Hematoporphyrin Derivative Rescue from Toxicity Caused by Chemotherapy or Radiation in a Murine Leukemia Model (L1210)

G. Canti, P. Franco, O. Marelli, L. Ricci, and A. Nicolin

Department of Pharmacology, School of Medicine, Università Degli Studi, Via Vanvitelli 32, 20129 Milan, Italy [G. C., P. F., O. M., L. R.], and Istituto Nazionale per la Ricerca su Tumori, Viale Benedetto XV, 10, Genoa, Italy [A. N.]

ABSTRACT

Hematoporphyrin [1,3,5,8-tetramethyl-2,4-bis(hydroxyethyl)-porphin-6,7-dipropionic acid dihydrochloride derivative] (HPD) is a compound that was studied in a number of laboratories because of its cytotoxic activity after activation by light. Modification of immune function seen during the photochemotherapeutic studies prompted attempts to determine the effect of HPD on the immune and hemopoietic systems. Splenic hyperplasia as well as marrow hypercellularity were noted in mice treated with HPD. *In vitro* phytohemagglutinin or lipopolysaccharide stimulation of spleen lymphocytes caused normal or scant increases in blast transformation compared to the stimulation index for lymphocytes from untreated animals. HPD treatment did not significantly alter production of antibody to sheep red blood cells, as evaluated by hemagglutination or hemolytic assay. In contrast, HPD treatment did promote an increased number of spleen colonies in lethally irradiated mice transfused with syngeneic bone marrow. The capacity of HPD to increase the number of bone marrow and spleen cells has been exploited to accelerate the recovery from peripheral leukopenia induced in animals by previous drug or radiation treatment. The time for full return from severe leukopenia induced by an antimetabolite compound (5-fluorouracil) or an alkylating agent (cyclophosphamide or X-rays) was significantly shorter in mice treated with HPD than in controls. Furthermore, improved survival was demonstrated in irradiated mice after HPD treatment. Finally, HPD treatment of L1210 leukemic mice did not affect the antitumor activity of cyclophosphamide.

If the properties described here are confirmed, HPD might contribute to recovery of leukopenic cancer patients.

INTRODUCTION

The efficacy of cancer chemotherapy is limited by the small difference between the cytotoxic susceptibilities of normal and neoplastic tissues (10, 23). Because of this poor selectivity, chemotherapeutic agents are often administered in suboptimal doses in order to avoid serious toxicity to the host (6, 7). More effective treatment might be achieved if toxic effects produced in the normal host tissues by antineoplastic treatment could be reduced without compromising the antitumor activity of the drug. Various efforts were made to enhance the therapeutic indices of chemical and physical treatments (3, 43). One approach is to give antitumor agents in combination with agents that limit toxicity but that do not themselves interfere with antitumor activity. In the literature, a few compounds that reduce the host toxicity of antineoplastic therapy are described (4, 5, 31, 39, 41, 42). Unfortunately, clinical trials using such "rescue" strategies have not demonstrated clearly an improved therapeutic index (18). Moreover, even under the most favorable circumstances, most compounds which reduce host toxicity are active after a limited range of treatment conditions by anticancer drugs or radiation (1, 26).

Perhaps the greatest limitation in the use of antineoplastic drugs is their effects on hematopoiesis (24, 27, 29). Lymphohematological impairment associated with the antineoplastic therapy causes defects in coagulation and susceptibility to infections that, in turn, require decreasing or discontinuing effective chemotherapy (25, 36).

HPD (17), a compound studied in our laboratory (8, 9, 19) and those of others (14, 16) because of its cytotoxic activity after light activation, was found to modify *in vitro* immunological assays and spleen volumes in HPD-treated mice. A program was designed to study the effect of HPD on the immune and hemopoietic systems (20).

In this paper, the effects of HPD on the lymphohematopoietic system in normal mice, their rescue from iatrogenic leukopenia, and protection after lethal panirradiation are reported.

MATERIALS AND METHODS

**Animals and Tumor.** Eight- to 10-week-old male DBA/2 x BALB/c F₁ (hereafter called D2CF₁) mice from Charles River (Calco, Italy) were used. L1210 murine leukemia was routinely maintained in the laboratory by weekly passage i.p. in DBA/2 mice (33).

