Featured Article

Hypoxia and Photofrin Uptake in the Intraperitoneal Carcinomatosis and Sarcomatosis of Photodynamic Therapy Patients

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ABSTRACT

Purpose: Response to photodynamic therapy depends on adequate tumor oxygenation as well as sufficient accumulation of photosensitizer in the tumor. The goal of this study was to investigate the presence of hypoxia and retention of the photosensitizer Photofrin in the tumors of patients with intra-abdominal carcinomatosis or sarcomatosis.

Experimental Design: Tumor nodules from 10 patients were studied. In nine of these patients, hypoxia was identified in histological sections of biopsied tumor after administration of the hypoxia marker 2-[(2-nitroimidazol-1-yl)yl]-N-(2,2,3,3,3-pentafluoropropyl)acetamide (EF5). In separate tumor nodules from 10 patients, Photofrin uptake was measured by fluorescence after tissue solubilization.

Results: Hypoxia existed in the tumors of five patients, with three of these patients demonstrating at least one severely hypoxic nodule. Physiological levels of oxygen were present in the tumors of four patients. An association between tumor size and hypoxia was not evident because some tumor nodules as small as ~2 mm in diameter were severely hypoxic. However, even these tumor nodules contained vascular networks. Three patients with severely hypoxic tumor nodules exhibited moderate levels of Photofrin uptake of 3.9 ± 0.4 to 3.9 ± 0.5 ng/mg (mean ± SE). The four patients with tumors of physiological oxygenation did not consistently exhibit high tumor concentrations of Photofrin: mean ± SE drug uptake among these patients ranged from 0.6 ± 0.8 to 5.8 ± 0.5 ng/mg.

Conclusions: Carcinomatosis or sarcomatosis of the i.p. cavity may exhibit severe tumor hypoxia. Photofrin accumulation in tumors varied by a factor of ~10× among all patients, and, on average, those with severe hypoxia in at least one nodule did not demonstrate poor Photofrin uptake in separate tumor samples. These data emphasize the need for reconsideration of the generally accepted paradigm of small tumor size, good oxygenation, and good drug delivery because this may vary on an individual tumor basis.

INTRODUCTION

Carcinomatosis or sarcomatosis of the peritoneal cavity is characterized by the presence of tumor nodules on serosal surfaces throughout the abdomen. Tumors that may spread in this pattern include gynecological and gastrointestinal malignancies (1) and sarcomas such as gastrointestinal stromal tumors [GISTs (2)]. Overall, the prognosis is poor for such patients, and surgery alone is generally ineffective in their treatment. Several multimodal therapies are under investigation, including intraoperative or postoperative i.p. chemotherapy (3), hyperthermic i.p. intraoperative chemotherapy (4), and intraoperative i.p. photodynamic therapy [PDT (5)].

PDT may be well suited for the treatment of superficial or microscopic disease remaining after surgical debulking. Feasibility of this approach was demonstrated in a Phase I study of intraoperative Photofrin PDT after debulking surgery of i.p. disease (6), and a Phase II trial has recently been completed (5). The efficacy of this modality is determined by the simultaneous presence of the required components for PDT: photosensitizer; oxygen; and light. However, little to no investigation of these crucial parameters has been conducted for patients with i.p. disease. The presence of pretreatment hypoxia in these nodules is of concern for several reasons. First, hypoxia itself has been found to be a useful prognostic factor for predicting poor outcome to cancer treatment (7), especially radiotherapy (8–10). Although a similar clinical study has not been conducted for PDT, it is likely to be important, given the oxygen dependence of currently used PDT agents, as shown in preclinical studies (11, 12). Secondly, hypoxia can develop or become more severe during illumination due to photochemical oxygen consumption and the vasoconstrictive effects of PDT (11, 13). In one study on patients with basal cell carcinomas undergoing PDT, significant treatment-induced hypoxia has been documented (14). Finally, the presence of hypoxia may indicate an insufficient vascular network that could limit photosensitizer delivery.

