We evaluated the potential role of photoradiation therapy with a benzoporphyrin derivative, monoacid ring A (BPD-MA), and dehematoporphyrin ether (DHE), for the ex vivo purging of residual tumor cells from autologous bone marrow (BM) grafts. BPD-MA and DHE photosensitizing activity was tested against two human large-cell lymphoma cell lines and colony-forming unit-leukemia (CFU-L) derived from patients with acute myelogenous leukemia (AML). In mixing experiments, 4-log elimination of tumor cell lines was observed after 1 hour of incubation with 75 ng/mL of BPD-MA or 30 minutes of treatment with 12.5 μg/mL of DHE followed by white light exposure. By comparison, using the same concentration of BPD-MA, the mean recovery of normal BM progenitors was 4% ± 0.8% (mean ± SD) for granulocyte-macrophage colony-forming unit (CFU-GM) and 5% ± 0.8% for burst-forming unit-erythroid (BFU-E). Similarly, DHE treatment resulted in the recovery of 5.2% ± 2% and 9.8% ± 3% of CFU-GM and BFU-E, respectively. Furthermore, equivalently cytotoxic concentrations of both DHE and BPD-MA and light were found not to kill normal pluripotent stem cells in BM, as demonstrated by their survival in two-step long-term marrow culture at levels equal to untreated controls. The T-lymphoblastic leukemia cell line CEM and its vinblastine (VBL)-resistant subline CEM/VBL, along with the acute promyelocyte leukemia cell line HL-60 and its vincristine (VCR)-resistant subline HL-60/VCR, were also tested. BPD-MA at 75 ng/mL was able to provide a greater than 4-log elimination of the drug-sensitive cell lines, but only a 34% and 55% decrease of the drug-resistant HL-60/VCR and CEM/VBL cell lines, respectively. On the contrary, 12.5 μg/mL of DHE reduced the clonogenic growth of all the cell lines by more than 4 logs. Further experiments demonstrated decreased uptake of both BPD-MA and DHE by the resistant cell lines. However, all the cell lines took up more DHE than BPD-MA under similar experimental conditions. Our results demonstrate the preferential cytotoxicity of BPD-MA and DHE toward neoplastic cell lines and CFU-L from AML patients. In addition, DHE was slightly more effective in purging tumor cells expressing the p-170 glycoprotein. These results suggest that photoradiation with DHE would be useful for in vitro purging of residual drug-resistant leukemia and lymphoma cells.

**MATERIALS AND METHODS**

**Human cell lines.** LY-16 and SK-DHL-2 are diffuse large-cell lymphoma B-cell lines that were established from two patients at Memorial Sloan-Kettering Cancer Center (MSKCC).9 HL-60 is a human promyelocytic leukemia cell line9 and CEM is a T-lymphoblastic leukemia cell line.1 CEM/VBL and HL-60/VCR cell lines were kindly provided by Dr E. Berman of our institution. Both of these drug-resistant cell lines express high levels of the mdr 1 gene product (p-glycoprotein).10 The cell lines were maintained in exponential growth conditions in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% fetal calf serum (FCS; Hyclone Labs, Logan, UT), 1% penicillin-streptomycin-neomycin (GIBCO, Chagrin Falls, OH), and 1% L-glutamine at 37°C in a fully humidified atmosphere of 5% CO2 in air. Cell viability was greater than 95% at the time of the study and the cell lines were free of mycoplasma contamination. The clonogenic efficiencies of the cell lines in semisolid medium ranged from 15% to 40%.