**Drug Treatments.** HPD (Sigma Chemical Co., St. Louis, MO), derivatized as described (14), was diluted to the desired concentrations in Hanks' balanced salt solution and kept in the dark. The doses of HPD and the schedules of administration are indicated in the tables and charts. CY and FUra were obtained from the Drug Research and Development Program, National Cancer Institute, Bethesda, MD, dissolved in saline (0.9% NaCl solution) immediately before use, and administered i.p.

**Cell Manipulation.** Cell numbers in peripheral blood, femoral bone marrow, spleen, and thymus were counted by an electronic cell counter. Cells were obtained from the retroorbital sinus by gentle squeezing of the spleens or the thymuses or by needle washing of the femoral cavity (36).

**Radiation.** Animals were irradiated (whole body) by a Securix Compact CGD machine (200 R; 30-cm distance; filter, 0.5 mm Al and 0.5 mm Cu; 200 kilowatts; 12 min; 10 min and 3 sec).

**Mitogen Stimulation.** Spleen cells were checked for viability by trypan blue dye exclusion and counted in a Bürker chamber under a light microscope. Cells were then suspended in RPMI 1640 medium, supplemented as reported previously (21), seeded (5 × 10⁵ cells/200 μl) in microplate wells (Linbro Chemical Co., New Haven, CT) with phytohe-
magglutinin (Burroughs Wellcome and Co., Research Triangle Park, NC) or lipopolysaccharide (Sigma), and incubated 48 hr at 37° in a moist atmosphere of 95% air and 5% CO₂. Twenty μl containing 0.8 μl [³H]thymidine (26 Ci/mmol; CEA, France) were added 18 hr before cultures were harvested onto glass fiber filters with a multiple cell harvester (Skatron, Norway). Experimental results were expressed in cpm ± S.E. and as

Stimulation index = \( \frac{\text{cpm in experimental wells}}{\text{cpm in control wells}} \)

Spleen Colonies. Panirradiated animals (800 rads) were inoculated i.v. with \( 5 \times 10^6 \) syngeneic bone marrow cells from the femurs of virgin donors, treated daily with HPD, and killed on Day 9. Spleen colonies were scored macroscopically by visual examination (32).

Hemagglutination and Hemolytic Titer. Five animals/group were immunized with \( 3 \times 10^9 \) SRBC on Day 0, i.p. Seven days later (Day +7), the mice were killed, and the sera were evaluated individually by the hemagglutination test (44). The hemolytic titer were assayed by mixing serum samples with SRBC and guinea pig complement (35). The titer represents the arithmetic mean for serum concentrations with 50% activity.

RESULTS

Spleens and thymuses from D2CF₁ mice treated previously i.p. with HPD were weighed, and the cell contents were counted, as were also the femoral bone marrow cells. Schedules and doses of HPD treatment and experimental results are shown in Table 1. Obvious splenomegaly was observed macroscopically in all groups of treated animals except one that was given HPD 24 hr before it was killed. Spleen enlargement was correlated with both increased absolute and relative spleen weights. In some groups of mice (75 mg/kg, 7 or 3 days before killing, or 8 mg/kg daily), the spleen weights and the number of spleen cells were more than double those of the control groups. Table 1 also shows a marked cellular hyperplasia of femoral bone marrow in 2 groups of animals treated 7 days before with 75 or 25 mg/kg daily. In contrast, volume, weight, and cell number were not altered in the thymuses of mice treated with HPD (data not shown).

Functional activity of the spleen cells was studied, and the findings are reported in Charts 1 and 2. Chart 1 shows the

