Intraperitoneal Photodynamic Therapy of Human Epithelial Ovarian Carcinomatosis in a Xenograft Murine Model

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ABSTRACT

The objective of this investigation was to determine the efficacy of i.p. photodynamic therapy (PDT) against solid, multifocal ovarian carcinoma using a newly described NIH:OVCAR-5 induced murine model. PDT was initiated when diffuse microscopic disease and small multifocal tumor nodules were present, similar to the extent of residual carcinoma that may persist clinically after laparotomy and tumor debulking. The photosensitizer, benzoporphyrin derivative monoacid ring A (BPD-MA), was administered in a dose of 0.25 mg/kg body weight i.p. 90 min prior to light exposure. An argon-pumped dye laser was used to deliver low intensity light (20 J) i.p. through a cylindrically diffusing fiberoptic tip. Treatment regimes consisted of a series of three to five treatments at 3–7-day intervals, with the extent of macroscopic disease or death from disease being the evaluable outcome parameters for tumoricidal and survival studies, respectively. The mean tumor burden at necropsy for treated animals was 0.034 ± 0.014 g compared to 0.379 ± 0.065 g in untreated controls (P < 0.001). Survival studies were initiated in two groups at day 7 and day 14 following cell inoculation. The first group received either three or five treatments at 5-day intervals, and both had a significant increase in median survival compared to untreated controls (57 and 53 days, respectively, compared to 43 days, P < 0.05). The second group was treated every 7 days until death and also had a significant survival advantage over controls (57 days compared to 47 days, P < 0.05). These studies suggest that benzoporphyrin derivative mono acid ring A-mediated PDT is a feasible, well-tolerated, experimental treatment approach that elicits a tumoricidal response against diffuse, solid i.p. disease in tumor-bearing mice, with concomitant prolongation of survival and needs careful optimization. PDT utilizes a combination of low-intensity illumination and tissue-localized photoactivatable chemicals (photosensitizers) to produce photochemical effects leading to tumoricidal effects (3). The photosensitizer is generally administered systematically and has some degree of preferential accumulation in malignant tissues. After a specified time for optimal biodistribution (increased contrast between tumor and surrounding normal tissue), the target tissues are irradiated at the absorbance maximum of the photosensitizer (4, 5). Phototoxic effects occur due to the formation of reactive intermediates such as singlet oxygen and free radicals from the activated photosensitizer (6). This results in the disruption of cellular and vascular structures, cell death, and tissue necrosis. PDT is well suited for the treatment of ovarian cancer due to the relatively easy access throughout the peritoneal cavity via fiberoptic techniques during laparoscopy or laparotomy. However, the advantages of the ready accessibility of the peritoneal cavity are mitigated by its geometric complexity, which makes light dosimetry a challenge. PDT circumvents the lack of specificity of conventional therapies to some extent due to its inherent dual selectivity: (a) increased selectivity is obtained by localization of the photosensitizer to the tumor, and (b) toxicity is confined due to spatial control of illuminated areas. Prior applications of PDT have most often used the administration of a free photosensitizer. In the case of ovarian cancer, where the disease is disseminated throughout the peritoneal cavity, it is important to have improved methods of photosensitizer localization. This may be achieved by binding photosensitizers to carrier systems such as liposomes or antibodies (4). Reasonably promising results using antibody-conjugated photosensitizers have been reported by Goff et al. (7–9) in a murine xenograft model, and the present study investigates i.p. PDT with liposomally encapsulated BPD-MA. BPD-MA is in Phase I–III clinical trials for cutaneous diseases (10). Initial laboratory and clinical studies suggest that BPD-MA is an efficient long wavelength-absorbing photosensitizer which lacks the side effects (prolonged cutaneous photosensitivity) of PF, the only PDT agent with regulatory approval in some countries. Almost all of the studies using BPD-MA-mediated PDT in oncology have been focused on either s.c. tumor models in the laboratory (11, 12) or cutaneous diseases clinically (10, 13). The purpose of this investigation was to explore the tumoricidal effect of PDT with liposomal BPD-MA in diffuse multifocal i.p. disease, which presents additional challenges for both uptake of photosensitizer and light delivery. A new xenograft model that more closely parallels the human disease process was developed and utilized in these experiments. The specific aim of the study was to evaluate the destruction of this small volume disease to demonstrate the efficacy and feasibility of PDT with liposomal BPD-MA as a potential adjunctive therapy in the treatment of ovarian carcinoma after debulking surgery.
MATERIALS AND METHODS

