The Addition of Bryostatin 1 to Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (CHOP) Chemotherapy Improves Response in a CHOP-resistant Human Diffuse Large Cell Lymphoma Xenograft Model

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ABSTRACT

The incidence of non-Hodgkin’s lymphoma has been increasing at a rate of 4% per year since 1950; more than 62,000 cases will be diagnosed in the United States in 2000. Diffuse large cell lymphoma (DLCL) is the prototype of curable non-Hodgkin’s lymphoma. Empirically designed chemotherapy regimens did not increase the cure rate of 30–40% achieved by the original four-drug regimen introduced in the 1970s [cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP)]. We studied the antitumor effects of the CHOP regimen alone or in combination with a unique protein kinase C activator, bryostatin 1, on a xenograft model for resistant DLCL in mice with severe combined immune deficiency (WSU-DLCL2-SCID). In this model, the efficacy of bryostatin 1 given at 75 μg/kg, i.p., alone for 1 or 2 days [B(1) and B(2)] was compared with the efficacy of CHOP alone, bryostatin 1 + CHOP (B+CHOP) given concurrently, bryostatin 1 for 1 day followed by CHOP on day 2 [B(1)+CHOP], and bryostatin 1 for 2 days followed by CHOP on day 3 [B(2)+CHOP]. CHOP doses were as follows: (a) cyclophosphamide, 40 mg/kg, i.v.; (b) doxorubicin, 3.3 mg/kg, i.v.; (c) vincristine, 0.5 mg/kg, i.v.; and (d) prednisone, 0.2 mg/kg, every day for 5 days, p.o. Tumor growth inhibition (T/C), tumor growth delay (T−C), and log10 kill for B(1), B(2), CHOP, B+CHOP, B(1)+CHOP and B(2)+CHOP were 49%, 39%, 25.8%, 15.1%, 14.6%, and 12%; 6, 7, 16, 25, 12, and 15 days; and 0.6, 0.5, 2.2, 3.6, 1.7, and 2.0, respectively. To begin elucidating the mechanism whereby bryostatin 1 potentiated the effects of CHOP in the mouse model; we studied the effect of bryostatin 1 on Bax, Bel-2, and poly(ADP-ribose) polymerase proteins in vitro and in vivo. Bax protein increased in a time-dependent manner without any measurable change in Bel-2 expression. However, significant cleavage of the preapoptotic marker poly(ADP-ribose) polymerase was not recorded, and the percentage of apoptotic cells detected by flow cytometry increased only slightly (~8%) after 96 h of bryostatin 1 exposure. The in vitro and in vivo results emphasize the superiority of combining bryostatin 1 with the CHOP regimen against the WSU-DLCL2 model. One possible mechanism may be the modulatory effects of bryostatin 1 on the Bax:Bel-2 family of apoptosis-regulatory proteins. The use of this combination should be further explored clinically in the treatment of lymphoma.

INTRODUCTION

NHL is a group of heterogenous diseases resulting from malignant proliferation of lymphocytes. These malignancies have been increasing in incidence at a rapid rate of 4% per year during the past four decades (1–4). More than 62,000 cases will be diagnosed in the United States in 2000 (5). NHL is the second and third leading cause of death from cancer in males ages 15–34 and 35–54 years, respectively (6). DLCL is the most frequently occurring type of NHL, accounting for 31% of all lymphomas (7). DLCL is also the prototype of curable lymphoma. The four-drug combination of CHOP cures 30–40% of unselected patients with DLCL (8). This regimen has become the standard treatment because more modern regimens have not been found to be superior to CHOP in randomized cooperative group clinical trials (9). The newer, alternative regimens to CHOP such as methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone (m-BACOD) or prednisone, doxorubicin, cyclophosphamide, etoposide, cytarabine, bleomycin, vincristine, and methotrexate (ProMACe-CytaBOM), contained more drugs. The development of such combination chemotherapy regimens was considered rational in that agents were selected based on nonoverlapping toxicity and/or evidence of activity in hematological malignancies when tested as single agents. Moreover, CHOP itself was developed

1 The abbreviations used are: NHL, non-Hodgkin’s lymphoma; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; DLCL, diffuse large cell lymphoma; SCID, severe combined immunodeficient; PARP, poly(ADP-ribose) polymerase; CHO, cyclophosphamide monophosphate, doxorubicin, and oncovin; MTD, maximum tolerated dose; Td, tumor doubling time; 7AAD, 7 amino-actinomycin D; B+CHO, bryostatin 1 + CHO; B+CHOP, bryostatin 1 + CHOP.