The presence of hypoxia in i.p. nodules can be determined by labeling with a hypoxia marker such as the 2-(2-nitroimidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)acetamide (EF5). In preclinical studies, tumor hypoxia as labeled by EF5 directly...
correlates with radiation resistance (15). A Phase I trial of EF5 has been completed to detect hypoxia in tumors from patients, including those with sarcomas (16), squamous cell carcinomas (17), and other tumor types (18). Data from Phase II trials suggest that hypoxia, as measured by EF5 binding, correlates to other clinically relevant end points, such as tumor necrosis and cytokine regulation (18–20).

The purpose of the present study is to quantify levels of hypoxia and Photofrin in i.p. tumor nodules of patients entered into a Phase II i.p. PDT trial. Data on EF5 binding and Photofrin uptake are reported for multiple tumor nodules per patient in cases where >1 sample could be obtained; however, hypoxia and photosensitizer content were evaluated in separate tumor nodules from each patient. Our data show that a range in EF5 binding and Photofrin uptake is present in tumor specimens. Severe hypoxia could be detected in even the smallest nodules (~2 mm) evaluated. In patients with severely hypoxic tumor nodules, moderate levels of tumor Photofrin uptake were found, whereas low Photofrin uptake was found in the tumors of some patients with nodules of physiological oxygenation.

MATERIALS AND METHODS

Human Subjects. Tissues from 10 patients were studied for Photofrin uptake; 9 of these patients were also studied for tumor hypoxia. Patients were either enrolled separately on the Phase I EF5 trial and the Phase II i.p. PDT trial or enrolled on a trial of EF5 specifically designed for patients undergoing i.p. PDT (alternately, some patients were treated off-study as a compassionate exception to the i.p. PDT trial). The Phase I trial of EF5 was initiated in February 1998, and the EF5 trial for patients undergoing i.p. PDT was initiated in October 2002. The Phase II trial of PDT for disseminated i.p. malignancies began in April 1997. All trials were approved by the Institutional Review Board of the University of Pennsylvania and the Clinical Trials Scientific Review and Monitoring Committee of the University of Pennsylvania. The i.p. PDT trial was approved under an investigator-sponsored IND with the United States Food and Drug Administration. The EF5 trials were approved by Cancer Therapy Evaluation Program, National Cancer Institute. Exclusion and inclusion criteria for these trials have been published previously (5, 16). Patients provided separate written informed consent for each trial. For the patients enrolled separately on the Phase I EF5 and Phase II i.p. PDT trials, EF5 biopsy was performed at a laparoscopy for staging and assessment for treatment with i.p. PDT. Therefore, the time between EF5 biopsy and Photofrin injection was 3 weeks (patient 5), 8 weeks (patient 2), and 14 weeks (patient 1). In all other patients, who were enrolled on the joint EF5 and i.p. PDT trial, EF5 and Photofrin were administered on the same day: biopsy and PDT treatment took place 48 h later. Biopsies for EF5 and Photofrin analysis were collected before illumination was begun.

Drugs Administered. EF5 was supplied by Cancer Therapy Evaluation Program, National Cancer Institute, in vials containing 300 mg of drug plus 100 ml of water with 5% dextrose and 2.4% alcohol. EF5 solution was administered via a peripheral i.v. catheter at a rate no greater than 350 ml/h. Total infusion time was 1–1.5 h, depending on the total dose (12 or 21 mg/kg) administered. Tissue biopsy for EF5 binding was performed 24–48 h later. Binding was corrected for dose and time of drug exposure as described below.

Photofrin was supplied by Axcan Pharma Inc. (Mont-Saint-Hilaire, Quebec, Canada) and administered i.v. at 2.5 mg/kg. Surgical debulking and intraoperative PDT were performed 48 h later. Full treatment details on the i.p. PDT trial have been published previously (5).