Tumor Cells. NIH:OVCAR-5 cells were obtained from the Fox Chase Cancer Institute (Philadelphia, PA). Cells were grown in RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum and maintained in an incubator at 37°C in an atmosphere of 5% carbon dioxide. Cells were harvested for injection during the log growth phase, when they were 80—85% confluent. For tumor harvest and transplantation, cells were trypsinized (trypsin-EDTA: GIBCO, Grand Island, NY), centrifuged at 1000 rpm for 10 min (model 6000B: Sorvall Centrifuges, Dupont, Wilmington, DE), and resuspended in PBS without Ca2+ or Mg2+ (GIBCO) and counted on a hemocytometer plate. The suspension volume was adjusted to a concentration of 17.5 x 10⁶ cells/ml. In previous studies, it was shown that a 2.0-ml suspension containing 35 x 10⁶ cells resulted in reproducible disease progression in vivo.

Animal Model. A xenograft model for human epithelial ovarian carcinoma was developed in our laboratory and utilized in these experiments. In brief, 6-8-week-old Swiss athymic nude mice (Cox Breeding Laboratories, Cambridge, MA) were given injections i.p. with 35 x 10⁶ cells from the established NIH:OVCAR-5 cell line. Within 7 days, diffuse granular carcinomatosis is apparent, and within 14 days the animals develop small multifocal tumor nodules throughout the peritoneal cavity. Within 28—35 days after inoculation, animals develop significant macroscopic tumor burden in the abdomen and pelvis.

Mice received proper care and maintenance in accordance with institutional guidelines. They had continual access to food and water, which was taken ad libitum. Throughout the experiments, mice were housed in laminar flow racks under specific pathogen-free conditions and were monitored daily for general health status. Cancer growth was made evident by the presence of abdominal distention and palpable tumor masses in the peritoneal cavity. Animals were sacrificed when they became moribund or had an evident excessive tumor burden.

Photosensitizer. Liposomal BPD-MA was a generous gift from Quadra Logic Technologies, Inc. (Vancouver, British Columbia, Canada) and was stored in powder form and kept refrigerated. Samples were prepared immediately prior to use by dissolution of BPD-MA in D5W solution and diluted with sterile PBS for injection. All work involving the use of photosensitizer was performed in subdued lighting.

Phototoxicity. PDT was performed 28-35 days after i.p. injection of 35 x 10⁶ NIH:OVCAR-5 cells. This time point was chosen to provide a more severe criterion for determining dose-limiting toxicity than earlier time points when toxicity was found to be similar. Mice (n = 5-7/group) were given i.p. injections of the photosensitizer (0.25-2.0 mg/kg BPD-MA) 90 min prior to light exposure. Two ml of a 0.1% v/v intralipid solution (Intralipid, soybean oil emulsion for i.v. use; Kabi Pharmacia, Inc., Clayton, NC) was injected i.p. prior to treatment to enhance light scattering (14). Animals were anesthetized by i.p. administration of 0.02—0.04 ml ketamine cocktail (ketamine, 30 mg/ml; xylazine, 5 mg/ml; and atropine, 0.5 mg/ml). An argon-pumped dye laser (Coherent, Palo Alto, CA), focused through a x 10 microscope objective onto the proximal end of an optical fiber, was used to deliver 690 nm light i.p. via a 8.0 mm x 0.4-mm cylindrically diffusing fiberoptic tip (built-in-house). This fiber was inserted into the peritoneal cavity of a supine anesthetized mouse through a centrally placed 22-gauge catheter that traversed the abdominal wall. One fourth of the total light energy was delivered to each i.p. quadrant over equivalent time periods. At the conclusion of treatment, mice recovered in an animal warmer until they awoke and resumed normal activity. No mice died during treatment. Toxicity thresholds were established, and treatment parameters were defined based on the percentage of animals alive 72 h after treatment. Pilot experiments established that any deaths related to the effects of PDT occurred within 48 h of light administration.

Photodynamic Therapy in Vivo. Mice were inoculated i.p. with 35 x 10⁶ NIH:OVCAR-5 cells, given a numeric ear tag for identification, and then randomly divided into two groups (treatment and control groups). Control groups consisted of animals that received no treatment, those that received an i.p. injection of intralipid and i.p. light delivery, and those that received i.p. BPD-MA and intralipid but no light. No significant difference in tumor weight or survival was seen between any of these control groups (data not shown). I.p. disease was confirmed in all mice (treatment and control) prior to therapy by the presence of positive peritoneal cytology.