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**Dye-Mediated Photolysis Is Capable of Eliminating Drug-Resistant (MDR+) Tumor Cells**

By Roberto M. Lemoli, Tadahiko Igarashi, Marianne Knizewski, Luis Acaba, Anna Richter, Ashok Jain, David Mitchell, Julia Levy, and Subhash C. Gulati

PHOTORADIATION therapy using light-activated photosensitizing compounds is a promising new approach to cancer treatment.1,2 The capacity of some photosensitizing dyes to sensitize leukemic and lymphoma cells to a greater extent than normal hematopoietic progenitor cells has also suggested their potential use for ex vivo treatment of autologous bone marrow (BM).3,4 In vivo and in vitro applications of photodynamic therapy have centered on the use of hematoporphyrins (HP), especially dehematoporphyrin ether (DHE)4,5 or merocyanine-540 (MC-540).3,6 Benzoporphyrin derivatives (BPD) are photosensitizers derived from HP that have shown a selective uptake by tumor cells and are activated by light at 690 nm to cause singlet oxygen release that ultimately leads to tumor cell killing. Preliminary studies have demonstrated the superiority of BPD over HP for photoradiation therapy. BPD has higher cytotoxicity and is also able to better absorb light than HP at the 700 nm wavelength, which is known to be more effective in penetrating human tissue.5 Moreover, among BPD, four structural analogs have been studied and compared for their activity in vitro. The results of the study showed that the analog carrying a cyclohexadiene ring fused at ring A and one acid group attached at ring C of the porphyrin (BPD, monoacid, ring A, or BPD monoacid ring A [BPD-MA]) was associated with a higher uptake and subsequently greater elimination of tumor cells.6

Based on these premises, we compared the dose-dependent cytotoxic effects of BPD-MA and DHE on two cell lines of lymphoid origin and colony-forming unit-leukemia (CFU-L) derived from acute myelogenous leukemia (AML) patients. Moreover, we evaluated the clonogenic growth inhibition and cellular uptake of BPD-MA and DHE on two multidrug resistant (MDR+1) leukemia cell lines, HL-60/vincristine (HL-60/VCR) and CEM/vinblastine (CEM/VBL), and their parental (MDR−) cell lines. To simulate purging conditions, tumor cells were mixed with irradiated mononuclear or buffy coat BM cells and the results were compared with those of normal BM progenitor cells.

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BM cells. BM was aspirated from the posterior iliac crest of healthy volunteers after obtaining written informed consent. Buffy coat cells were collected after sedimentation with 6% hydroxyethyl starch (HES; a kind gift of DuPont Pharmaceutical, Wilmington, DE), which reduced the hematocrit to 9.4% ± 1.5% SD and the hemoglobin to 3.2 ± 0.5 g/dL (mean ± SD). The mononuclear light-density fraction was collected by centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals Co, Piscataway, NJ) (1.077 g/mL).

Leukemic samples. Leukemic cells were obtained from the peripheral blood (PB) or BM of four AML patients (Table 1). The diagnosis of AML was based on morphologic criteria and cytochemical staining and by surface-marker analysis and the leukemic specimens were subclassified according to the French-American-British (FAB) classification system. The leukemic samples were processed, cryopreserved, and thawed at the time of the study as previously reported.

Reagents. BPD-MA (a kind gift from QLT Inc, Vancouver, British Columbia, Canada) was diluted in dimethyl sulfoxide (DMSO) to the concentration of 1 mg/mL and stored frozen in a light protected container at −20°C until use. DHE (Photofrin II; QLT) was diluted in sterile water at 2.5 mg/mL and stored frozen. For each experiment, stock solutions of both compounds were diluted in serum-free IMDM immediately before their use.

Light source. Cells were exposed to light using a white light box (supplied by QLT) containing 16 tungsten light bulbs (100 W). The spectrum of light generated ranged from 400 to 900 nm, with a peak around 630 nm. The light intensity measured by an IL 1400 photodetector (International Light Inc, Newburyport, MA) was 9.25 ± 0.1 mW/cm². The temperature at the level of the exposed samples was 27°C ± 1°C during the treatment.

