In Vitro and in Vivo Light Dose Rate Effects Related to Hematoporphyrin Derivative Photodynamic Therapy

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ABSTRACT

In vitro and in vivo experiments were performed to evaluate the parameter of light dose rate as it relates to the efficiency of hematoporphyrin derivative (HPD)-induced photosensitization. Exponentially growing Chinese hamster ovary cells were incubated with HPD (25 µg/ml) and were then exposed to red light (630 nm) delivered at different dose rates. A total of five dose rates (0.5, 5.0, 15, 23, and 60 milliwatts/sq cm) were examined following a 1-hr HPD incubation, two dose rates (1 and 20 milliwatts/sq cm) were examined after a 12-hr HPD incubation, and three dose rates (0.4, 4, and 40 milliwatts/sq cm) were examined following a 16-hr incubation and a 30-min serum wash protocol. The effect of light dose rate was determined from cell survival curves obtained by standard clonogenic colony formation assays. Similar levels of cellular toxicity were obtained when cells from each HPD incubation group were treated with equal doses of red light delivered at different dose rates. For in vivo experiments, albino mice were given injections of HPD (7.5 mg/kg) and 24 hr later the right hind leg of each mouse was treated with localized red light (630 nm). A total dose of 270 J/sq cm was delivered to the right hind leg at dose rates of 5, 25, or 125 milliwatts/sq cm. The resulting acute skin damage induced by HPD photosensitization was scored over a 30-day period, and skin response curves for the three dose rates were obtained. Comparable levels of damage were induced in each of the three experimental groups. The results obtained from both in vitro and in vivo studies indicate that the photosensitizing efficiency of HPD photodynamic therapy is not affected by nonthermal variations in clinically relevant dose rates of delivered light.

INTRODUCTION

HPD PDT is used clinically in the treatment of various types of solid tumors (8, 9, 12, 25). The in situ localization of HPD in tumor tissue and the photodynamic induction of tumor tissue toxicity are properties responsible for the effectiveness of this treatment modality (15, 26, 31). Clinical utilization of HPD PDT began 8 years ago, and was used initially for palliative treatment following the failure of conventional therapies (7, 9, 10, 22). HPD PDT is currently being used for both palliative therapy and as a primary modality in the treatment of certain bladder, lung, skin, and eye tumors (3, 4, 6, 8, 21).

HPD PDT combines drug administration and visible light irradiation, and therefore many of the clinical procedures and parameters used in PDT are similar to those used in conventional radiation therapy and chemotherapy. Since HPD PDT is a relatively new treatment modality, there are still several parameters which need to be examined. These variables include: (a) dose of HPD administered; (b) time interval between HPD administration and PDT treatment; (c) wavelength of light used in treatment; (d) dose of delivered light; and (e) dose rate of delivered light. In general, HPD is administered as a single i.v. bolus at a dose of 2.0 to 5 mg/kg (7). Photodynamic treatment is normally initiated 48 to 72 h after HPD administration, although at least one clinical group has treated patients as early as 3 h following HPD administration (6). Tissue-penetrating red light at 630 nm can be generated by a tunable dye laser and is used to photoactivate HPD (14). The dose of delivered light for individual treatments ranges from 20 to 50 J/sq cm for cutaneous and s.c. lesions to 100 to 400 J/sq cm for pulmonary and ocular lesions (3, 4, 6, 10). The dose rate of delivered light ranges from 20 to 200 milliwatts/sq cm. While several of the procedures related to HPD PDT are loosely standardized for various treatment sites (8), it is clear that the parameters related to HPD PDT still need to be studied and documented.

Improvements in the efficacy of HPD PDT are likely to be obtained by optimizing the clinical parameters described above. Clinical randomization of HPD PDT variables would be one method of addressing the question of treatment optimization. However, it would not be appropriate at this early stage in the development of HPD PDT to depend primarily on this procedure for obtaining needed information. Fortunately, preclinical and basic studies can be utilized to examine several of the parameters listed above. The objective of our study was to examine the parameter of delivered light dose rate. Specifically, experiments were performed to evaluate the efficiency of varying light dose rates in conjunction with HPD PDT-induced phototoxicity. The effect of light dose rate was documented by in vitro cell killing and in vivo normal skin toxicity.

MATERIALS AND METHODS

Drugs. HPD was obtained from Photofrin Medical, Inc., Cheektowaga, NY, as a sterile solution (5 mg/ml) dissolved in 0.9% NaCl solution.

Light Source. A Model 375 dye laser (using Kiton red dye) pumped by a Model 184 5-watt argon laser (Spectra-Physics, Inc., Mountain View, CA), and a Model 600 dye laser (DCM dye) pumped by a Model 150 5-watt argon laser (Cooper Laser Sonics, Santa Clara, CA) were the sources of monochromatic red light (630 nm) used in this study. A 400 µm quartz fiberoptic cable was interfaced with the output of the dye laser, and a microinoc was fitted on the end of the fiber to broaden and even out the intensity of delivered light (14). The light wavelength was...
documented with a scanning monochromator (Model H-20, American ISA, Metuchen, NJ), and the light dose rate was measured with a thermopile (Model 210; Coherent, Inc., Palo Alto, CA).