Immunohistochemistry. Individual tumor nodules were excised (intact, if possible), put in iced EXCELL 610 media (JRH Biosciences, Leneka, KS) with 15% FCS, transported to the laboratory, and frozen in Tissue Tek OCT (Sakura Finetek USA, Inc., Torrence, CA). Cryosectioning and immunohistochemistry were performed as described previously (21). Briefly, 10-µm sections were fixed on slides from some nodules, in which each series was from a different level of the nodule. Sectioning levels were separated by at least 0.5 mm, with each successive level cut at greater distances from the tumor periphery. In addition to the regularly stained sections, within each slide series two sections were stained without antibody to assess endogenous tissue fluorescence (“no stain”). To assess nonspecific antibody binding, sections were stained with an antibody solution containing 0.5 mM EF5, whereby the free EF5 in the solution competed with tissue-bound EF5 for available antibody (“competitive staining”). Some tissue sections were additionally stained for CD31 to determine tumor vascularization. Slides to be exposed to CD31 antibody were blocked overnight in PBS containing 1.5% albumin and 0.3% Tween 20 (“antibody carrier”) supplemented with 5% skim milk and 5% rat serum. Staining (1.5 h at room temperature) in primary antibody was carried out with mouse antihuman CD31 (PharMingen, San Diego, CA) diluted 1:100 in antibody carrier. After rinses in PBS with 0.3% Tween 20, secondary antibody staining (1:100 in antibody carrier) was carried out at room temperature for 45 min with Cy5-conjugated rat antimeg IgG (Jackson ImmunoResearch, West Grove, PA). Sections were rinsed in PBS with 0.3% Tween 20, followed by fixation of CD31 labeling for 20 min in 4% paraformaldehyde. Staining for EF5 was then performed as described above.

Fluorescence Photography. Fluorescence microscopy (LabPhot microscope with a 100-W high-pressure mercury arc lamp and Photometrics Quantix charge-coupled device digital camera) was carried out as described previously (17). An automatic stage (Ludl Electronic Products) and IP Lab Spectrum software (Scanalytics, Fairfax, VA) precisely controlled stage movement for the purpose of photography of the entire section. Immediately before photography, slides were flooded with a solution of Hoechst 33342 (20 µM in PBS) to label the DNA of individual cells in the photographed images. Photographs were taken using filter cubes appropriate for Cy3 [EF5 or 2-(2-nitroimidazol-1-[H]-yl)-(3,3,3-trifluoropropyl)acetamide (EF3)], Cy5 (CD31), and Hoechst 33342. At the beginning and conclusion of each camera session, an image of hemacytometer-loaded calibration dye (a standard concentration of Cy3 in 1% paraformaldehyde) was taken. The fluorescence of this dye was defined as “1000.” The fluorescence of other images was then
Hypoxia and Photofrin in Intraperitoneal Tumors

Determination of Cube Reference Binding. Cube reference binding was used to determine the maximum possible EF5 binding in tumor tissue from each patient. This was performed as described previously (17). Briefly, tissue cubes were dissected from the fresh biopsy and incubated in vitro in the hypoxia marker EF3 (200 μM for 3 h) under low oxygen concentration (0.2%). Hypoxia marker binding values in the reference cubes were multiplied by the ratio of total drug exposure in individual patients. Total drug exposure was calculated (in μM·h) based on the area under the curve plotted from drug concentration at the time of EF5 infusion and at the time of tissue biopsy (22). Final values for in situ EF5 binding were obtained by subtracting the value for nonspecific binding (competed stain) in an adjacent section from that of regular stain binding.

Analysis of Photofrin Uptake. Photofrin data, we carried out a one-way ANOVA to determine whether there were statistically significant differences in EF5 binding among patients (24). This model took into account the correlation among tumors within individual patients (interspecimen variability) and the correlation among sections within individual tumors (intraspecimen variability). For the EF5 data, we also modeled the variance both within and between tumors as an increasing function of the mean EF5 uptake per subject. This approach was based on initial exploratory analyses. For the Photofrin data, we carried out a one-way ANOVA to determine whether there were differences between individuals. Statistical tests were two-sided, with statistical significance declared if the P for a test was <0.05.

RESULTS

Patient Information. All patients had carcinomatosis or sarcomatosis of the i.p. cavity. Histological diagnoses included GIST (n = 2; patients 1 and 2), ovarian carcinoma (n = 2; patients 3 and 4), colon carcinoma (n = 3; patients 5–7), small bowel carcinoma (n = 1; patient 8), and appendiceal carcinoma (n = 2; patients 9 and 10). In patient 10, tumor nodules were analyzed for Photofrin uptake but not for EF5 binding because of difficulties in obtaining the blood samples necessary to determine total EF5 exposure (see “Analysis of in Situ EF5 Bind-
For small changes in pO₂. Thus, severe hypoxia encompasses a large range of EF5 binding values, i.e., 30–100%.