<table>
<thead>
<tr>
<th>HPD (mg/kg)</th>
<th>Days on which treatment was given</th>
<th>Bone marrow cells × 10⁶/femur*a</th>
<th>Cells × 10⁶/spleen*b</th>
<th>Spleen wt (g)b</th>
<th>Relative spleen wt (g)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>-7</td>
<td>3.2 ± 0.9e</td>
<td>120 ± 20</td>
<td>0.098 ± 0.006</td>
<td>0.39</td>
</tr>
<tr>
<td>75</td>
<td>-3</td>
<td>9.6 ± 2.6e</td>
<td>289 ± 5.4e</td>
<td>0.222 ± 0.014</td>
<td>0.88</td>
</tr>
<tr>
<td>75</td>
<td>-1</td>
<td>3.6 ± 1.1</td>
<td>311 ± 5.8e</td>
<td>0.224 ± 0.027</td>
<td>1.04</td>
</tr>
<tr>
<td>75</td>
<td>Alternate (-7/-5/-3/-1)</td>
<td>4.3 ± 0.9</td>
<td>158 ± 16</td>
<td>0.128 ± 0.11</td>
<td>0.51</td>
</tr>
<tr>
<td>25</td>
<td>Daily (-7 to -1)</td>
<td>3.1 ± 0.9</td>
<td>180 ± 2.4e</td>
<td>0.150 ± 0.015</td>
<td>0.62</td>
</tr>
<tr>
<td>25</td>
<td>Daily</td>
<td>10.6 ± 1.9e</td>
<td>224 ± 15e</td>
<td>0.255 ± 0.019</td>
<td>1.06</td>
</tr>
<tr>
<td>8</td>
<td>Daily</td>
<td>2.1 ± 0.4</td>
<td>291 ± 21e</td>
<td>0.212 ± 0.032</td>
<td>0.84</td>
</tr>
</tbody>
</table>

*a Data for 8 mice/group killed on Day 0.

*b Arithmetic mean.

Relative spleen weight = \( \frac{\text{Mean spleen weight}}{\text{Mean mouse weight}} \) × 100.

Mean ± S.E.

p < 0.05 by the Student t test.

Chart 1. Mitogen stimulation of spleen cells from D2CF₁ mice treated with HPD. Three mice/group were treated with HPD, 75 mg/kg i.p., on the days indicated in the columns (-7, -3, -1). On Day 0, animals were killed, and spleen cells were assayed for mitogenic stimulation. A, phytohemagglutinin (PHA) stimulation; B, lipopolysaccharide (LPS) stimulation; a, p < 0.05 by the Dunnet t test for multiple comparison on cpm ± S.E.
susceptibility to mitogenic stimulation of spleen cells from mice treated with HPD, compared with the blastogenic responses of virgin cells. At the HPD doses given, reactivity to T-lymphocyte mitogen phytohemagglutinin, as evaluated by $[3H]$thymidine incorporation into the nucleus, was essentially the same as in control cells.

Significant radioactivity was incorporated by stimulated cells from recently treated animals (75 mg/kg, 1 day before). A similar pattern of responsiveness was shown by cells after stimulation with the B-lymphocyte mitogen lipopolysaccharide. Lymphoid activity was further evaluated in in vivo studies of antibody production by mice treated with HPD primed with suboptimal amounts of SRBC. Humoral immune response to SRBC by mice treated with HPD (Chart 2), as evaluated by hemagglutination and hemolytic assays, was not impaired in any group of animals. Moreover, the increased antibody titer observed in some groups of mice treated with HPD was not statistically significant. Although the data in Charts 1 and 2 do not exclude, in principle, minor functional alterations in HPD-treated spleen cells, they do exclude gross modifications of lymphocyte properties, and we conclude that complex immunological reactions dependent on lymphocyte subpopulations and macrophage interactions have not been impaired.

The influence of HPD treatment on the number of spleen colonies in lethally irradiated D2CF1 recipients of syngeneic bone marrow cells was also studied. As shown in Table 2, HPD treatment of recipients, 25 mg/kg on Day −9 or −7, was able to increase significantly over that in the controls the number of macroscopic spleen colonies in syngeneic bone marrow-transfused animals. Yet, both 8 mg/kg daily and 75 mg/kg in a single HPD inoculum had no effect on colony formation.

The effects of HPD treatment on leukopenia induced by drugs or radiation in D2CF1 mice were studied, and the results were reported in Tables 3 to 5. Leukopenia was produced by treatment with the antimetabolite compound FUra or with the alkylating agent Cy or by total-body radiation. Four days after the FUra treatment, the number of WBC in the mice treated with HPD was not modified; i.e. it was as low as in the controls. Seven days after treatment, the WBC number had increased significantly over that in the controls and reached basal values by the 11th day, the nadir time for the control animals. HPD was active over a range of doses. Daily HPD treatment of less than 0.25 mg/kg/mouse had very little activity, whereas a single treatment of 75 mg/kg or 25 mg/kg daily caused toxic manifestations (see Discussion).

