three different lasers. Temperature rise due to the presence of Photofrin Ⅱ was also measured.

An optical-thermal model provided estimation of fluence rate and temperature. The optical portion

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of the model estimated the distribution of light using a diffusion approximation of the transport equation for light propagation [6]. The temperature response due to the absorption of the laser light was computed using a finite difference model of the bio-heat conduction equation [7].

2. MATERIALS AND METHODS

The C3H mice were inoculated with radiation-induced fibrosarcoma (RIF) tumors.

Tumors were a grown subcutaneously on the flanks of mice. When the experiments were performed, tumors were about 10 days old with a diameter size of 12-16 mm. Mice were anesthetized with 95 mg/kg body weight i.p. sodium pentobarbital and the tumor area was shaved with skin intact. Photofrin Ⅱ (PF Ⅱ) of 2.5 mg/kg body weight was injected intravenously. The intravenous injection of PF Ⅱ induces maximal tumor concentration within about 3 hours [2]. A previous fluorescence study showed a rapid uptake of PF Ⅱ in the same RIF tumor [8]; fluorescence signal induced by PF Ⅱ was strong during the first three hours after injection and showed a gradual decrease afterwards. Thus, in these experiments, laser irradiation was started between one and two hours after the PF Ⅱ injection.

An argon-pumped dye laser and Metalaser, Inc. gold vapor laser were used as light sources. A laser beam was delivered through a 400 μm flat-end fiber and subcutaneous tumors were irradiated externally. The thermal responses due to irradiation from the argon-dye (628nm), gold vapor (pulsed 628 nm) and multi-line argon (488/514.5 nm) lasers were compared. 630 nm has been a popular choice for photodynamic therapy since this wavelength matches one of the drug absorption peaks, and light at this wavelength penetrates deeper in the biological tissue than at other peaks of the PF Ⅱ spectrum which are at shorter wavelengths. In this work, 628nm was chosen so that the thermal response of the tumor to the gold vapor laser radiation operating in a pulsed (30 ns), high repetition (about 8 kHz) mode could be compared with the continuous argon-dye laser irradiation at the same wavelength. A thermal camera (Inframetrics 600, 3-5 μm band) provided measurements of surface temperature distributions at 30 images per second. Continuous data of about 15 minutes was recovered to ensure that steady-state temperatures were measured.

The e^{-2} beam diameter was set to 6.0 mm at the tumor surface, except for a couple of cases where the diameter was 10.0 mm. The average irradiance [W/cm^2] was calculated as power divided by area. Laser power was adjusted to provide average irradiances of 100,300 and 500 mW/cm^2 for each source. However, irradiance profile at the surface was Gaussian, so the peak irradiance at the center of the spot was twice the average irradiance. The exact irradiance profile was used in the computer model that computed fluence rate and transient and steady state temperature fields as a function of depth for wavelengths of 500 and 628 nm.

2.1 Computer Modeling of Temperature Rise

Measurement of the internal temperature is very important. A micro-thermocouple can be used. For instance, temperature rise in the monkey's retina irradiated by laser was measured by an inserted micro-thermocouple [9]. However, it poses several problems for small tumors. First, the thermocouple ususally used with a guiding solid tude perturbs the measurement system and this insertion induces bleeding often ending up with small blood clots. Secondly, it is difficult to locate the exact position of the thermocouple tip inside the tumor. Direct absorption of laser beam by thermocouple metals overestimates temperature.

In this paper, computer models were applied to study the characteristics of internal light and heat distributions. The light intensity, i.e., fluence rate, f (r)[W/cm^2] was computed based on radiative transfer theory [10]. However, the general solution is not known and accurate solutions are limited to

simple conditions. The assumption of highly scattering media was made. This technique, called the delta-Eddington approximation [6], is valid since most of biological tissues behave as highly scattering media at the visible and near infra-red wavelengths. This optical model was implemented to solve a 2-D axially symmetric geometry case.

Temperature rise was computed numerically based on the bio-heat conduction equation [7]. Absorbed light functions as heat source which develops temperature field. Thermal conduction in the medium and convection at the surface occur. The finite difference method was implemented for this purpose. The heat source term or the rate of heat generation, s, [W/cm^3] is computed from the optical model and given by the product of the fluence rate and local absorption coefficient, μ_a;that is, $s=f(r)\mu_a(r)$.