Substantial variability in EF5 binding was evident in some patients, as measured by the range of recorded values both within levels of the same specimen (intrasurem) and among specimens from the same patient (interspecimen; Table 1). In general, patients with lower levels of median EF5 binding demonstrated similar intrasurem and interspecimen variability evidenced by the range in binding, whereas patients with higher binding levels generally exhibited greater overall interspecimen ranges in binding as well as higher interspecimen than intrasurem variability. The increased interspecimen variability is reflected in the estimated SEs of the mean, based on the mixed effects model. Mean differences in EF5 binding among patients varied greatly, ranging from 0.4 to 26.2, but were not statistically significant (P = 0.541), likely because of the large interspecimen variability in those with high EF5 binding.

Low to negligible levels of EF5 binding were found in the tumors of patients with GIST (patients 1 and 2) and small bowel (patient 8) and appendiceal (patient 9) cancer (Table 1). Here, little binding variability was found among different tumor nodules from the same patient or between different levels of the same nodule. The EF5 binding level corresponded to physiological oxygenation of these tumors (Fig. 1). We have previously reported that EF5 binding at each level is a percentage of the maximum hypoxia marker binding in tissue from the same patient. Reference binding values were not available for patients 4, 7, and 9. For these patients, the percentage of reference binding was calculated based on the average reference binding level in other patients of the same disease cohort.

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### Table 1 EF5 binding in intraperitoneal malignancies

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Histology</th>
<th>Cohort</th>
<th>Specimen</th>
<th>Sectioning levels</th>
<th>% of reference binding at each level&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Intraspecimen median (range)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Interspecimen median (range)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Interspecimen mean (SE)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>GIST&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Sarcoma</td>
<td>A</td>
<td>1, 2, 3, and 4</td>
<td>0.3, 0.1, 0.2, and 0.6</td>
<td>0.3 (0.5)</td>
<td>0.2 (0.3)</td>
<td>0.4 (0.5)</td>
</tr>
<tr>
<td>2</td>
<td>GIST</td>
<td>Sarcoma</td>
<td>A</td>
<td>1, 2, and 3</td>
<td>1.4, 1.4, and 1.1</td>
<td>1.4 (0.3)</td>
<td>1.4</td>
<td>1.4 (1.7)</td>
</tr>
<tr>
<td>3</td>
<td>Ovarian CA</td>
<td>Ovarian</td>
<td>A</td>
<td>1 and 2</td>
<td>6.3 and 4.7</td>
<td>5.5 (1.6)</td>
<td>5.5 (6.2)</td>
<td>5.1 (2.8)</td>
</tr>
<tr>
<td>4</td>
<td>Ovarian CA</td>
<td>Ovarian</td>
<td>A</td>
<td>1</td>
<td>3.2</td>
<td>3.2 (67.5)</td>
<td>26.2 (11.0)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Colon CA</td>
<td>GI</td>
<td>A</td>
<td>1</td>
<td>6.0</td>
<td>6.0 (32.8)</td>
<td>15.0 (7.5)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Colon CA</td>
<td>GI</td>
<td>A</td>
<td>1</td>
<td>50.0</td>
<td>29.7 (35.9)</td>
<td>29.2 (10.1)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Colon CA</td>
<td>GI</td>
<td>A</td>
<td>1</td>
<td>23.2 and 54.9</td>
<td>38.7 (32.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Small bowel CA</td>
<td>GI</td>
<td>A&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1, 2, and 3</td>
<td>2.3, 0.2, and 1.3</td>
<td>1.3 (2.1)</td>
<td>1.3</td>
<td>1.4 (1.7)</td>
</tr>
<tr>
<td>9</td>
<td>Appendiceal CA</td>
<td>GI</td>
<td>A&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1</td>
<td>0.3</td>
<td>0.3 (1.5)</td>
<td>1.0 (1.3)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> EF5 binding is expressed as a percentage of maximum hypoxia marker binding in tissue from the same patient. Reference binding values were not available for patients 4, 7, and 9. For these patients, the percentage of reference binding was calculated based on the average reference binding level in other patients of the same disease cohort.