Photosensitizer incubation and photoradiation. Tumor cells and normal BM cells were incubated with different concentrations of BPD-MA or DHE for 1 hour or 30 minutes, respectively, at 37°C in the dark. The cells were resuspended in IMDM containing 1% FCS. To simulate ex vivo BM purging conditions, neoplastic cells were also incubated together with irradiated (3,000 cGy) BM cells (1:20 ratio, 2 × 10⁶ BM cells/mL, final concentration). After incubation with BPD-MA or DHE, the cells were washed twice, resuspended in IMDM supplemented with 10% FCS, and then exposed for 20 minutes to light. The experiments were performed in dim light to protect the cells from prematurely activated drug. After photoradiation, cells were counted and assayed for colony formation. In control experiments, cells were exposed to the light without the photosensitizer. Other controls included samples incubated with BPD-MA or DHE but not exposed to the light and samples neither incubated with the photosensitizing agents nor exposed to the light.

Clonogenic assay. After incubation, tumor cell lines, alone or in mixture with irradiated BM cells, were plated in 35-mm plastic Petri dishes (Miles Lab, Naperville, IL) in quadruplicate. The culture medium consisted of 1.1% methylcellulose (Methocel A4M; Dow Chem Co, Midland, MI) in IMDM containing 20% FCS, 1% antibiotics, and 1% L-glutamine. The number of cells plated in 1 mL of medium was 500 untreated and 5 × 10⁴ treated. Cells were incubated at 37°C in a fully humidified atmosphere of 5% CO₂ in air and scored for colonies (>50 cells) after 7 days of incubation using an inverted microscope.

After treatment, normal BM cells were assayed for colonies derived from CFU–granulocyte-macrophage (CFU–GM) and burst-forming units-erythroid (BFU-E) as already described. Culture medium consisted of 1 mL of IMDM supplemented with 24% FCS, 0.8% bovine serum albumin (BSA; Sigma Chemical Co, St Louis, MO), 10⁻⁴ mol/L of 2-mercaptoethanol (Sigma), 1 U of partially purified erythropoietin (Epo; Toyobo Inc, New York, NY), 10% of a selected lot of MoCM, and 0.2 mmol/L bovine hemin (Sigma). Methylcellulose final concentration was 1.32%. Quadruplicate cultures were incubated as described above and colonies were scored after 14 days of incubation. Clonogenic assay for CFU-L was the same as used for normal BM progenitors without the addition of Epo and bovine hemin. A total of 10² irradiated autologous cells were added per milliliter of culture. The number of cells plated was adjusted to approximately 100 colonies/mL of culture in the control samples; the treated samples were cloned at a higher cell concentration. Colonies (>20 cells) were scored after 10 to 12 days of incubation.

Two-step long-term BM culture. Materials included 12.5% fetal bovine serum (FBS), 12.5% horse serum, penicillin (density, 1.077 g/mL), McCoy’s 5A tissue culture medium, sodium bicarbonate (7.5% wt/vol), 100 mmol/L sodium pyruvate, and Corning tissue culture flasks (Corning, NY). Media additions included vitamins (100× concentrate), essential amino acids (50× concentrate), and glutamine (200 mmol/L), as provided by GIBCO. Also, an antibiotic-antimycotic solution (containing 10,000 U penicillin, 10,000 U streptomycin, and 25 mg amphotericin B per milliliter and 275 mmol/L of hydrocortisone in DMSO) were added to the long-term marrow culture (LTMC) medium.

Primary LTMC were established according to the method of Keating and Toor. Cells were washed by centrifugation for 10 minutes at 1,500 rpm, followed by resuspension in 10 mL of LTMC medium. Cultures were maintained in 25-cm² Corning flasks in a 10 mL volume at 37°C, 5% CO₂ in humidified incubator for 7 days and then moved to a 33°C incubator for 3 weeks, at which time a good adherent layer was formed. At that time, adherent layers were irradiated in a 400 gamma radiation source (1,000 cGy), after which the supernatant was removed. BM cells for secondary culture were suspended in LTMC medium and placed over the irradiated adherent layers, after which they were incubated at 33°C. Every week throughout the culture time, half the culture supernatant was removed and replaced with LTMC medium. Cells from the supernatant were counted and plated in the standard progenitor cell assay as discussed. Results were expressed as the number of colonies arising per 10⁶ cells plated.