The optical properties of skin dermis and the thermal properties of water were used for a homogeneous, semi-infinite slab. For the optical properties, the delta-Eddington coefficients for of skin dermis were used [11]; μ_a (*absorption coefficient) = 1.4/cm, μ_s (scattering coefficient) = 90/cm, g(anisotropic factor) = 0.4 at 628 nm and μ_a = 3.4/cm, μ_s = 120/cm, g = 0.1 at 500 nm. The tissue index of refraction was 1.4. The thermal properties of water were used, i.e., 0.0016 cal/cm-sec-℃ for the thermal conductivity and 1.0 cal/cm^3-℃ for the volumetric specific heat. The incident beam was assumed to be Gaussian with a diameter of 6.0 mm and the average irradiance was 500 mW/cm^2.

3. RESULTS

Non-irradiated surface temperatures of the tumors varied between 29~33 °C. Results are reported as temperature rise from the initial temperature of the tumor. A typical temperature response at the center (r=0) of the irradiation is shown in Figure 1 where the evolution of maximum temperature rise was plotted for irradiation of 300 mW/cm^2 with the gold vapor laser. As shown in Figure 1,

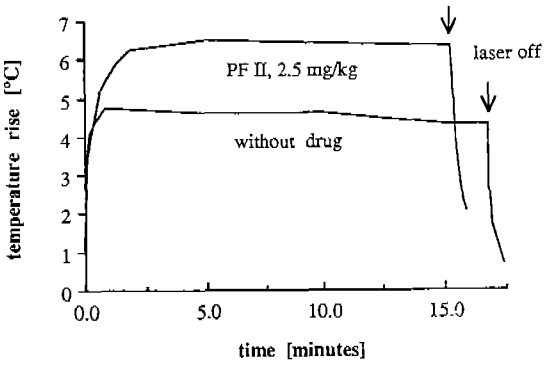

Fig. 1 Laser-induced surface temperature rise of tumor for drug free and mouse injected with the PF Ⅱ. Measurements were made at the beam center with a thermal camera. A beam size of 6.0 mm and an average irradiance of 300 mW/cm^2 from the gold vapor laser was used

surface temperature increased rapidly after the laser was on, reaching steady state in less than a minute when no drug was injected. On the other hand, it took about two and a half minutes to reach a stabillized temperature with PF Ⅱ injected. PF Ⅱ increased the maximum temperature rise about 50 % in this case but it took longer to reach to the steady-state temperature. After the laser was turned off, temperature dropped sharply, followed by a slow decay indicative of the volume of tissue heated. In general, temperature distributions on the tumor surfaces were uniform, showing minor fluctuations depending on the surface conditions.

Steady state temperature rises for three lasers as a function of irradiance are presented in Table 1. A beam size of 6.0 mm and laser powers of 28.3 mW, 84.8 mW and 141.4 mW provided irradiances of 100,300 and 500 mW/cm^2 respectively. Table 1 shows surface temperatures at the beam center and at 2.25 mm from the center. Temperature rise as expected increased with the irradiance; variations in the table were mainly due to individuality of tumors since each treatment used a different mouse. The PF Ⅱ injection increased temperature signi-

Table 1 Surface temperature rise at the beam center (r=0) and at 2.25 mm from the beam center. Temperatures were measured about 15 minutes after laser onset to ensure the steady state temperature. Values are for a beam diameter of 6.0 mm. Values for 10.0 mm beam are marked by *. PF Ⅱ injection was 2.5 mg/kg body weight

Average irradiance [mW/cm²]		Argon laser 488/515 nm		Argon-dye CW 628 nm		Gold vapor pulsed 628 nm	
		control	PF Ⅱ	control	PF Ⅱ	control	PF Ⅱ
r = 0	100	1.5 3.6*	2.2	1.1	2.0	1.4	2.0
	300	4.8 11.4*	7.9	4.0	7.6	4.3	6.4
	500	7.9	13.7	7.1	11.7	6.5	10.5
r= 2.25mm	100	1.0 3.2*	2.0	0.5	1.1	0.4	1.0
	300	4.5 8.6*	6.5	2.7	4.6	3.4	5.0
	500	6.7	9.9	5.2	7.4	4.2	8.75

* beam size is 10mm, without photofrin Ⅱ

ficantly. An irradiance of 300 mW/cm² or induces hyperthermia in the surface region under our experimental settings. No distinct difference in temperature rises generated by the pulsed gold vapor and the continuous dye lasers was detected by the thermal camera operating at 30 frames per second.

Temperature distribution did not depend only on the irradiance. For a larger beam, the total power is greater and a larger volume is being heated resulting in higher maximum temperature by heat conduction. For the same average irradiance, for example, of 100 mW/cm², the laser power was 28.3 mW for the 6.0 mm beam and 78.5 mW for the 10.0 mm beam. The maximun temperature rise for the 10.0 mm beam was about 2.4 times higher. Both power density and beam size (or power and beam size) should be taken into account to examine light and heat distributions.