<sup>b</sup> Median and range (largest-smallest) of values for each specimen.

<sup>c</sup> Median and range (largest-smallest) of median values of specimens for each patient.

<sup>d</sup> The model-based mean and SE of specimens for each patient.

<sup>e</sup> GIST, gastrointestinal stromal tumor; CA, carcinoma.

<sup>f</sup> An additional three specimens from patient 8 were found by histological analysis to contain no tumor and are thus not included in these data.

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In our previous publication of EF5 binding in human sarcomas (16), tumor areas of highest EF5 binding were reported for the section demonstrating the most severe hypoxia in each patient. In the present report, EF5 binding was measured within all of the tumor tissue in a given section and reported separately for each specimen and sectioning level (Table 1).
ously reported (16) low levels of EF5 binding in small nodules of GIST from another patient. The patient was not included in the present study because he was not considered for PDT treatment. However, in this patient, high levels of EF5 binding were found in much larger tumor nodules (1.65 and 6 cm), suggesting that in this tumor type, size may contribute to the development of hypoxic regions. The limited number of GIST samples available precluded any analysis of tumor size versus hypoxia within this specific histology.

EF5 binding \( \leq 8\% \) of cube reference, corresponding to physiological oxygenation to modest hypoxia, was found in the majority of specimens of ovarian carcinoma from patients 3 and 4 (Table 1, Fig. 1). However, severe hypoxia was found in one of six specimens of ovarian cancer evaluated. Specimen B of patient 4 demonstrated cube reference binding \( > 30\% \), i.e., severe hypoxia, at both sectioning levels studied.

In addition to the single specimen of ovarian cancer, specimens of colon carcinoma from all three patients (patients 5–7) demonstrated high levels of EF5 binding (Table 1). Severely hypoxic tumors were detected in patients 4–6, and moderate hypoxia was detected in patient 7. These tumors demonstrated substantial intra- and intertumoral hypoxic heterogeneity compared with GISTs and the small bowel and appendiceal tumors that were well oxygenated. Oxygenation in the colon and ovarian cancers ranged from physiological levels to severely hypoxic (Fig. 1), but in general the intraspecimen variability was smaller than the interspecimen variability (see Table 1). Ovarian cancer (patient 4) demonstrated the broadest interspecimen hypoxic heterogeneity among all nine of the patients studied. However, colon cancer was the most likely histological type to contain moderate to severe hypoxia. Colon cancer, together with small bowel and appendiceal cancer, comprises the gastrointestinal cohort of patients treated on the i.p. PDT trial (5). The small sample of patients we examined, diseases of different histology within the gastrointestinal cohort demonstrated vastly different levels of hypoxia. These data suggest that it may be worthwhile to stratify future outcome analysis by specific histology, in addition to the more general disease cohort.

Throughout this study, every attempt was made to acquire tumor nodules of \( \leq 5 \) mm in thickness because PDT is only administered to patients when their disease is debulked to a size of 5 mm or less. However, in some patients, larger tumors were removed and available for analysis. Thus, the size of the nodules sampled was representative of the disease in each patient and varied among individuals. The smallest disease studied was \( \sim 1–2\) mm nodules of GIST and colon carcinoma. Specimens of \( \sim 30\)- or 45-mm bulky disease were taken of colon and ovarian cancer, respectively. The association between hypoxia and tumor size was evaluated, and no relationship between greatest tumor dimension and EF5 binding was found in tumors from 1 to 45 mm in diameter (Fig. 2). Using a polarographic needle probe (Eppendorf pO2 Histograph) to measure the oxygenation of tumors (\( \sim 10 \) mm diameter or larger) of the breast and the head and neck, others have demonstrated a correlation between decreasing tumor size and decreasing hypoxia in some studies (26), but not in all studies (27, 28). Notably, in the present study, the majority of tumors that we investigated were \( < 10 \) mm in maximum diameter. Eleven of the 12 sections that demonstrated EF5 binding of \( \sim 10\% \) or higher were from tumor nodules of \( \leq 10 \) mm in greatest dimension. In particular, the single ovarian biopsy that exhibited severe hypoxia was from a nodule 8 mm
in diameter, versus the oxic measurements in ovarian specimens measuring 45 mm. The lack of severe hypoxia in larger tumor nodules was not affected by the presence of necrotic tissue, which cannot metabolize EF5, because analysis was conducted only on viable malignant tissue identified by a pathologist on each section. Furthermore, very limited necrosis was detected in the tumors of this study.

