Systemic Toxicity in Mice Induced by Localized Porphyrin Photodynamic Therapy

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ABSTRACT

An unexpected high level of acute lethality has been documented following Photofrin II-mediated photodynamic therapy (PDT) treatments which were localized to the hind leg of normal and tumor-bearing mice. Doses of PDT which induced lethality (10 mg/kg Photofrin II, 200–500 J/cm²) were in the range of doses required to obtain murine tumor cures. The percentage of lethality was proportional to the total light dose but was inversely proportional to the dose rate of delivered light. Comparable levels of acute toxicity were observed in four pigmented mouse strains (C57BL/6J, C3H/HeJ, DBA/2J, and DBA/2) and in two albino mouse strains (BALB/c and Swiss Webster). Decreased sensitivity to PDT-induced lethality was observed in two pigmented mouse strains (B6D2OSN and B6D2NSN). The administration of warfarin, aspirin, indomethacin, or antagonists had significant protective effects in terms of decreasing PDT-induced lethality. However, injection of cobra venom factor (to deplete C3 and C5 of the complement system) did not alter the lethality mediated by PDT. Histological profiles obtained 24 h following PDT demonstrated vascular congestion in the liver, kidney, lung, and spleen. Significant decreases in removable blood volume, core temperature, and spleen weight were also observed within 24 h of localized PDT treatment. These results indicate that PDT-induced lethality is consistent with a traumatic shock syndrome and suggest that endogenous vasoactive mediators of shock such as prostaglandins, thromboxanes, and histamine are associated with the lethality induced by localized PDT in mice.

INTRODUCTION

Photodynamic therapy is the generalized term for the treatment of solid malignancies by using tissue-penetrating visible light following the administration of a tumor-localizing photosensitizer such as hematoporphyrin derivative or its porphyrin component Photofrin II (1, 2). The combination of drug uptake in malignant tissue and selective delivery of laser-generated light provides for an effective therapy with efficient tumor cytotoxicity and minimal normal tissue damage (3, 4). The photochemical generation of singlet oxygen and possibly other reactive oxygen species is responsible for the cytotoxicity induced by PDT (5, 6). Information regarding the biochemical, cellular, and preclinical parameters associated with PDT has recently been reviewed (6, 7). The clinical efficacy of PDT is currently being evaluated in Phase III trials for obstructive and partially obstructive carcinoma of the bronchus and esophagus as well as for transitional cell carcinoma of the bladder (8). In addition, PDT is being used on a limited basis to treat malignancies of the head and neck, skin, cervix, eye, and brain (2, 8).

Recently, we encountered high levels of acute lethality in mice treated with Photofrin II-mediated PDT during experiments designed to evaluate effects of light dose rate on tumor response. This observation was unexpected in that: (a) the PDT doses that were used (10 mg/kg Photofrin II, 200–500 J/cm²) were comparatively low and were in the range of doses required to produce tumor cures in mice (9); and (b) the area of light treatment was specifically localized to the hind leg in order to eliminate light exposure to all vital organs and structures. Lethal toxicity induced by various photosensitizers has been documented as early as 1911 (reviewed in Ref. 10), and systemic toxicity has been reported following whole body and abdominal light exposure of porphyrin PDT in mice (11). In this report, we describe the clinical, hematological, and histopathological responses of acute toxicity induced by Photofrin II-mediated PDT as a function of various treatment parameters and mouse strains.