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empirically by adding doxorubicin (Adriamycin) to the preexisting cyclophosphamide, vincristine, and prednisone regimen (8). Hence, such an approach continues to represent the state of the art in developing new therapeutic regimens in lymphoma. The availability of new xenograft animal models for human lymphoma may provide an important tool for evaluating new agents, combinations, or concepts in a preclinical in vivo setting. Our WSU-DLCL2-SCID xenograft model (10) is unique in several respects: (a) we have frozen aliquots of the cell line from which the model was established (WSU-DLCL2) at different passages to allow reinitiation of xenografts at any time (the take rate from cell line to mice is 80%); (b) when passaged in mice by serial transplantation, the take rate is 100%; (c) the WSU-DLCL2 cell line was established from a patient with lymphoma resistant to chemotherapy, radiation therapy, and bone marrow transplantation, and the WSU-DLCL2-SCID model is therefore a model for resistant lymphoma; xenograft tumors have also been shown to be resistant to standard chemotherapy agents; (d) the cell line and xenograft tumors have preserved the immunophenotypic and cytogenetic characteristics; it has the most common chromosomal translocation found in lymphoma, i.e., t(14; 18) (q32;q21); (e) the model is EBV negative; and (f) it overexpresses Bcl-2 and the multidrug resistance pump p-glycoprotein, which are currently regarded as two of the most important drug resistance molecules.

One approach to maximize the antitumor effect of certain regimens and possibly improve cure rates is to integrate new agents that have unique modes of action. The National Cancer Institute natural products program has identified a number of novel marine animal products with significant antilymphoid activity. The bryostatins represent one such group. Bryostatin 1, isolated from the marine bryozoan Bugula neritina (11), exhibited both in vitro and in vivo antitumor activity in a number of model systems (12, 13). In addition, bryostatin 1 has biological effects on both T and B lymphocytes as well as on the hematopoietic system (13). This agent has undergone preclinical evaluation against a variety of human lymphoid tumors and was found to have antitumor, immune modulating, and differentiating effects on a number of B-cell tumors including acute lymphoblastic leukemia (14), chronic lymphocytic leukemia (15), NHL (16), and Waldenström’s macroglobulinemia (17). In this study, we investigated the antitumor effect of the CHOP regimen alone and in combination with bryostatin 1 in a SCID mouse xenograft model bearing the WSU-DLCL2 cell line.

MATERIALS AND METHODS

WSU-DLCL2 Cell Line. The human DLCL cell line (WSU-DLCL2) was established in our laboratory at the Wayne State University School of Medicine (10). The cell line was maintained in RPMI 1640 containing 10% heat-inactivated fetal bovine serum, 1% l-glutamine, 100 units/ml penicillin G, and 100 μg/ml streptomycin. Cells were incubated in a humidified 5% CO2 atmosphere at 37°C. No growth factors, mitogens, or EBV were added to the cell culture medium at any time. The cell line has a doubling time of 18 h and preserved the natural B-cell phenotype of the original tumor in the patient.

Cell Growth. WSU-DLCL2 cells were plated in 24-well culture clusters (Costar, Cambridge, MA) at a density of 2 × 10^5 viable cells/ml/well. Triplicate wells were treated with bryostatin 1 (10 nM), cyclophosphamide monophosphate (C; 5.84 μM), doxorubicin (H; 1.5 μM), oncovin (O; 260 nM), or diluent (control). Prednisone (P) was used only in the in vivo studies. Plates were incubated at 37°C in a humidified incubator with 5% CO2. All cultures were monitored throughout the experiment by cell count and viability every 24 h for 4 days using 0.4% trypan blue stain (Life Technologies, Inc., Grand Island, NY) and a hemocytometer.