EF5 is a vascular-delivered drug; thus, one might hypothesize that avascular tumor nodules could exhibit limited EF5 binding as a consequence of poor drug delivery. We have previously demonstrated (29) the presence of vascular structure in tumor nodules as small as 1–2 mm from patients enrolled on the i.p. PDT trial. To confirm the vascularity of the nodules evaluated for EF5 binding, sections adjacent to those analyzed for EF5 were stained for both EF5 and CD31. Fig. 3 depicts photomicrographs of EF5-identified hypoxia and CD31-labeled vascular structure in representative small tumor nodules (~2 mm) with severe (Fig. 3A), modest (Fig. 3C), or physiological (Fig. 3E) hypoxia. The corresponding tissue areas, identified by Hoechst 33342 staining on each section, are shown in Fig. 3, B, D, and F, respectively. All nodules exhibit well-formed vascular structure, although the size of the vessels may differ between tumors of different histologies (compare colon cancer in Fig. 3, A and C, with GIST in Fig. 3E). CD31 staining does not demonstrate vascular perfusion, but it does indicate the presence of a vascular network in even the millimeter-sized nodules of this study. The adequate delivery of EF5 through this network is evidenced by the presence of EF5 binding in the most vascular-distant cells in micrograph (Fig. 3A). Furthermore, in this image, the pattern of increasing hypoxia as a function of distance to the nearest blood vessel (regardless of perfusion) suggests that diffusion-limited hypoxia, as opposed to perfusion-limited hypoxia, predominates. Such a distribution of hypoxia may be relevant to the PDT tumor response (30) because mechanisms of Photofrin PDT damage include insult to malignant cells, which may or may not be located in close proximity to a blood vessel, and damage of the blood vessels themselves.

**Photofrin Uptake in Tumor Nodules of Intraperitoneal Disease.** Photofrin concentration was measured in tumor nodules that were distinct from those used for EF5, but which were sampled from the same patients studied for EF5 binding. The nodules ranged in size from 1.8 to 20 mm in greatest diameter, and as with the EF5 binding, no association between tumor size and Photofrin uptake was evident (data not shown). The Photofrin uptake in each nodule is listed in Table 2. In contrast to EF5 binding, the variability in Photofrin uptake within patients...
was reasonably constant and did not vary in any obvious fashion with mean Photofrin level. In patient 7, interspecimen differences in Photofrin uptake were associated with site of biopsy; the first six nodules (uptake range, 0.38–0.91 ng/mg) were excised from the surface of the small bowel, whereas the last nodule (uptake, 4.27 ng/mg) was removed from the surface of the liver. Because normal liver is known to accumulate high levels of Photofrin, it might be expected that tumor nodules from this site would have high Photofrin uptake. Also, some variability in Photofrin uptake among specimens may be accounted for by heterogeneity in endogenous chromophore levels due to sample-to-sample variability in blood volume (23), although the mostly small biopsies of this study were nonhemorrhagic in nature. Overall, differences in Photofrin uptake among individuals were highly significant (P < 0.0001).

The highest levels of Photofrin uptake were found in nodules of small bowel cancer resected from the peritoneum of patient 8; average drug uptake was 5.8 ± 0.5 ng/mg. Patients 3 and 4 (ovarian cancer) and patients 5 and 6 (colon cancer) also demonstrated moderate Photofrin uptake with average levels of 2.7 ± 0.6, 3.9 ± 0.5, 3.9 ± 0.5, and 3.9 ± 0.4 ng/mg, respectively. The lowest levels of Photofrin uptake were found in patients 1 and 2 (GIST) and patient 9 (appendiceal cancer), in whom average drug levels were 1.3 ± 1.1, 1.9 ± 0.6, and 0.6 ± 0.8 ng/mg, respectively. These data suggest the presence of disease-dependent trends in Photofrin uptake. We will more rigorously examine the association between disease histology and Photofrin accumulation in a separate, future study that evaluates a larger number of i.p. PDT patients.