Tumoricidal Response. Treatment was initiated on postinoculation day 14 and administered as described above. Based on the toxicology studies described above, all treatment studies used 0.25 mg/kg body weight of BPD-MA and 20 J of 690 nm light administered i.p. Low-intensity light (20 J total, ~5 J/quadrant) was delivered in equivalent fractions to each i.p. quadrant. Minor adjustments in the duration of illumination were made based on energy output of the fiberoptic tip to keep the total fluence constant during each treatment session. Typical illumination times per quadrant were in the range of 70—80 s; total time of treatment was in the range of 280—320 s.

For tumoricidal studies, this sequence of steps was repeated every 72 h until three treatments were completed. Multiple treatments were given based on previous work in our laboratory in which there was no significant tumoricidal response to a single i.p. treatment using the aforementioned regimen. All mice were sacrificed for necropsy 72 h after the third treatment (approximately day 26 postinoculation). Prior to sacrifice, the treatment and control mice were grouped together and randomly selected in a blinded fashion for necropsy. Initial tumor resection was performed by the same investigator (K. M.), and complete resection of macroscopic disease was confirmed by a second investigator (M. B.). Tumor ex vivo were grouped according to anatomical location, and the wet tissue weights were obtained (Mettler AE 163; Mettler Instrument Corp., Highstown, NJ). Tumoricidal response was assessed by comparing the extent of gross residual disease in treated animals to the extent of disease in untreated controls. Tumoricidal PDT experiments were performed in duplicate. The differences in the means of resected tumor weights were tested for significance using the two-tailed Student t test.

A subset of resected tissue was fixed in 10% phosphate-buffered formalin (Mallinkrodt Inc., Paris, KY) and embedded in paraffin. Sections cut 5-μm thick were stained with hematoxylin and eosin for microscopic evaluation to confirm carcinoma as the predominant cell type in resected tissue specimens.

Survival Studies. For survival studies modeling treatment of advanced disease, mice were divided into two groups (treatment and controls). Treatment mice underwent initial PDT on postinoculum day 14 and subsequent treatments at 7-day intervals. Treatments were continued throughout the life span of the mice for a total of nine treatments. For survival studies modeling treatment of minimal residual disease, the mice were divided into three groups according to the number of treatments scheduled (3, 5, and untreated controls). Treatment was initiated on postinoculation day 7, and the sequence of BPD-MA injection followed by 690 nm illumination was repeated every 5 days until the appropriate number of treatments were completed. The end point was defined as death due to disease, which was confirmed in all mice at necropsy. Survival analysis was performed using the Kaplan-Meier method. Survival curves were compared, and differences in survival were tested for significance using the Wilcoxon signed rank test. A P < 0.05 was considered significant.

Biodistribution. The present studies used an irradiation time of 90 min following BPD-MA administration. For future work we were interested in establishing times for irradiation following photosensitizer administration which would give the optimum tumoricidal response. Preliminary biodistribution studies were therefore initiated.

Standard curves for the estimation of the concentration of BPD-MA in tissue extracts were obtained as follows. Serial dilutions of a 1.25 μg/ml solution BPD-MA in PBS containing 2% FCS were prepared in methanol, and fluorescence emission spectra were recorded (Fluorolog 2; Spex Industries, Edison, NJ) with an excitation wavelength of 430 nm (excitation maximum of BPD-MA), and the emission spectra were monitored between 600 and 800 nm (emission maximum of BPD-MA, 695 nm). A linear equation was determined from each standard curve and used to calculate the concentration of BPD-MA from the fluorescence spectrum of a sample.

BPD-MA was injected i.p. at a dose of 2.0 mg/kg body weight in 1.0 ml solution. At time points of 1, 2, 4, 8, 12, 24, and 48 h after injection (n = 5—7 mice/time point) animals were sacrificed by CO₂ inhalation. At necropsy the skin, peritoneum, liver, spleen, kidney, small intestine, heart, lung, adipose tissue, and tumor nodules were harvested. Wet tissue samples were weighed immediately upon resection (Mettler AE 163; Mettler Instrument Corp.) and frozen at −70°C. For extraction of the photosensitizer, the tissues were homogenized (homogenizer model PT 10/25; Brinkman Instruments, Westbury, NY) in 3.0 ml 100% MeOH (Fisher Scientific, Fair Lawn, NJ) and centrifuged at 17,000 rpm (Sorval RC-5B, refrigerated supernspeed centrifuge; Dupont Instruments) at 22°C for 10 min. The supernatant was collected by centrifugation and used for analysis.
This suspension was refrigerated for 4 h, centrifuged, and the supernatant fluid was aspirated and combined with that previously collected. The fluorescence of the supernatant fluid was measured with a spectrofluorometer as described for the standard solutions, and concentrations of BPD-MA were determined from the equations of the standard curves. The fluorescence values of tissue samples from untreated animals were used for background correction.