MATERIALS AND METHODS

Drugs. Photofrin II (dihematoporphrin-ether) was obtained from Photomedica, Inc., Ranirar, NJ, as a sterile solution at a concentration of 2.5 mg/ml. The drug was diluted with saline to obtain a working concentration of 1 mg/ml. A 10-mg/kg dose of Photofrin II was administered by i.p. injection 24 h prior to localized laser irradiation in PDT experiments. Sodium warfarin (Coumadin, DuPont Pharmaceuticals, Inc., Wilmington, DE), indomethacin (Indocin, Merck Sharp & Dohme, Inc., West Point, PA), and aspirin (acetylsalicylic acid, Sigma Chemical Co., St. Louis, MO) were administered in the drinking water to test mice. The concentration of warfarin was 5 mg/liter and mice were administered the drug from day −3 to day −1 prior to PDT light treatment (12, 13). Indomethacin was dissolved in 95% ethanol at a concentration of 10 mg/ml, and was then added to the drinking water for a final concentration of 20 mg/liter in 0.25% ethanol (14, 15). Indomethacin was administered to test mice from day −4 to day −2. Aspirin was added to the drinking water at a concentration of 625 mg/liter and was administered to mice from day −4 to day +2 (16). The antihistamine pyrilamine maleate (Histavet-P, Schering Corp., Kenilworth, NJ) was delivered to test mice by i.m. injection. A 0.5-mg/kg dose of antihistamine was administered immediately following PDT to the hind leg not involved in the PDT treatment (17). Cobra venom factor was obtained from Cordis Laboratory, Inc., Miami, FL, as a lyophilized powder. The drug was dissolved in water and administered by i.v. injection at a dose of 250 units/kg on day −2 (18).

Animals. Eight strains of female mice (8 to 12 weeks old) were utilized in various experiments. Pigmented mice included: C57BL/6J, C3H/HeJ (endotoxin resistant), DBA/2J (C5 proficient), DBA/2J (C5 deficient), B6D2OSN (C5 deficient) and B6D2NSN (C5 proficient). Albino mouse strains used in this study included Swiss Webster and BALB/c. All mice were purchased from The Jackson Laboratory, Bar Harbor, ME.

Tumor Models. Pigmented and nonpigmented B16 melanomas were used during initial experiments in this study (9). The tumors were originally obtained from the NIH Tumor Repository and they were maintained by serial passage in the hind flank of C57BL/6J mice. A 1-mm³ piece of tumor was transplanted s.c. via trochar injection in the shaved hind right leg of mice. Tumor volumes were determined 3 times/week and mice with tumors measuring 25–35 mm³ were entered into PDT experiments.

Photodynamic Therapy Protocols. An i.p. injection of Photofrin II (10 mg/kg) was administered 24 h prior to light treatment (19). A 1-cm diameter spot localized to the hind right leg was used as the treatment area for all light exposures. Red light at 630 nm was generated by an argon pumped dye laser (Spectra-Physics, Inc., Mountain-
view, CA). A 400-μm diameter quartz fiber was interfaced to the output of the dye laser and a microlens was attached to the distal tip of the fiber for uniform light delivery. The wavelength of delivered light was documented with a spectroscope (Cooper Lasersonics, Inc., Santa Clara, CA) and a power meter (Coherent Radiation, Palo Alto, CA) was used to measure light intensity. All mice undergoing PDT were restrained in holders without anesthesia.

**Histological and Hematological Evaluation.** Acute histological specimens were obtained for C57BL/6J mice by sacrificing the animals 24 h following PDT (10 mg/kg Photofrin II, 150 mW/cm², 500 J/cm²). Samples from the liver, spleen, lung, kidney, and skin were obtained and placed in 10% buffered formalin. The specimens were embedded in paraffin, sectioned, and processed by using hematoxylin-eosin staining or periodic acid-Schiff staining.

An electronic blood cell counter (ELT-8/DS, Orthodiagnetics Systems, Inc.) was used to obtain quantitative values for hematocrits, platelets, and WBC from C57BL/6J mice treated with PDT (10 mg/kg, 500 J/cm², 150 mW/cm²²). Blood samples were collected by heart puncture at the completion of the PDT light treatment (0 h) and at 1, 2, and 4 h, and 1, 3, 7, and 14 days. Control mice received either saline or Photofrin II (without light).