BM cells tested for photosensitivity before secondary LTMC were treated as follows. Cells were suspended at a concentration of 2 × 10⁶/mL in medium containing 5% human plasma (obtained from normal donor blood). DHE or BPD-MA were added at various concentrations for 1 hour, after which the cells’ plasma concentration was increased to 10%. Cells were then exposed to red light (15 J/cm²), after which they were washed by centrifugation, suspended in LTMC medium, and layered over irradiated adherent cells at a concentration of 2 × 10⁶/mL in a total volume of 10 mL.

Statistical analysis. All the experiments were performed a minimum of three times to obtain the mean ± standard deviation (SD) value for each experiment. Statistical analysis was calculated using Student’s paired t-test and the Wilcoxon rank sum test.

Uptake of DHE and BPD-MA by wild-type and MDR* cells. Cells were incubated with the drugs (at various concentrations) for 1 hour at 37°C in a humidified CO₂ incubator. After incubation and a brief wash with phosphate-buffered saline (PBS), one-half of the cells were

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>FAB*</th>
<th>% PE</th>
<th>% Blasts</th>
<th>% Promyelocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M2</td>
<td>1.63</td>
<td>78</td>
<td>19</td>
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<tr>
<td>2</td>
<td>M4</td>
<td>0.56</td>
<td>70</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>M5A</td>
<td>2.14</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>M5B</td>
<td>1.6</td>
<td>69</td>
<td>19</td>
</tr>
</tbody>
</table>

* FAB classification.
† The percentages of blasts and promyelocytes were obtained by counting 200 cells from May-Grünwald-Giemsa–stained cytospin smears after thawing.
SENSITIVITY OF MDR\(^+\) CELL LINES TO PHOTOLYSIS

Fig 1. BPD-MA (A) and DHE (B) toxicity on normal BM (○) CFU-GM and (○) BFU-E as compared with the clonogenic growth inhibition of (A) HL-60, (○) SK-DHL-2, and (○) LY-16. In all the experiments, cells were concentrated at \(2 \times 10^6\)/mL. Tumor cells were mixed with irradiated mononuclear light-density BM cells. Mean values from three or more separate experiments are given as the percentage of control samples (photoexposure in absence of BPD-MA or DHE).

used for quantitating the drug content immediately after incubation (designated as 0 hours). The remaining cells were kept at ambient temperature in medium containing 10% FCS for determination of drug content after a 30-minute leaching period (designated as 30 minutes). To determine the drug content in the cells, the cells were spun down in 1.7-mL Eppendorf tubes in a Microcentrifuge (Eppendorf Centrifuge 5415 C; Brinkmann Inst Inc, Westbury, NY) at 6,000 rpm for 5 minutes. The cell pellets were resuspended in PBS containing 2% Triton X-100 and freeze-thawed three times (using a dry ice-methanol mixture). After freeze-thawing, 0.5 mL of DMSO was added to tubes containing DHE and 0.5 mL of absolute methanol to tubes containing BPD. The tubes were immediately frozen at \(-70^\circ\text{C}\) until fluorescence was determined. Control experiments for both DHE and BPD were prepared by adding known amounts of drugs to cell lysates under conditions equivalent to the experimental conditions described above and provided the baseline for possible quenching of fluorescence by cell lysates.

The samples were spun at 14,000 rpm immediately before determination of fluorescence (microwell plate reader [Starna Cells, Inc, Stasco, CA] in a spectrofluorometer [Jasco Model FP-770; Jasco Inc, Easton, MD]). DHE fluorescence was determined at 400 nm excitation and 634 nm emission wavelengths. BPD fluorescence was determined at 439 nm excitation and 700 nm emission wavelengths. Protein content in the lysates was determined by Lowry's method using 10-μL aliquots of the samples in which fluorescence was determined. Results were calculated in units of drug per 10^7 cells and per milligram of cell protein.