RESULTS

Phototoxicology

Results of phototoxicology experiments are shown in Table 1. Survival was determined by the percentage of animals in each group that survived 72 h after PDT. More severe phototoxicity was observed with both increasing light exposure and photosensitizer dose. Toxicity, however, more closely paralleled incremental changes in the dosage of BPD-MA, as compared to changes in the total light energy delivered. Necropsy of animals that died due to phototoxicity revealed diffuse inflammation, hepatic congestion, and tissue edema, most notably in the bowel and peritoneum. Clinically, these animals had increasing abdominal distention and progressive lethargy. They became dehydrated, hypothermic, and appeared to develop shock, with death generally occurring 12–48 h following high-intensity PDT. Intestinal perforation and/or ischemic bowel was noted in several mice treated with higher doses of photosensitizer. Mice surviving phototoxicity testing suffered no prolonged untoward effects.

Photodynamic Therapy

Tumoricidal Response. i.p. disease was confirmed in all mice (treatment and control) before therapy by the presence of positive peritoneal cytology. A significant reduction in the overall solid tumor burden (Fig. 1) was noted. For treated animals, the mean weight of residual carcinoma at necropsy was 0.034 ± 0.014 g as compared to 0.379 ± 0.065 g in controls (P < 0.001). This overall tumor reduction collectively reflects the significant decrease in disease noted at all anatomical sites (Fig. 2). The greatest bulk of tumor in this xenograft model is in the pelvis, which was reduced to 0.015 ± 0.007 g in mice given PDT as compared to 0.187 ± 0.038 g in those not treated (P < 0.001). PDT effectively reduced the subgastric tumor volume to 0.009 ± 0.004 g in comparison to 0.075 ± 0.01 g (P < 0.001) in untreated controls. A comparable tumoricidal effect on i.p. carcinoma was also noted in the hepatic, splenic, and mesenteric areas. Small implants on the inferior surface of the diaphragm were not consistently present and accounted for the least volume of disease. Diaphragmatic studing was noted in six control mice (35%) with a mean weight of 0.012 +/− 0.007 g, while only one treated mouse (5%) had any visible implants, which weighed 0.002 g (not significant).

In the mice treated with PDT, there was a marked reduction in the frequency of macroscopic tumor implants at multiple anatomical sites (Fig. 3). Using gross dissection, the lower limit of visibly detectable tumor implants was ~1 mm in diameter. The frequency data shown in Fig. 3, therefore, represent any animal with at least one implant of >1 mm, at any specific anatomical location. All untreated mice had evidence of disease on gross inspection at necropsy. In comparison, 6 (27%) treated mice had no detectable macroscopic disease. The pelvic and subgastric regions most often demonstrated tumor growth and had the highest volumes of disease as well. A single mouse (6%) in the control group had disease limited to these two areas. In contrast, 10 mice (45%) treated with PDT had disease limited to the pelvis and subgastric regions, suggesting destruction of macroscopic disease at all other sites. In cases without overt evidence of carcinoma, some degree of subclinical disease most likely persisted. This was suggested by survival studies in which treated mice eventually succumbed to advanced disease.

There was only one prolonged PDT-related untoward effect on the mice. In mice that consistently survived PDT, significant weight loss occurred when treatments were administered at 72-h intervals. The mean weights of the two groups were not dissimilar prior to treatment,
Survival. Because of the marked weight loss observed with treatments administered at 72-h intervals, survival analysis was performed on mice treated at 5- and 7-day intervals. Sustained weight loss was not observed using these regimens, suggesting adequate time for recovery was allowed between treatments. In the assessment of PDT administered every 7 days and commencing 14 days after tumor cell inoculation (advanced disease model), the median survival for control animals (n = 9) was 47 days, and all controls died between 38 and 61 days. For the treated mice (n = 11), 45% were still alive after 61 days, and the median survival for this group was 57 days. Kaplan-Meier survival curves are shown in Fig. 4. Evaluation using the Wilcoxon signed rank test confirmed a significant survival advantage with i.p. PDT (P < 0.05). For the treatment group commencing at 7 days after tumor cell inoculation (minimal residual disease model) PDT was repeated either three or five times at 5-day intervals. Kaplan-Meier survival curves are shown in Fig. 5. Both of the courses consisting of three treatments and five treatments gave significant survival advantages (median survivals of 57 and 53 days, respectively) over untreated controls (median survival of 43 days, P < 0.05), but did not significantly differ from each other. The two survival studies have significantly different survivals for control mice (P < 0.05). The reason for these differences is not fully understood. Possibilities include variations in the immune status of the nude mice between batches, and increase in aggressiveness in the OVCAR-5 cell line with repeated in vitro passaging. Nevertheless, the treatment mice and the control mice in each survival experiment were directly comparable. Mice from the same batch each received the same number of cells from the same passage number.