**Statistical Analysis.** The χ² test was used to evaluate mouse lethality responses and the Student’s t test was used to evaluate the mean values for hematological determinations.

**RESULTS**

The percentage of acute lethality induced by localized PDT in C57BL/6J mice with either pigmented or nonpigmented B16 melanomas is shown in Table 1. PDT-induced lethality is documented as a function of both total light dose (200-500 J/cm²) and dose rate of delivered light (150 or 600 mW/cm²). In all cases, the site of light exposure covered the tumor and was confined to a 1-cm-diameter spot on the hind right leg. An increase in total light dose corresponded to a concomitant increase in the percentage of PDT-induced deaths for both mouse-tumor models. However, increasing the dose rate of delivered light produced a decrease in lethality for mice treated at equal total light doses.

Acute lethality in normal C57BL/6J mice following localized PDT directed to the hind leg is summarized in Table 2. These experiments were performed to determine whether the high degree of PDT-mediated lethality described in Table 1 was due to the fact that the first group of treated mice had tumors. The percentage of lethality described in Table 2 is documented as a function of both total light dose and light dose rate. The percentage of lethality was again observed to be directly proportional to the total light dose and inversely proportional to the dose rate of delivered light. Light exposure without prior administration of Photofrin II did not induce any lethality even though local temperature rises of 6°C (150 mW/cm²) and 21°C (600 mW/cm²) were produced at the site of light exposure.

The effects of localized PDT on different strains of mice are documented in Table 3. The PDT parameters (10 mg/kg, 150 mW/cm², 500 J/cm²) were comparable to dose parameters required to achieve cures in most mouse tumor models. Similar levels of PDT-induced lethality were observed in all mouse strains except for the B10D2/OSN and B10D2/NSN strains, which exhibited significantly decreased lethality following PDT.

Table 4 describes the effect of various pharmacological agents on the lethality induced by PDT in normal C57BL/6J mice. The combination of PDT and either warfarin, aspirin, indomethacin, or antihistamine produced a protective effect in terms of decreased lethality and the protection was observed at all light doses analyzed (300-500 J/cm²) and in each case the reduction in lethality was approximately 50%. These drugs (which function by inhibiting cyclooxygenase, histamine, and coagulation) were administered by using previously reported protocols (12-18). Cobra venom factor (which depletes C3 and C5 of the complement system) did not alter the lethality induced by PDT. Experiments described in Tables 3 and 4 were performed approximately 3 months apart and therefore separate groups of positive controls (PDT, 500 J/cm², 150 mW/cm²) were used.

**Histological profiles obtained from PDT-treated C57BL/6J**

**Table 3** PDT-induced lethality as a function of mouse strain

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>No. of mice treated</th>
<th>% of lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>26</td>
<td>76.92</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>20</td>
<td>70.00</td>
</tr>
<tr>
<td>B10D2/OSN</td>
<td>20</td>
<td>20.00</td>
</tr>
<tr>
<td>B10D2/NSN</td>
<td>20</td>
<td>0.00</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>9</td>
<td>77.78</td>
</tr>
<tr>
<td>DBA/2F</td>
<td>10</td>
<td>80.00</td>
</tr>
<tr>
<td>B874/15</td>
<td>10</td>
<td>100.00</td>
</tr>
<tr>
<td>Swiss Webster</td>
<td>10</td>
<td>70.00</td>
</tr>
</tbody>
</table>

* The PDT treatment consisted of a 10 mg/kg i.p. injection of Photofrin II administered 24 h prior to localized hind leg exposure to 630 nm light at a dose of 500 J/cm² and delivered at a light dose rate of 150 mW/cm². Lethality was observed within 2 days of PDT treatment.

* Endotoxin resistant.

* C5 deficient.

* C5 proficient.

* Albino.