In nine patients (patients 1–9) who were evaluated for both Photofrin uptake and EF5 binding, we examined whether patients who had severely hypoxic tumors also demonstrated lower Photofrin uptake in separate nodules of their disease. The presence of diffusion-dependent hypoxia, such as that shown in Fig. 3, could lead to limitations in photosensitizer delivery. Fig. 4 plots the mean concentration of Photofrin in all tumor biopsies from a given patient versus the level of hypoxia detected in each patient. Low average Photofrin accumulation was not apparent in patients with severely hypoxic tumors. In fact, among the highest mean Photofrin levels (>4 ng/mg) detected in the tumor nodules of patients 4–6, who exhibited severely hypoxic tumors (EF5 binding > 30% of cube reference binding). In patients with physiological oxygenation, mean Photofrin uptake ranged from <1 to ~6 ng/mg (Fig. 4), suggesting that low accumulation of Photofrin may be found in minimally hypoxic disease. The low cellularity of some specimens, such as those of appendiceal cancer, may account for some of these findings. Whereas a low tumor cell density minimally affects EF5 analysis, which is based on histologically identified malignancy, low tumor cell density among stroma tissue will affect the Photofrin analysis, which is based on the weight of the excised nodule. As a consequence, among tumor nodules with high stroma content,

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**Table 2** Photofrin uptake in intraperitoneal malignancies

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Histology</th>
<th>Cohort</th>
<th>Specimen</th>
<th>Photofrin uptake (ng/mg)</th>
<th>Interspecimen median (range)</th>
<th>Interspecimen mean (SE)</th>
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<tbody>
<tr>
<td>1</td>
<td>GISTa</td>
<td>1</td>
<td></td>
<td>1.26</td>
<td>1.26</td>
<td>1.3 (1.1)</td>
</tr>
<tr>
<td>2</td>
<td>GIST</td>
<td>1, 2, 3</td>
<td></td>
<td>3.28, 1.34, and 0.96</td>
<td>1.34 (2.32)</td>
<td>1.9 (0.6)</td>
</tr>
<tr>
<td>3</td>
<td>Ovarian CA</td>
<td>1, 2, 3</td>
<td></td>
<td>1.99, 3.14, and 2.91</td>
<td>2.91 (1.15)</td>
<td>2.7 (0.6)</td>
</tr>
<tr>
<td>4</td>
<td>Ovarian CA</td>
<td>1, 2, 3, 4</td>
<td></td>
<td>4.44, 5.26, 4.30, and 1.72</td>
<td>4.37 (3.54)</td>
<td>3.9 (0.5)</td>
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<tr>
<td>5</td>
<td>Colon CA</td>
<td>GI</td>
<td>1, 2, 3</td>
<td>4.63, 2.79, and 4.12</td>
<td>4.12 (1.84)</td>
<td>3.9 (0.5)</td>
</tr>
<tr>
<td>6</td>
<td>Colon CA</td>
<td>GI</td>
<td>1, 2, 3</td>
<td>3.32 and 4.49</td>
<td>3.84 (1.68)</td>
<td>3.9 (0.4)</td>
</tr>
<tr>
<td>7</td>
<td>Colon CA</td>
<td>GI</td>
<td>1, 2, 3</td>
<td>3.06, 4.44, and 3.50</td>
<td>4.74, 3.32, 3.84, and 4.36</td>
<td>3.91 (1.36, and 0.39)</td>
</tr>
<tr>
<td>8</td>
<td>Small bowel CA</td>
<td>GI</td>
<td>1, 2, 3</td>
<td>0.66, 0.39, and 0.87</td>
<td>0.91, 0.38, 0.77, and 4.27</td>
<td>0.77 (3.89)</td>
</tr>
<tr>
<td>9</td>
<td>Appendiceal CA</td>
<td>GI</td>
<td>1 and 2</td>
<td>5.30, 4.79, and 6.40</td>
<td>6.67 and 5.80</td>
<td>5.8 (0.5)</td>
</tr>
<tr>
<td>10</td>
<td>Appendiceal CA</td>
<td>GI</td>
<td>1, 2, 3</td>
<td>0.98 and 0.13</td>
<td>0.68, 0.78, and 0.68</td>
<td>0.55 (0.85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4, 5, 6, 7</td>
<td>3.91, 1.36, and 0.39</td>
<td>4, 5, 6, and 7</td>
<td>0.78 (3.52)</td>
</tr>
</tbody>
</table>