**Animals.** Eight- to 12-week-old female Swiss albino mice were obtained from Bantin-Kingman, Inc., Fremont, CA. The mice were used to examine light dose rate effects of HPD PDT in normal skin, and were maintained on standard mouse food and water ad libitum.

**Cell Culture Procedures.** CHO fibroblasts were used in all in vitro experiments (14, 17, 18). The cells were grown in F-10 medium supplemented with 10% FCS and antibiotics (penicillin and streptomycin). Prior to treatments, appropriate numbers of exponentially growing CHO cells were plated onto either 35- or 60-mm plastic Petri dishes and incubated at 37 °C for 4 h to allow for cell attachment. The growth medium in the dishes was removed and the cells were rinsed once with serum-free F-10 medium. The cells were then incubated in the dark for either 1, 12, or 16 h in F-10 medium containing HPD (25 μg/ml) and FCS (1 or 5%). Cells incubated with HPD for 16 h also received an additional 30-min incubation in fresh F-10 medium containing 10% FCS (washout protocol) immediately after the 16-h incubation. Following the various HPD incubation protocols, the medium was removed and the cells were rinsed with serum-free F-10 medium. The lids of each dish were removed and the cells were exposed to red light at 630 nm. The delivered light doses ranged from 0 to 5400 J/sq m, and the dose rates of delivered light varied from 0.4 to 60 milliwatts/sq cm. The cells were then incubated for 8 to 10 days at 37°C. The surviving fraction of cells was determined from colony formation (18). Three dishes were treated at each dose point in each experiment, and experiments were repeated 3 to 5 times.

**Normal Skin Photodamage Treatment.** Mice received a localized PDT treatment to their right hind leg 24 h following an i.p. injection of HPD (7.5 mg/kg). The mice were placed in styrofoam holders with their right hind leg extending out of the holder. The treatment leg was shaved prior to light exposure, and the mice were not anesthetized during the treatment. Total light doses of 270 J/sq cm were delivered over a 1.5-sq cm area of exposed leg. The light dose rate during treatment was either 5, 25, or 150 milliwatts/sq cm. A total of 10 animals were utilized in each treatment group. Examination and scoring of the skin response was started 2 days following treatment, and was carried out 3 times a week. A numerical scoring system, similar to that used to quantitate skin responses following PDT (1, 16) was utilized to document skin responses following PDT (Table 1). The skin responses were scored by 2 observers, and the results for each animal were averaged.

**Temperature Measurements.** Temperature measurements were obtained s.c. in a second group of mice which was treated in a manner identical to that previously described. A 30-gauge hypodermic copper-constantan thermocouple probe and a Model 400-A digital temperature indicator (Omega Engineering, Inc., Stamford, CT) were used to document the temperature in mice. The thermocouple probe was inserted s.c. in the area to be irradiated with red light. Base-line readings were obtained, and then the hind leg of each mouse was exposed to 630 nm light for 5 min. The dose rate of delivered light varied from 100 to 400 milliwatts/sq cm. Temperature readings were recorded at intervals of 15 s during the 5-min light exposure and during the subsequent 4 min.

**RESULTS**

Charts 1 to 3 illustrate survival curves for CHO cells incubated with HPD under 3 different protocols and then exposed to 630 nm red light delivered at various light dose rates. These charts represent the surviving fraction of treated cells as a function of the dose of delivered light. Five different dose rates (0.5, 5.0, 15.0, 23.0, and 60.0 milliwatts/sq cm) were examined following a 1-h HPD incubation, as shown in Chart 1. Two light dose rates (1.0 and 20.0 milliwatts/sq cm) were examined following a 12-h HPD incubation, as shown in Chart 2. Three light dose rates (0.4, 4.0, and 40 milliwatts/sq cm) were examined following a 16-h HPD incubation and a 30-min wash, as shown in Chart 3. The photosensitizing efficiency of HPD PDT (as measured by the level of cell killing for a given light dose) was similar at all light dose rates examined for each incubation protocol. The pretreatment plating efficiencies for the different experiments ranged from 68 to 88%.

Chart 4 shows the s.c. temperature measurements obtained in nonanesthetized mice during light exposure. The individual curves demonstrate temperature levels during and immediately following localized leg exposure to 630 nm light delivered at dose rates ranging from 100 to 400 milliwatts/sq cm. A plateau in the temperature rise was observed within 3 to 4 min of light exposure, and the s.c. temperature levels returned rapidly to pretreatment exposure levels following the light treatment. Light dose rates of 100 and 200 milliwatts/sq cm induced s.c. temperature rises of less than 4°C. Light dose rates from 300 to 400 milliwatts/sq cm induced s.c. temperature rises which ranged from 6 to 10°C.