**Table 4** Lethality in C57BL/6J mice treated with exogenous pharmacological agents

<table>
<thead>
<tr>
<th>Drug treatment</th>
<th>% of lethality</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDT alone</td>
<td>44.44 (36)</td>
</tr>
<tr>
<td>PDT + warfarin</td>
<td>61.00 (31)</td>
</tr>
<tr>
<td>PDT + indomethacin</td>
<td>35.00 (20)</td>
</tr>
<tr>
<td>PDT + aspirin</td>
<td>35.00 (20)</td>
</tr>
<tr>
<td>PDT + antihistamine</td>
<td>35.00 (20)</td>
</tr>
<tr>
<td>PDT + cobra venom factor</td>
<td>ND</td>
</tr>
</tbody>
</table>

* The PDT treatment consisted of a 10 mg/kg i.p. injection of Photofrin II administered 24 h prior to localized hind leg exposure to 630 nm light at a dose of 500 J/cm² and delivered at a light dose rate of 150 mW/cm². Lethality was observed within 2 days of PDT treatment.

* Numbers in parentheses, number of mice.

* ND, not determined.
mice were consistent with responses seen during a systemic shock reaction (20). Vascular congestion was present in the liver, kidney, lung, and spleen 24 h following PDT (data not shown). Hematological profiles (WBC, platelets, and hematocrit) in C57BL/6J mice following PDT are shown in Figs. 1–3 for time periods ranging from 1 h to 14 days following treatment. Fig. 1 shows that the WBC initially increased from a base-line level of 10,000/mm³ to over 40,000/mm³ during the first 4 h following PDT. The WBC values were still over 30,000/mm³ at 24 h posttreatment. However, by 3 days posttreatment the WBC levels had decreased to 2,900/mm³ which was significantly below control levels (P < 0.01). Animals surviving the PDT treatment had WBC levels which returned to control values by 7 days posttreatment. Platelet levels in PDT-treated mice were lower than control values for the initial 24 h following treatment as shown in Fig. 2. The platelet count increased to levels significantly higher than control levels (P < 0.01) at 7 and 14 days posttreatment. Hematocrit levels are shown in Fig. 3.

**DISCUSSION**

Results from this study demonstrate that systemic toxicity in the form of acute lethality is induced following Photofrin II-mediated PDT in both normal and tumor-bearing mice. The phenomenon of acute toxicity (lethality) in experimental animals following photosensitizer-initiated photodynamic action has been reported previously (10, 11), but there have not been any prior reports on lethality induced by porphyrin PDT associated with localized tumor treatments. The PDT dose response experiments (combining various pharmacological agents), together with the histopathological observations and hematological responses obtained in this study, strongly suggest that...
localized PDT elicits systemic toxicity in the form of a traumatic shock syndrome (20). Traumatic shock can be produced by tissue injury which in turn leads to inadequate peripheral perfusion. Despite a wide variety of inducers of shock, the final pathway appears to be circulatory collapse (20). The etiology of irreversible shock includes induction of significant levels of ischemia or hypoxemia, the release of prostaglandins and kinins, as well as production of large amounts of cell necrosis. Indomethacin and aspirin are antiinflammatory agents which can inhibit cyclooxygenase and therefore decrease prostaglandin and thromboxane synthesis. Warfarin acts as an anticoagulant and antihistamine actions are primarily related to antagonizing the effects of histamine. Each of these agents can reduce the action of endogenous mediators of microcirculatory disruption which is characteristic of traumatic shock. Our observations are therefore in agreement with previous reports describing the generation of eicosanoids and antihistamine following porphyrin photosensitization (17, 21). A separate study has also shown that inhibitors of cyclooxygenase (indomethacin and aspirin) can significantly reduce the vasoconstriction and vascular stasis induced by PDT (22).