RESULTS

Effect of photoradiation with BPD-MA and DHE on tumor cells. In all the experiments, tumor cells or normal BM cells were resuspended in IMDM with 1% FCS. The low FCS concentration was chosen for our study after preliminary experiments showed an inverse correlation between serum content and the photosensitizer activity (data not shown). Moreover, the cytotoxic activity of BPD-MA and DHE varied depending on the cell concentration. When the tumor cells were mixed with irradiated mononuclear BM cells at the final concentration of \(2 \times 10^6\) cells/mL (as compared with \(2 \times 10^7\) cells/mL), the colony growth inhibition of the cell lines was reduced by 2 logs or more. Twenty or 30 minutes of light exposure did not induce statistically different results, therefore only the data of the shorter time of treatment are reported. Figure 1 details the dose-response curves of BPD-MA and DHE on the lymphoma cell lines SK-DHL-2 and LY-16 and the leukemic cell line HL-60 in presence of irradiated BM cells. Incubation with BPD-MA at 75 ng/mL followed by exposure to the light resulted in the reduction of clonogenic tumor cells beyond the limit of accurate detection of our assay (ie, >4 logs). Similarly, SK-DHL-2 and HL-60 colony growth was reduced by more than 4 logs after treatment with 8 or 12.5 μg/mL of DHE, respectively.

To evaluate whether red blood cell (RBC) contamination might interfere with the activity of the photosensitizing agents, additional experiments were performed by mixing SK-DHL-2 and LY-16 cell lines with irradiated BM buffy coat cells. Under these experimental conditions, using 200 ng/mL of BPD-MA, 3.1 and 3.7 logs of LY-16 and SK-DHL-2 cell kill was obtained, respectively. The hematopoietic toxicity was also reduced; 200 ng/mL of BPD-MA resulted in the recovery of 10% ± 5% (mean ± SD) of CFU-GM and 13% ± 3.5% SD of BFU-E (Fig 1A). In control experiments, HL-60 and LY-16 cells that were exposed to the light alone showed similar clonogenic efficiency to that of the cells neither preincubated...
with the photosensitizing compounds nor exposed to the light. SK-DHL-2 had an 11.6%±13% colony-forming inhibition, which was not statistically significant. BPD-MA or DHE preincubation in the dark (up to 200 ng/mL or 15 µg/mL, respectively) not followed by photoradiation did not decrease the plating efficiency of the three cell lines or normal BM progenitor cells.

Four samples derived from AML patients (Table 1) were also tested with photoradiation treatment and the colony-forming inhibition of CFU-L was evaluated in vitro (Fig 2). No CFU-L (ie, ≥3 logs of leukemic cell elimination) were observed after the combined treatment with BPD-MA (50 ng/mL) or DHE (10 µg/mL) and light exposure.

**Effect of phototherapy on hematopoietic progenitor cells.** The mean recovery of normal BM progenitor cells after phototherapy is shown in comparison with the colony-forming inhibition of the cell lines in Fig 1 and the AML samples in Fig 2. Light alone reduced the plating efficiency of CFU-GM and of BFU-E 13.9%±14.9% and 9%±14%, respectively (P>.2). Photoradiation with 50 ng/mL of BPD-MA resulted in the mean recovery of 21.5%±5% of CFU-GM and 15%±11% of BFU-E. When BPD-MA was used at 75 ng/mL, a mean recovery of 4%±0.8% of CFU-GM and 5%±0.8% of BFU-E was observed. After the sequential treatment of BM mononuclear cells with 10 µg/mL of DHE (a concentration capable of reducing >3 logs of CFU-L) and light exposure, we obtained a mean recovery of 10.6%±2% and 26.6%±3% for CFU-GM and BFU-E, respectively. The survival of 5.2%±2% of CFU-GM and 9.8%±3% of BFU-E was induced by the photoradiation treatment with 12.5 µg/mL of DHE.

**Effect of phototherapy on early hematopoietic progenitor cells.** BM cells were treated with either BPD-MA or DHE and placed in secondary LTMC to determine the effect of phototherapy on early progenitors. Representative results are shown in Fig 3.