The WBC in mice with leukopenia induced by FUra showed
that HPD was able to accelerate the recovery from leukopenia but was ineffective in preventing leukopenia induced by drugs (Table 3).

An HPD effect on WBC recovery in leukopenia induced by CY was also found. In keeping with the data in Table 3, HPD did not prevent the fall in WBC, but WBC well above the basal level were obtained by Day 7, significantly higher than in the spontaneously recovered control animals (Table 4). Panirradiation also caused marked leukopenia which was not counteracted by HPD, as evidenced by the WBC counts on Day +4 (Table 5). However, partial recovery on Day +7 and full recovery on Day +11 were quite obvious, in sharp contrast with the lowest WBC counts (nadir) seen at those times in the control group and in the group given the lowest dose of HPD. Moreover, probably because of the weakness of the irradiated mice, toxicity and poor recovery from leukopenia were the overwhelming effects of the highest dosage of HPD. Normal mice treated only with the highest dose of HPD (25 mg/kg on alternate days) showed a moderate increase on WBC counts, which was not statistically significant (data not shown).

Table 6 reports the results of the studies designed to ascertain the effects of HPD treatment on lethally irradiated mice. In the study reported here, most of the control animals did not survive X-ray irradiation, nor did the mice treated with the lower HPD dosage (0.025 mg/kg daily) or with the largest dosage (25 mg/kg on alternate days). HPD treatment was quite effective in the groups of mice treated with 0.25 or 2.5 mg/kg daily. The large majority, 8 of 10 and 7 of 10, were actually "rescued" from lethal irradiation and survived indefinitely.

The rate of tumor growth and the chemotherapeutic activity of CY were not modified by HPD treatment. In fact, the results reported in Table 7 did not demonstrate any differences in survival between the group treated with CY only and the group treated with CY and HPD. Furthermore, these studies confirm the lack of antitumor activity of HPD alone as reported previously (19).

**DISCUSSION**

For the large majority of anticancer drugs, whether administered singly or in combination, bone marrow suppression is the most important dose-limiting factor. Moreover, resident and circulating lymphocytes and platelets are also highly susceptible to the toxic effects of antineoplastic agents (25). Efforts to mitigate severe marrow impairment and leukopenia were pursued in a number of ways including blood transfusion, autologous bone marrow transplantation, alternative schedules and selection of drug treatment, and supportive agents that might either prevent or rescue from marrow toxicity (11, 13, 22, 28).

In this paper, circulating white cells and cells resident in hemolymphopoietic organs from virgin and immunodepressed animals were studied after treatment with HPD. Hematoporphyrins, a family of compounds to which HPD belongs, in contrast to the related iron-containing hemins, do not stimulate the hemolymphopoietic apparatus (40).

Our data show that HPD did induce spleen and bone marrow hyperplasia, did not cause immunological impairment, protected

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**Table 4**

<table>
<thead>
<tr>
<th>HPD (mg/kg)</th>
<th>Days on which treatment was given</th>
<th>WBC/cu mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day −1</td>
<td>Day +4</td>
</tr>
<tr>
<td>0.025 Daily</td>
<td>8324 ± 728</td>
<td>938 ± 156</td>
</tr>
<tr>
<td>0.25 Daily</td>
<td>9182 ± 616</td>
<td>1296 ± 214</td>
</tr>
<tr>
<td>2.5 Daily</td>
<td>7827 ± 612</td>
<td>1149 ± 238</td>
</tr>
<tr>
<td>25 Alternate</td>
<td>8803 ± 919</td>
<td>981 ± 106</td>
</tr>
</tbody>
</table>

* Treatment from Day +1 to Day +6.

**Table 5**

<table>
<thead>
<tr>
<th>HPD (mg/kg)</th>
<th>Days on which treatment was given</th>
<th>WBC/cu mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day −1</td>
<td>Day +4</td>
</tr>
<tr>
<td>0.025 Daily</td>
<td>8780 ± 868</td>
<td>1320 ± 559</td>
</tr>
<tr>
<td>0.25 Daily</td>
<td>8430 ± 541</td>
<td>860 ± 169</td>
</tr>
<tr>
<td>2.5 Daily</td>
<td>9580 ± 328</td>
<td>1660 ± 828</td>
</tr>
<tr>
<td>25 Alternate</td>
<td>7630 ± 522</td>
<td>2100 ± 647</td>
</tr>
</tbody>
</table>

* Treatment from Day +1 to Day +6.