**Biodistribution.** The tissue concentrations of BPD-MA in resected i.p. organs and tumor are shown in Table 2. The maximum concentration of BPD-MA in each tissue is reached within 2 h after i.p. injection of 2.0 mg/kg. The highest levels are present in the tumor

![Fig. 3. Comparison of frequency of tumor in anatomical sites between treated (■) and control (□) mice. The mice that had been treated in Fig. 1 were classified for the percentage of treated mice that showed macroscopic tumor (>1 mm) at each anatomical site.](image)

![Fig. 4. Kaplan-Meier survival curves for treated (——) and control (——) mice (advanced disease model). Treatment mice underwent initial PDT 14 days postinoculum and were treated every 7 days until death.](image)

yet there was an average weight loss of 1.9 g after three treatments as compared to a mean weight gain of 0.7 g in controls (P < 0.05). The extent of weight loss is several times greater than can be explained by reduction in tumor burden alone, suggesting some manifestation of systemic stress. The extent of weight gain in control mice is not unexpected in the context of normal growth of immature mice and tumor progression.

Four mice died during treatment: two from excess anesthesia sedation, one from i.p. hemorrhage due to a liver laceration, and one from undetermined causes. Two control mice also died during the same time interval of undetermined causes.

**Table 2 Mean concentrations (μg/g tissue) of BPD-MA in peritoneal organs of tumor-laden mice**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Intestines</th>
<th>Spleen</th>
<th>Liver</th>
<th>Kidney</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.35 ± 0.85</td>
<td>1.94 ± 0.56</td>
<td>2.92 ± 0.42</td>
<td>1.02 ± 0.16</td>
<td>4.51 ± 1.18</td>
</tr>
<tr>
<td>2</td>
<td>2.18 ± 0.36</td>
<td>0.94 ± 0.14</td>
<td>2.59 ± 0.75</td>
<td>0.73 ± 0.2</td>
<td>1.76 ± 0.45</td>
</tr>
<tr>
<td>4</td>
<td>1.17 ± 0.28</td>
<td>0.92 ± 0.27</td>
<td>1.79 ± 0.31</td>
<td>0.54 ± 0.07</td>
<td>1.01 ± 0.22</td>
</tr>
<tr>
<td>8</td>
<td>1.02 ± 0.39</td>
<td>0.65 ± 0.33</td>
<td>2.11 ± 0.89</td>
<td>0.83 ± 0.29</td>
<td>0.71 ± 0.18</td>
</tr>
<tr>
<td>12</td>
<td>0.88 ± 0.26</td>
<td>0.05 ± 0.02</td>
<td>0.35 ± 0.07</td>
<td>0.19 ± 0.1</td>
<td>0.38 ± 0.17</td>
</tr>
<tr>
<td>24</td>
<td>0.32 ± 0.15</td>
<td>0.07 ± 0.03</td>
<td>0.03 ± 0.1</td>
<td>0.21 ± 0.08</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>48</td>
<td>0.09 ± 0.05</td>
<td>0.01 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.05 ± 0.02</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

*Swiss nude mice (n = 5–7) with human ovarian carcinoma were given liposomal BPD-MA (2 mg/kg i.p.) and sacrificed after varying times. Samples of organs were dissected, weighed, homogenized, and extracted with methanol. The fluorescence signals were compared with standard curves to generate tissue concentrations of BPD-MA.*

*Mean ± SE.*

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Fig. 6. Tumor: normal tissue ratios in mice given i.p. BPD-MA measured after 1 h (●), 4 h (□), and 24 h (●). Tumor-bearing mice were given liposomal BPD-MA (2 mg/kg i.p.) and sacrificed after varying times. Samples of organs were dissected, weighed, homogenized, and extracted with methanol. The fluorescence signals were compared with standard curves to generate tissue concentrations of BPD-MA. Values given are μg BPD-MA/g tissue divided by μg BPD-MA/g tissue.