![Figure 2](image1.png)

**Fig 2.** Comparison of BPD-MA-mediated (A) and DHE-mediated (B) mediated phototoxic effects on CFU-L derived from four AML patients versus normal BM progenitor cells. See legend to Fig 1 for details. (♀) AML 1; (♂) AML 2; (♀) AML 3; (♂) AML 4; (♀) CFU-GM; (♂) BFU-E.

![Figure 3](image2.png)

**Fig 3.** Presence of colony-forming cells (CFC) arising from secondary LTMC treated with either light alone (♀), 22.5 µg/mL DHE and light (♂), or 225 ng/mL BPD-MA and light (♂). Cells for CFC assay were harvested immediately after treatment (time), or at 1, 2, or 3 weeks after the initiation of secondary LTMC.
shown in Fig 3. In this experiment in BM cells treated with 225 ng BPD-MA/mL, no committed progenitors survived (shown at time 0). However, in weeks 1, 2, and 3, colony formation in BPD-MA–treated materials was equivalent to that observed in control cultures. These results indicate that, even under conditions that eliminate all committed progenitors, BPD-MA and light do not eliminate early progenitors. In this experiment, DHE at 22.5 µg/mL inhibited colony formation by committed progenitors, but did not exhibit any inhibition of early progenitors.

Cytotoxic activity of phototherapy on MDR+ cell lines. Preliminary experiments showed almost complete resistance of HL-60/VCR and CEM/VBL to the treatment with various drugs, including doxorubicin, daunorubicin, VBL, VCR, and VP-16, whereas the parent cell lines were inhibited by 3 to 4 logs. The alkylating agent 4-hydroperoxycyclophosphamide (4-HC; 30 µg/mL) induced a colony-forming inhibition of 3.5 logs, as compared with the 4 logs observed when the MDR- cell lines were studied (data not shown).

The colony growth reduction obtained after photoradiation with BPD-MA and DHE on HL-60, CEM, and their MDR+ sublines is shown in Fig 4. BPD-MA, at 75 ng/mL, was able to eliminate more than 4 logs of the drug-sensitive cell lines, but only 34% and 55% of HL-60/VCR and CEM/VBL, respectively. Conversely, DHE resulted in the inhibition of the clonogenic capacity of all the cell lines beyond the lower limit of detection of our assay (Fig 4B).

Measurement of fluorescence to determine the concentration of the drugs in cell lysates immediately after a 1-hour incubation of the wild-type cells and their MDR+ counterparts with three different concentrations of DHE and BPD showed that the MDR+ cells took up an average 53% less of either BPD or DHE than did their wild-type counterparts (Table 2). Because the cell sizes among the four cell lines are different, it was felt that relating the amount of drug taken up by the cells to the cell protein would be more appropriate than relating the amount to the cell number. The drug uptake by the cells was generally proportional to the concentration of the drug in the incubation medium. There was no obvious difference in the rate of leaching of the drugs during the 30-minute period between wild-type and MDR+ cell lines. Sim-

---

**Table 2. Uptake of DHE and BPD-MA by Wild-Type and MDR+ Cells**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Incubation Concentration (µg/mL)</th>
<th>DHE</th>
<th>BPD-MA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>30 min</td>
<td>0 h</td>
</tr>
<tr>
<td>HL-60</td>
<td>2.0 (2.5)</td>
<td>0.100</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td>4.0 (6.0)</td>
<td>0.283</td>
<td>0.236</td>
</tr>
<tr>
<td></td>
<td>8.0 (10.0)</td>
<td>0.693</td>
<td>0.760</td>
</tr>
<tr>
<td>HL-60-VR</td>
<td>2.0 (2.5)</td>
<td>0.069</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>4.0 (6.0)</td>
<td>0.133</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>8.0 (10.0)</td>
<td>0.400</td>
<td>0.301</td>
</tr>
<tr>
<td>CEM</td>
<td>2.0 (2.5)</td>
<td>0.112</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>4.0 (6.0)</td>
<td>0.278</td>
<td>0.179</td>
</tr>
<tr>
<td></td>
<td>8.0 (10.0)</td>
<td>0.710</td>
<td>0.455</td>
</tr>
<tr>
<td>CEM-VR</td>
<td>2.0 (2.5)</td>
<td>0.061</td>
<td>0.047</td>
</tr>
<tr>
<td></td>
<td>4.0 (6.0)</td>
<td>0.133</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>8.0 (10.0)</td>
<td>0.310</td>
<td>0.276</td>
</tr>
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</table>