Chart 5 shows the daily skin response to albino Swiss mice...
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Chart 2. Surviving fraction of CHO cells as a function of delivered light dose. Cells were incubated for 12 h in F-10 medium containing HPD (25 μg/ml) and 5% FCS prior to treatment. Cells were exposed to 630 nm light at dose rates of 1.0 or 20 mW/cm². Bars, SE.

The total light dose to the leg of each experimental mouse was 270 J/sq cm, and the light was delivered at dose rates of 5, 25, or 150 J/sq cm. The treatments induced moist desquamation encompassing approximately one-third of the treatment field. Similar results were obtained for each of the 3 light dose rates examined. Damage was not observed in the legs of mice given injections of HPD and placed in holders (without light treatment), or in mice treated with red light (without prior HPD administration).

DISCUSSION

HPD PDT has been in clinical use for 8 years, and has produced effective responses when used in the treatment of malignant tumors of the skin, bronchus, bladder, and eye (3, 4, 6, 8, 21). However, the majority of patients which have undergone HPD PDT have been considered late stage, and thus the follow-up period in most studies is relatively short. As with all new therapeutic modalities, many of the treatment parameters have not been fully examined or optimized. The current experiments were designed to examine light dose rate as it relates to the cytotoxic effectiveness of HPD PDT. In vitro studies using HPD-labeled CHO cells demonstrated that a large range of light dose rates were equally effective in inducing phototoxicity. This effect was observed in cells following both short (1 h) and extended (12 or 16 h) HPD incubation conditions. Since the subcellular localization of porphyrins can change as a function of incubation time (23, 27) it was decided to examine the in vitro effect of light dose rate following a large range of HPD incubation protocols. The short incubation procedure may induce PDT damage primarily to the plasma membrane, while the extended incubation procedures may induce damage primarily to various subcellular compartments (27). Results from the in vivo experiments were in agreement with those from in vitro experiments, and demonstrated that comparable levels of acute skin toxicity were induced at all light dose rates examined (28).

Numerous in vitro and in vivo radiation biology studies have documented that the dose rate of delivered low-linear energy transfer radiation can be a significant factor in the effectiveness of a given treatment (2, 13, 19). It was therefore of both clinical and scientific importance to determine whether similar results would be observed for HPD PDT. In the case of ionizing radiation, the ability of treated cells to accumulate and then repair sublethal damage is enhanced under conditions of low dose rates (19). The range of dose rates for maximal variations in cytotoxicity is reported to be between 1 and 100 rads/min (19). There are several possible reasons why variations in effectiveness of HPD PDT were not observed for treatments performed at different light dose rates. First, HPD PDT may follow the fundamental photochemical reciprocity law of Bunsen-Roscoe (5). This law states that the intensity of radiation is inversely related to the time of exposure needed to produce a given effect; i.e., the delivered fluence induces the same response regardless of the fluence rate. If this law holds true for HPD PDT in biological systems, it would indicate that repair of sublethal damage does not occur following HPD PDT. A second reason for the lack of a dose rate effect in our studies may be that the lowest dose rates examined (0.4 milliwatt/sq cm for in vitro studies and 5.0 milliwatts/sq cm for in vivo studies) were too high for the documen-
depletion of oxygen or differential photobleaching of HPD.

In vivo light dose rates higher than 150 milliwatts/sq cm were not examined, in order to avoid possible artifacts due to effects of hyperthermia (14, 24). Results obtained in our study demonstrated that exposure of albino mouse skin to 630 nm light at fluence rates between 100 and 200 milliwatts/sq cm causes a s.c. temperature rise of approximately 3–4°C. However, light dose rates of 300 and 400 milliwatts/sq cm induce temperature rises in s.c. tissue in mice of 7 and 10°C, respectively. While skin heating at 43°C for 1 h does not produce an observable skin reaction in mice (20), a synergistic reaction following HPD PDT and moderate increases in skin temperature may be possible. A synergistic response of HPD PDT and heat (40.5–45.5°C) has been reported in the treatment of mouse tumors (29, 30), and therefore a similar phenomenon may occur in skin tissue. However, while light dose rates which can induce clinically significant hyperthermia were not examined in this study, it may occasionally be advantageous to utilize high light dose rates in order to exploit any synergistic interactions of HPD PDT and hyperthermia.

Finally, the results of our study are consistent with clinical observations, in which tumor response following HPD PDT appears to be independent of light dose rates in the range from 20 to 80 milliwatts/sq cm (7). The information on light dose rate therefore suggests that this parameter of HPD PDT can be changed for various treatment conditions without altering the effectiveness of the overall procedure. This would suggest that large lesions can be treated with a single treatment at reduced dose rates, while smaller lesions can be equally treated for shorter periods of time at higher dose rates. However, this would be true only for lesions having the same thickness, because of the significant attenuation of visible light by tissue (8).

REFERENCES

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