The tumor:tissue ratios for these organs are shown in Fig. 6. Initially, at 1-h postinjection, the tumor:tissue ratios are >1 for intestine, spleen, liver, and kidney. At 2 h postinjection, the tumor:tissue ratios become <1 for the liver and intestine. These higher concentrations, relative to that of tumor, persist throughout the 72-h period of investigation. The tissue concentrations of BPD-MA in adipose tissue, the abdominal wall (skin and peritoneum), and thoracic cavity (heart and lungs) are shown in Table 3. The maximum concentration of BPD-MA in each of these organs, except the skin, is also reached within 2 h after i.p. administration. The maximum concentrations of photosensitizer in the skin are found at the 2-h time point. The highest concentrations are noted in the peritoneum (6.88 ±/− 0.73 μg/g) and adipose tissue (3.70 ±/− 0.88 μg/g). Initially, at 1 h postinjection, the tumor:tissue ratios are >1 for skin, adipose, lung, and heart. The concentration of BPD-MA in tumor is consistently higher than those in the heart and lungs throughout the 72-h period of investigation. At 24 h postinjection, the tumor and tissue concentrations are approximately equivalent in the skin and adipose tissue, with tumor:tissue ratios of 1.08:1 and 1.29:1, respectively. The photosensitizer is consistently more concentrated in the peritoneum during the first 12 h, but at 24 h a tumor:tissue ratio of 1.20:1 is observed.

DISCUSSION

This study demonstrates the feasibility, tumoricidal activity, and survival benefit of BPD-MA-mediated PDT directed against diffuse i.p. ovarian carcinoma. Previous in vivo investigations of PDT for ovarian carcinoma have primarily focused on the treatment of ascitic or s.c. tumors. Tochner et al. (15) effectively demonstrated selective destruction of ascitic tumor cells and enhanced survival. Tumors from s.c. implantation of NIH:OVCAR-3 cells into athymic mice have been completely eradicated using PF-based PDT (16).

Although there was an almost 10-fold decrease in tumor burden following PDT therapy, the survival advantages seen in the present study were not dramatic. It can be seen from Fig. 3 that the number of mice which had no detectable tumor was very small. We believe that the relatively poor survival benefits can be explained by the fast rate of regrowth of tumors that had been substantially reduced but not eliminated. We believe the findings are important to the present body of information for several reasons. First, the xenograft model utilized in these experiments manifests tumor derived from human ovarian carcinoma cells and has been described in detail. In contrast to models expressing primary murine malignancies, these tumors possess many of the inherent biological properties of human disease. Advanced disease is characterized by extensive solid tumor burden and ascites, with parenchymal invasion, lymphatic metastases, and vascular dissemination. Individual tumor nodules exhibit developing neovasculature characterized by the absence of mature basement membrane. Second, the anatomical site of carcinoma is within the peritoneal cavity and presents a more realistic set of challenges than the treatment of s.c. tumors. Third, the focus of treatment is against solid tumors, in contrast to the more frequently studied ascitic tumor models for ovarian cancer. Fourth, a tumoricidal effect is demonstrated against diffuse i.p. carcinomatous implants at a time when the extent of disease is similar to the amount of residual disease that may persist following laparotomy and tumor debulking. Light at 690 nm may penetrate tissues 5–10 mm; because of the limited penetration, a substantial reduction in massive tumor burden is not expected using PDT. This modality, however, is an appealing adjunctive, therapeutic option to follow aggressive surgical debulking, for the treatment of microscopic implants and small volume residual disease, especially since it has been shown to be phototoxic to human ovarian cancer cells that are platinum resistant (7, 17). Delaney et al. (18), and Sindelar et al. (19) have demonstrated the feasibility of delivering PDT to the peritoneal cavity at the time of staging laparotomy in patients with disseminated intraperitoneal tumors.

Benzoporphyrin derivatives belong to the group of compounds often referred to as second-generation photosensitizers (20) which have higher absorption coefficients associated with their activation wavelengths in the 650–800 nm range and enhanced target specificity (14). These relatively new molecules are proposed as possible alternatives to the first-generation porphyrin mixtures, PF and HPD. BPD-MA is a synthetic chlorin (17) with an absorbance peak at 690 nm (ε = 33,000 m−1 cm−1 in MeOH (21)). BPD-MA has been shown to be a potent photosensitizer both in vitro (22) and in vivo (23). Treatment effect varies with the wavelength of light that activates a specific sensitizer, and second-generation sensitizers are activated at longer wavelengths, allowing for a 30% increase in treatment depths (17). The BPD-MA used in the present experiment was encapsulated in liposomes, which has been reported to increase the tumor specificity when administered i.v. (4, 24). A clinical advantage is offered by the lack of significant cutaneous photosensitization associated with BPD-MA.