The concentration of the drugs in cell lysates was determined by fluorescence immediately after 1 hour of incubation (0 h) with three different concentrations of each drug, and after a 30-minute leaching period (30 min). The results were expressed in micrograms of drug per milligram of cell protein. DHE concentrations are in parentheses.
ilar results were observed when the concentration of BPD-MA in cell lysates was determined by radioactivity (data not shown). However, it is of interest that cells took up more DHE than BPD-MA under equivalent conditions (Table 2).

**DISCUSSION**

HP derivatives are currently used for cancer photodynamic therapy in vivo. Although their mechanism of action is not fully understood, it appears that the cellular damage is mediated by the generation of reactive oxygen and hydroxyl radicals upon stimulation with light. The preferential toxicity of DHE against myeloid and lymphoid cell lines, as compared with normal hematopoietic progenitors, has also been shown in vitro and therefore DHE has been proposed as a purging agent for minimal residual disease in autologous BM transplantation (ABMT). HP derivatives have been synthesized and preliminary results in vitro have shown their potential usefulness for cancer therapy. Specifically, BPD-MA compared favorably with HP because of their 10 to 70 times higher cytotoxicity against tumor cells and maximum light absorption at the wavelength (around 700 nm) with which human tissue attenuation is the least and light penetration is the most effective. More recently, the preferential uptake of BPD-MA by leukemic cells versus normal cells and the selective toxicity of photoradiation with BPD-MA on chronic myelogenous leukemia (CML) clonogenic cells have been demonstrated.

In this report, we assessed the potential role of BPD-MA and DHE, coupled with light exposure, for ex vivo treatment of autologous BM grafts. We report experiments designed to evaluate the capability of phototherapy to purge malignant cells that express the MDR phenotype. MDR is a mechanism of drug resistance that has been described in tumor cell lines exposed in vitro to anthracyclins, vinca alkaloids, and podophyllotoxin. MDR is related to the activity of a 170-Kd glycoprotein (p-170) that is encoded by the multidrug resistance gene mdr 1 and functions as a transmembrane drug efflux pump. High expression of the mdr 1 gene and its product has been reported in several human tumors and has been correlated with a poor clinical response.

One of the major concerns in ABMT is the potential contamination of autologous grafts by leukemic and lymphoma cells. Usually, autologous hematopoietic stem cell (AHSC) (using BM or PB stem cells [PBSC]) transplantation is performed when a patient is in remission. Reliable methods to prove that a subsequent relapse after AHSC transplantation occurred because of the concomitant infusion of leukemic cells with thawed stem cells are not available. Furthermore, true clinical benefit for various purging methods is also not properly established. Review of recent literature suggests comparable survival for patients with AML receiving a purified BM transplant or an unpurged PBSC transplant. Comparable results for selected patients were also observed for patients receiving unpurged ABMT. Because ABMT is frequently performed after exposure to chemotherapy agents that may lead to the selection of cells with high MDR expression, the use of a purging technique directed against drug-resistant tumor cells would be beneficial.