---

**Table 6**

<table>
<thead>
<tr>
<th>HPD (mg/kg)</th>
<th>Days on which treatment was given</th>
<th>Mean survival time (days) D/T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.</td>
<td>D/T</td>
</tr>
<tr>
<td></td>
<td>Daily</td>
<td>11.5 (9-13)</td>
</tr>
<tr>
<td>0.025</td>
<td>Daily</td>
<td>(25-40)</td>
</tr>
<tr>
<td>0.25</td>
<td>Daily</td>
<td>(17-50)</td>
</tr>
<tr>
<td>2.5</td>
<td>Alternate</td>
<td>26.4 (20-51)</td>
</tr>
</tbody>
</table>

* Treatment from Day +1 to Day +10.

---

**Table 7**

<table>
<thead>
<tr>
<th>CY (120 mg/kg)</th>
<th>Days on which treatment was given</th>
<th>Mean survival time (days) D/T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.E.</td>
<td>D/T</td>
</tr>
<tr>
<td></td>
<td>Daily</td>
<td>(9-12)</td>
</tr>
<tr>
<td></td>
<td>Alternate</td>
<td>(9-13)</td>
</tr>
<tr>
<td></td>
<td>Daily</td>
<td>(20-41)</td>
</tr>
<tr>
<td></td>
<td>Daily</td>
<td>(50)</td>
</tr>
<tr>
<td></td>
<td>Alternate</td>
<td>(39-42)</td>
</tr>
</tbody>
</table>

* Treatment on Day +1.

### Notes:
- **a** D/T, dead mice/treated mice after 60 days.
- **b** Numbers in parentheses, range (days) of survival time.
- **c** p < 0.05 by x² test.
mice from lethal irradiation, increased rescue from iatrogenic leukopenia, and did not modify the rate of tumor growth or the effectiveness of chemotherapy. As for hyperplasia induced by HPD in the spleen and bone marrow of normal mice, the cellular subpopulations were not directly analyzed. However, indices such as mitogenic stimulation, antibody production, and bone marrow spleen colonization in irradiated hosts were normal or moderately increased. Since the above activities require collaboration of different cell types (12, 34), non-gross modification of cell compartments is likely to have been induced by HPD. The acute toxicity of HPD in normal mice is low (50% lethal dose, 500 mg/kg i.p.). Reversible photosensitization was described in human patients (14, 15). As regards iatrogenic leukopenia in mice, this side effect has not yet been studied in detail but was observed at HPD dosages (75-mg/kg single dose or 25 mg/kg daily) far above those able (0.25 mg/kg daily) to rescue them from leukopenia or irradiation deaths.

Hemopoietic hyperplasia induced by HPD, possibly an undesirable event in normal mice, might prove therapeutically useful in leukopenic animals. When there is leukopenia induced by chemotherapy or radiation, pharmacological manipulations as described in this paper might be of therapeutic benefit.

Shorter time of recovery from leukopenia was obtained regardless of how the leukopenia was induced; and while spontaneous recovery from Cytoxan-induced toxicity is rapid, this recovery is even more rapid in the presence of HPD.

Radiation has toxic effects on a variety of cells and organs, primarily on the lymphohemopoietic system. The mechanism by which lethally irradiated animals survive following HPD treatment is not known. However, the therapeutic activity might be at least partly related to the ability of HPD to accelerate host marrow recovery. During some photochemotherapeutic studies, HPD given to tumor-bearing animals (19) or to cancer patients (25) had no effect on the rates of tumor growth. In keeping with these observations, the mean survival time of L1210-bearing mice was not modified nor was the antitumor activity of CY impaired. In the past, other compounds that protect against the toxic effects of antineoplastic drugs or radiation were described. When several of these compounds were given to tumor-bearing hosts, they either counteracted the antitumor activity of drugs (37) or radiation (2) or stimulated the growth rate of tumors (30). Although compromised mice have increased susceptibility to HPD toxicity, nontoxic amounts of HPD exhibited rescue properties that can improve the therapeutic indices of radiotherapeutic treatment and antineoplastic drugs and are worth further study in other experimental tumor systems and possibly in cancer patients.

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REFERENCES


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