Phototoxicology studies, following treatment of the entire peritoneal surface, have demonstrated significant toxicity associated with either high light or photosensitizer doses (18, 19). Results from our animal studies concur with these findings. PDT-induced injuries of the peritoneal surface would be expected to cause a burn-like reaction that could lead to major fluid and electrolyte disturbances, prolonged paralytic ileus, and elevated liver enzymes (15). In Phase II clinical trials using i.p. PDT with dihematoporphyrin ethers, major morbidity was seen in 9 (23%) of 39 patients (18) and 5 (22%) of 23 patients (19) who underwent surgery plus PDT. Serious complications included gastric and small bowel perforations, intestinal fistulas, intra-abdominal abscesses, ureteral leak and urinoma, necrotizing pancre-
atysis, and postoperative hemorrhage. Additionally, third spacing of fluids, greater than expected during normal postoperative course, was noted in the majority of patients. Delaney et al. (18) showed that 59% of patients developed pleural effusions and 15% required thoracentesis, transient edema, and ileus. One patient developed a pericardial effusion.

Animal studies using PF-mediated PDT have suggested that acute PDT-induced lethality is consistent with a traumatic shock syndrome (25). Veenhuizen et al. (26) have studied lethal toxicity after i.p. PDT and draw a distinction between death due to PDT-mediated intestinal damage over a period of 2 weeks and death due to a shock syndrome within 20 h. Lethal shock can be caused by sepsis (27), hemorrhage (28), severe burns (29), or trauma (28), and is thought to involve a common cascade of cytokines, eicosanoids, and other mediators (30).

Central to this cascade is the production of TNF-α by macrophages (31), especially peritoneal macrophages. TNF-α then causes release of vasoactive substances which mediate the drop in blood pressure and disseminated intravascular coagulation typical of multiorgan failure (32). Peritoneal macrophages have been shown (33) to accumulate large amounts of photosensitizers, and PDT causes them to release TNF-α (34). Other authors have suggested that macrophages play a role in the tumoricidal effect of PDT either by becoming photodynamically activated (35) or by being more cytotoxic toward cells which have mild PDT injuries (36). Two factors apply in the present case which make this mechanism for systemic toxicity likely. First, the BPD-MA was administered in liposomal form. It is well known (37, 38) that peritoneal macrophages phagocytose i.p. administered liposomes. Hunt et al. (39) have shown that mouse peritoneal macrophages take up liposomal BPD-MA in vitro and, on photoactivation, release nitric oxide. Macrophages activated with IFN-γ were more sensitive to this effect than those activated with lipopolysaccharide. It has also been shown (40) that after i.v. administration of PF to S.C. tumor-bearing C3H mice, peritoneal macrophages took up the photosensitizer nine times more than the malignant cells. Second, athymic nu/nu mice have been shown (41) to have a 25-fold enhanced phagocytosis capacity of peritoneal macrophages over their nu/+ and +/+ litter mates. This is thought (41) to be one way in which they compensate for their immune defect. It would seem therefore that in this model, peritoneal macrophages may be significantly involved in both tumor destruction at modest light doses and in lethal shock at high light doses.

A major obstacle in the i.p. treatment of tumors with PDT is uniform light delivery to all visceral and parietal peritoneal surfaces, without producing significant toxicity. The confinement of i.p. organs with vastly different optical properties [i.e., liver, absorption coefficient at 630 nm, μα ~0.23–0.65 mm⁻¹, aorta μα ~0.056–0.23 mm⁻¹, and bladder μα ~0.14 mm⁻¹ (42)] presents a substantial challenge for uniform light delivery and accurate dosimetry (43). Therefore, despite a reasonable selectivity of the photosensitizer for tumor, damage of normal tissues at high fluence regions proximal to the source, and insufficient treatment of asites, or carcinoma distant from the source are observed (44). Cancer cells implanted along the dorsal peritoneum and around the liver or diaphragm are difficult to treat due to being shielded from direct light exposure by intraabdominal organs. To reduce this problem, multiple treatments have been given in some studies (9, 45) at 48–72-h intervals, allowing redistribution of the asites in the peritoneal cavity between treatments. Light delivery and dosimetry must be optimized to all peritoneal surfaces, however, to reach microscopic and macroscopic foci of carcinoma that are not redistributed by peritoneal circulation (14).