The results of our study showed that neoplastic cells of myeloid (HL-60) and lymphoid (SK-DHL-2, LY-16, and CEM) origin were eliminated by our photoradiation models beyond the lower limit of accurate detection of our assay (>4 logs of clonogenic growth reduction). The same phototherapy protocols induced a 3-log growth inhibition of CFU-L from four patients with AML. These data are particularly relevant because purging protocols using alkylating agents, alone or when combined with other chemotherapeutics, failed to detect any differential activity between CFU-L and normal hematopoietic progenitor cells, suggesting that the results obtained with cell lines may not be predictive for clinical studies. Similar results were previously reported by our group using a combination of complement-mediated lysis by monoclonal antibodies (MoAbs) and chemotherapeutics. It is recognized that the sparing of committed progenitors when a potential purging procedure is being evaluated is not sufficient evidence to indicate that the purging agent has validity. It is generally accepted that early progenitor survival is key to the success of long-term BM reconstitution. For this reason, experiments were undertaken to determine whether phototherapy conditions that decreased or eliminated committed progenitors would significantly effect survival of early progenitors. We have shown (Fig 3) that conditions that either reduced or eliminated committed progenitors had no noticeable effect on colonies arising from nonadherent cells in long-term culture from 1 to 3 weeks. These results suggest that early progenitors are probably less sensitive to either BPM-MA or DHE than are committed progenitors. Further studies with a murine leukemia purging model have shown that when L1210 murine leukemia cells are mixed with normal murine progenitors and treated with BPD-MA and light, a 4-log reduction of leukemia cells was effected while full hemopoietic reconstitution of lethally irradiated recipients was achieved (Jamieson et al, submitted for publication). However, our immunopharmacologic purging method resulted in the nearly complete depletion of the committed BM progenitors pool. In this study, the satisfactory recovery of CFU-GM and BFU-E using BPD-MA or DHE concentrations capable of eliminating ≥3 logs of CFU-L further supports the possible role for photoradiation therapy in marrow purging programs. Moreover, to provide an indirect comparison between the two ex vivo treatments, the four AML samples evaluated here were among the eight specimens previously tested.

However, despite similar antitumor activity, BPD-MA and DHE differed markedly in their capacity to eliminate MDR cells. In fact, BPD-MA reduced the clonogenic capacity of HL-60/VCR and CEM/VBL by 0.5 logs or less, whereas DHE was equally effective on HL-60 and CEM cell lines and their MDR + sublines (ie, >4 logs killing). Several investigators have been successful in purging MDR+ tumor cells by using specific MoAbs directed against the p-170. A more effective killing was achieved when three rounds of complement-mediated lysis were combined with VP-16 treatment. In addition, recent data have shown the expression of the p-170 on early hematopoietic progenitor cells. Further investigations are needed to understand the importance of cells weakly positive for MDR at diagnosis. An ongoing investigation in our laboratory is addressing the question of whether the mechanism
of resistance to BPD-MA involves the p-glycoprotein. The mechanism of phototherapeutic cytotoxicity is not mediated through MDR interactions; therefore, no increased toxicity to early progenitors is expected and was not observed in studies by J. Levy et al (unpublished observation, University of British Columbia, Vancouver, Canada). Preliminary data from our laboratory show that the combination of the calcium channel blocker verapamil with BPD-MA is only able to partially reverse the resistance to the photosensitizer. Moreover, the assessment of BPD-MA and DHE concentrations in cell lysates did not show substantial differences between the two drugs in terms of cellular uptake and retention (Table 2), further adding support to the fact that mechanisms of action other than the p-glycoprotein are involved in the resistance to BPD-MA. It is interesting that cells took up more DHE than BPD-MA under equivalent conditions. Finally, in view of the clinical use of phototherapy, we have also performed experiments to evaluate the role of serum, RBC contamination, and cell concentration in the cell suspension. Our results confirmed that the activity of BPD-MA and DHE was affected by different experimental conditions.8 Serum components as well as RBC (present in the BM buffy coat fraction) may compete with the target cells for the photosensitizer. However, the mechanism of photosensitization and selective killing of neoplastic cells remained unchanged. Although further studies are needed to investigate the specific role of dye-mediated photolysis, our results suggest that phototitation with DHE or BPD-MA may be useful for the ex vivo treatment of drug-resistant tumor cell-contaminated BM grafts.

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REFERENCES


30. DeCormick E, Tamayo E, Herve P: In vitro chemosensitivity of leukemia progenitor cells (AML-CFU) to a combination of mafosfamide lysine (ASTA-2 7654) and etoposide (VP-16-213). Bone Marrow Transplant 5:13, 1990


Dye-mediated photolysis is capable of eliminating drug-resistant (MDR+) tumor cells

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