The argon laser at 488 nm/514.5 nm induced higher temperature than the dye laser (628 nm) or gold vapor laser (628 nm). This was expected since biological tissues have a higher absorption at the argon wavelengths. However, the difference in the steady-state temperature between the blue/green and red wavelengths was far less than that in the absorption coefficients. This was due to heat condution in tissue and can be illustrated by computer simulations.

The computed light distribution and the heat source for 500 nm and 628 nm radiation are presented in Figure 2; the resulting temperature response at the center of the beam at 0.4 second and 2.5 minutes shown in Figure 3. 628 nm light penetrated deeper, but its magnitude of the heat source at the surface was only about 40% of that at 500 nm primarily due to a higher absorption coefficient at 500 nm. The temperature is proportional to the amount of heat absorbed, i.e. the heat source, just after laser irradiation when the heat conduction effect is negligible. The temperature distributions at

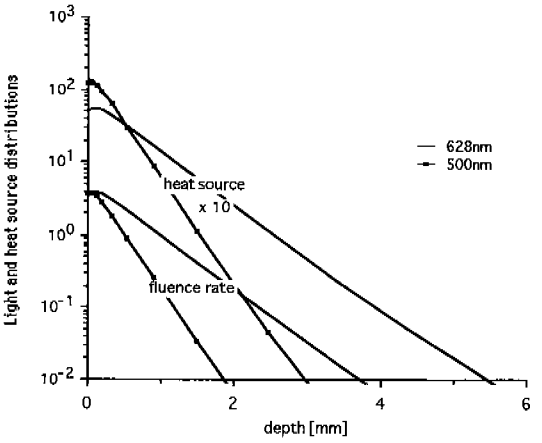

Fig. 2 Computer simulations for wavelengths of 628 and 500 nm. Fluence rate 91W/cm²] and heat source [W/cm³] were shown as functions of depth. The Gaussian beam with a diameter of 6.0mm was assumed and the average irrqdiance was 500 W/cm². Heat source values were muliplied by a factor of 10 to distinguish from fluence rates

0.4 second resemble those of the heat sourcee. However, 2.5 minutes after laser-on, the maximum surface temperature at 628 nm increased up to 73% of that at 500nm (Figure 3). As time progressed, heat was conducted to deeper layers and temperature field was extended far beyond the extent of light penetration.

Predicted surface temperatures showed good correlations with measured values. The computed temperature rise (using a power of 500 mW at the beam center) at 628 nm was 6.52℃. This was very comparable with 7.1℃ by the dye laser and 6.5℃ by the gold vapor laser (see Table 1). At the multi -line argon wavelengths, the computed temperature rise, 8.87℃ deviated more from measured 7.9℃. This might be partly because the model simplified the argon multiple wavelengths to one wavelength, i.e., 500nm.

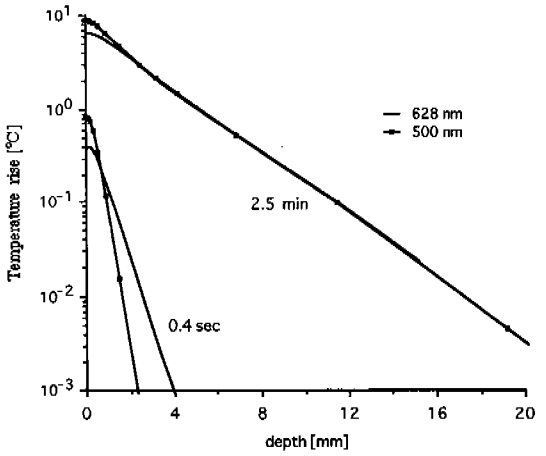

Fig. 3 Computer simulations of temperature rise [℃] along the beam center. The incident beam was assumed to be Gaussian with a diameter of 6.0 mm and the average irradiance was 500W/cm^2

4. DISCUSSION

When tissue or a tumor is illumined by light, energy is absorbed by the molecules and heat is generated. Heat transfers mostly through conduction to cooler regions in tissue and by convection at the surface. A photosensitizer behaves as an added absorber, which increases the overall absorption coefficient; decreases the fluence rate; yet increases the heat source. The drug distribution and the heat conduction mechanism from drug to tissue is very difficult to determine. However, salient features are observed in terms of surface temperature. The experimental parameters used in this report were close to those used for PDT research on deterring the mouse tumor growth [1,2,12]. The PF Ⅱ injection (2.5 mg/kg) enhances absorption and causes a substantial rise in temperature during irradiance compared to drug free tissue. Undoubtedly the increased temperatures influence PDT results. The degree of influence can be estimated by surface temperature measurements and computer simulations of the temperature distribution with depth.

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