In the NIH:OVCAR-5-induced ovarian cancer model, the majority of macroscopic disease develops in the pelvic and subgastric regions. These areas are readily accessible by direct illumination during PDT, as administered in the experiments reported in the present study. Accordingly, a significant tumor reduction occurred in both areas as a result of treatment. Tumors along the bowel serosa may be shielded somewhat from direct light due to the extensive length of the bowel, with numerous folds and layers within the peritoneal cavity. Similarly, direct illumination of the splenic tumors is hindered by the dorsal location of the spleen. Carcinomatous implants along the inferior surface of the liver and diaphragm are obscured by the stomach and proximal small bowel, and liver, respectively. The unexpected result from this study was the significant degree of tumor reduction in these anatomical locations that, in all probability, were suboptimally illuminated as was reported by Lilge et al. (14), who measured the light distribution with implanted optical fiber fluorescence rate detectors. Among the possible reasons for this are the high therapeutic efficacy of BPD-MA-mediated PDT, or that the combination of the cylindrically diffusing fiberoptic tip and i.p. intralipid solution may contribute substantially to enhanced uniform light delivery. Other effects such as enhanced oxygen supply to these areas may well play a role. Our study suggests that for optimal tumoricidal effect, PDT may need to be administered in multiple treatments. Similar results were noted by Tochner et al. (15) who used HPD for PDT to treat a murine ovarian cancer model.

Although mechanisms for the preferential accumulation of liposomally encapsulated photosensitizers administered i.v. have been proposed (46, 47), little is known about the routes of uptake into the tumor or other tissues after i.p. administration. No comparison between liposomal and nonliposomal BPD-MA has yet been made for i.p. tumors. The pharmacokinetics and biodistribution of liposomally encapsulated drugs are generally thought to depend mainly on the size of the liposomes, but also on the constituents of the phospholipid bilayer which affect parameters such as surface charge and membrane rigidity. In a comparison of free and liposomally encapsulated cefoxitin administered i.p., it was found (48) that the liposomal formulation persisted longer in the peritoneum and gave higher concentrations in the liver and spleen, while the free formulation was more quickly

**Table 3 Mean concentrations (μg/g tissue) of BPD-MA in tumor-laden mice**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Skin</th>
<th>Lung</th>
<th>Heart</th>
<th>Adipose</th>
<th>Peritoneum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.58 ± 0.12b</td>
<td>0.98 ± 0.23b</td>
<td>0.45 ± 0.09b</td>
<td>3.70 ± 0.88b</td>
<td>6.88 ± 0.73b</td>
</tr>
<tr>
<td>2</td>
<td>0.71 ± 0.13</td>
<td>0.41 ± 0.09</td>
<td>0.28 ± 0.08</td>
<td>2.35 ± 1.07</td>
<td>1.88 ± 0.38</td>
</tr>
<tr>
<td>4</td>
<td>0.39 ± 0.11</td>
<td>0.34 ± 0.11</td>
<td>0.14 ± 0.02</td>
<td>2.80 ± 0.63</td>
<td>2.36 ± 0.55</td>
</tr>
<tr>
<td>8</td>
<td>0.35 ± 0.05</td>
<td>0.42 ± 0.22</td>
<td>0.19 ± 0.09</td>
<td>1.32 ± 0.41</td>
<td>1.63 ± 0.54</td>
</tr>
<tr>
<td>12</td>
<td>0.20 ± 0.08</td>
<td>0.1 ± 0.06</td>
<td>0.07 ± 0.06</td>
<td>0.19 ± 0.11</td>
<td>0.17 ± 0.08</td>
</tr>
<tr>
<td>24</td>
<td>0.22 ± 0.07</td>
<td>0.04 ± 0.02</td>
<td>0.01 ± 0.01</td>
<td>0.12 ± 0.06</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>48</td>
<td>0.17 ± 0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Swiss nude mice (n = 5–7) with human ovarian carcinoma were given liposomal BPD-MA (2 mg/kg i.p.) and sacrificed after varying times. Samples of organs were dissected, weighed, homogenized, and extracted with methanol. The fluorescence signals were compared with standard curves to generate tissue concentrations of BPD-MA.

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**Mean ± SE.**
PHOTODYNAMIC THERAPY

Intraperitoneal Photodynamic Therapy of Human Epithelial Ovarian Carcinomatosis in a Xenograft Murine Model

Kelly L. Molpus, Daniel Kato, Michael R. Hamblin, et al.


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