a Drug uptake was measured by fluorescence in whole tumor nodules after tissue solubilization.

b Median and range (largest-smallest) of values of specimens for each patient.

c The model-based mean (SE) of specimens for each patient.

d GIST, gastrointestinal stromal tumor; CA, carcinoma; GI, gastrointestinal.

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**Fig. 4** The mean concentration of Photofrin (nanograms/milligram) versus the oxygenation status of the most hypoxic specimen detected in each patient. Photofrin uptake and EF5 binding were measured in separate tumor nodules. Error bars are SE. Patients include those with gastrointestinal stromal tumor (△), ovarian carcinoma (○), colon carcinoma (●), small bowel carcinoma (□), or appendiceal carcinoma (◇).
DISCUSSION

These data demonstrate substantial inter- and intratumoral hypoxic heterogeneity among different histological tumors that can manifest themselves as carcinomatosis or sarcomatosis. An important conclusion of our investigations is that even a ≤5 mm tumor nodule may be substantially hypoxic because high levels of hypoxia marker binding were found in colon cancer nodules, which were among the smallest nodules evaluated in this study. These data are potentially important to the efficacy of i.p. PDT because PDT is an oxygen-dependent process. Furthermore, because many of the colon cancer nodules demonstrated moderate Photofrin uptake of ~3–5 ng/mg, PDT itself may further contribute to tumor hypoxia through photochemical-induced oxygen depletion. Others have demonstrated that oxygen depletion can occur during PDT of basal cell cancer nodules thought to contain Photofrin in the range of only 0.29–1.59 ng/mg (14).

Vascular networks were present in even the smallest and most severely hypoxic tumors, suggesting a mechanism for tumor uptake of i.v. delivered cancer drugs, such as Photofrin. Vascular networks were also detected in nodules demonstrating low levels of hypoxia, suggesting that EF5 (or Photofrin) delivery was not limited by drug access, although we cannot demonstrate the presence of blood flow in these patient samples. The finding of biopsies that demonstrate low Photofrin uptake in patients who have little EF5 binding in their disease brings to light the possibility that delivery of both agents may have been limited. However, extensive preclinical evaluation has established that under long incubation times, sufficient concentration of hypoxia marker are delivered to label even low perfusion areas (31–35). Additionally, we always see EF5 binding adjacent to regions of necrosis. In control studies of perfusion-created limitations in hypoxia marker delivery, drug inaccessibility was visible in distinct binding patterns, characterized by high binding areas delineating well-defined “holes” (30). No such binding patterns were found in the clinical samples of the present study, suggesting that EF5 delivery was not a limiting factor.

Technical limitations prevented the examination of Photofrin uptake and EF5 binding in the same tumor nodule. However, in patients with severely hypoxic tumor nodules (patients 4–6), separate tumor specimens demonstrated substantial Photofrin uptake. Based on these data, we suggest that the currently accepted paradigms of the relationships among tumor size, vascular distribution, drug delivery, and hypoxia must be reconsidered because they may be much too general. The possibility of the presence of well-photosensitized but hypoxic disease in the i.p. cavity requires further investigation, and patients with such disease may perhaps benefit from the augmentation of tumor oxygenation during i.p. PDT. These findings strongly support the addition of methods to measure tumor hypoxia and photosensitizer uptake to future i.p. PDT trials to permit the investigation of a larger patient population and correlation with treatment outcome.

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REFERENCES