It is significant to note that the doses of PDT used in the current study were comparable to those used previously in tumor cure experiments (20). Equally important is the fact that the site of PDT treatment in the current set of experiments was specifically limited to the hind leg in order to avoid illumination of all vital organs. Interestingly, the lethality observed in mice following PDT is not unique to Photofrin II. We have observed comparable effects with chlorin photosensitizers and other investigators have observed lethality following PDT by using phthalocyanines. Fortunately, the type of acute systemic shock reaction described in this study has not been observed in humans undergoing clinical PDT. In addition, larger animals (rats and rabbits) treated with the highest doses of Photofrin II-mediated PDT used in the current study did not exhibit any systemic toxic reaction (data not shown). These observations would suggest that the relationship of PDT treatment area to total body area may be an important parameter in the induction of acute lethality.

The lethality induced in mice by localized PDT appears to be a generalized phenomenon and not limited to tumor-bearing mice (which may have compromised immune systems) or to any one particular strain of mouse. In our study, similar levels of PDT-induced lethality were observed for pigmented and albino mice as well as for mice with transplanted melanomas. The only exception to the high level of PDT-induced lethality occurred in the two B6D2 mouse strains. It is currently unclear why these two mouse strains exhibited resistance to PDT-induced lethality. However, the resistance does not appear to be related to complement activation since both C5-proficient and C5-deficient strains of B6D2 mice were resistant to the toxic effects of PDT. Interestingly, these two strains were produced by an initial cross between C57BL/10 mice and DBA/2 mice (23). The C57BL mouse is proficient for C5 while the DBA/2 mouse lacks C5 and yet both C57BL and DBA/2 mice exhibited high levels of PDT-induced lethality. In previous studies, cutaneous phototoxicity was shown to be associated with a decrease in total complement activity and the phototoxic reaction was suppressed in complement-depleted animals (24, 25). In the current study, the administration of cobal venous factor (to deplete C3 and C5) had no effect on PDT lethality in C57BL mice. However, there may be differences in porphyrin pharmacokinetics in the B6D2 mouse strains which could influence the degree of systemic toxicity observed following PDT but this has not yet been evaluated. It is also unknown whether the B6D2 mouse strains are inherently more resistant to traumatic shock than the other mouse strains used in this study.

The inverse relationship between PDT-induced lethality and the dose rate of delivered light was unexpected but may be associated with physiological properties of the treatment. The highest light dose rate (600 mW/cm²) produced a 21°C localized temperature rise 1 mm below the surface at the treatment site. This thermal condition could have induced a complete and localized vascular closure with a concomitant decrease in the release to prostaglandins or other endogenous toxic substances. In addition, the volume of blood flowing through the PDT treatment area would be 4 times lower for exposures performed at 600 mW/cm² compared to those performed at 150 mW/cm². Nevertheless, it is apparent that the addition of local hyperthermia did not potentiate PDT-induced lethality even though heat has been shown to enhance PDT-induced tumor destruction (6, 26).

Results from this study indicate that PDT-induced lethality in mice will have to be taken into account when screening new tumor photosensitizers. Quantitative dose response experiments (using tumor cure as an end point) may be extremely difficult to complete as a consequence of the high level of lethality which may accompany the treatment. In addition, possible systemic factors involved with tumor responses in PDT-treated mice (i.e., eosinoid and/or cytokin release as well as physiological factors involved with shock induction) may not be relevant in PDT treatment in larger animals or in humans. The positive effects that indomethacin, aspirin, warfarin, and antihistamine had on reducing PDT-associated lethality strongly suggest that endogenously produced vasoactive mediators can influence generalized PDT responses. The doses of PDT currently used in larger animals and humans (2) do not elicit any observable systemic toxicity, and therefore it is possible that systemic factors associated with PDT in mice will be greatly reduced or eliminated following treatment in larger animals and humans.

In summary, the PDT-induced lethality documented in the current study appears to be comparable to a traumatic shock syndrome and is associated only with small animal models. The acute lethality observed in mice does not appear to be clinically relevant but the phenomenon should be taken into account during the in vivo screening of new tumor photosensitizers using mouse models.

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REFERENCES

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