Apoptosis. For light microscopic examination, WSU-DLCL2 cells were seeded in 24-well culture plates as described above. Briefly, untreated (control) and cells treated with bryostatin 1, CHO, and B+CHO were set in three replications. Aliquots from cell cultures were cytocentrifuged using a CytoSpin II centrifuge (Shandon Southern Instruments, Sewickley, PA). Cell smears were air dried, stained with tetrachrome at full concentration for 5 min, and then stained at 50% dilution with distilled H2O for another 5 min. Slides were analyzed under light microscopy (Nikon, Garden City, WY). Three hundred cells were counted and analyzed under high power for viability, mitosis, apoptosis, and death. Features of apoptosis looked for included nuclear chromatin condensation, formation of membrane blebs, and apoptotic bodies.

WSU-DLCL2-Xenografts. Four-week-old female ICR-SCID mice were obtained from Taconic Laboratory (Germantown, NY). The mice were adapted, and WSU-DLCL2 xenografts were developed as described previously (10). Each mouse received 107 WSU-DLCL2 cells (in serum-free RPMI 1640) s.c. in each flank area. When s.c. tumors developed to approximately 1000 mg, mice were sacrificed, and tumors were dissected and mechanically dissociated into single-cell suspensions. Mononuclear cells were separated by Ficoll-Hypaque density centrifugation and washed twice with RPMI 1640. These cells were subjected to phenotypic analysis for comparison with the established tumor cell line to insure the human origin and its stability. After formation of s.c. tumors, serial propagation was accomplished by excising the tumors, trimming extraneous material, and cutting the tumors into fragments of 20–30 mg that were transplanted s.c. using a 12-gauge trocar into the flanks of a new group of mice.

Efficacy Trial Design. For the subsequent drug efficacy trials, small fragments of the WSU-DLCL2 xenograft were implanted s.c. and bilaterally into naive, similarly conditioned mice, as described previously. Mice were checked three times per week for tumor development. Once transplanted WSU-DLCL2 fragments developed into palpable tumors (100–200 mg), groups of five animals were removed randomly and assigned to different treatment groups.

To further extend our work in using bryostatin 1 as an antilymphoma and biological modulating agent, we combined it with CHOP using our WSU-DLCL2-SCID model. Bryostatin 1 (NSC 339555) was provided to us by the National Cancer Institute Division of Cancer Treatment and Diagnosis. In this model, the efficacy of bryostatin 1 given at 75 μg/kg, i.p., alone for 1 or 2 days was compared with that of CHOP alone, B+CHOP given concurrently, bryostatin 1 for 1 day followed by CHOP on day 2, and bryostatin 1 for 2 days followed by CHOP on day 3. CHOP doses were as follows: CHOP was given at MTD times one injection (i.e., cyclophosphamide, 40 mg/kg,
Activity of CHOP in Lymphoma

i.v.; doxorubicin, 3.3 mg/kg, i.v.; vincristine, 0.5 mg/kg, i.v.; and prednisone, 0.2 mg/kg, p.o.] every day for 5 days. Mice were observed for measurement of s.c. tumors, changes in weight, and side effects of the drugs. The s.c. tumors were measured three times per week. Animals were euthanized when their total tumor burden reached 1500 mg to avoid discomfort. All studies involving mice were performed under Institutional Review Board-approved protocols. Tumor weights in SCID mice were plotted against time on a semilog sheet with the growth pattern resembling an S shape. Td is the time (in days) required for the tumor to double its weight during the exponential growth phase.

Assessment of Tumor Response. The end points for assessing antitumor activity were established according to standard procedures used in our laboratory (10, 15, 17) and are as follows: (a) tumor weight (mg) = (A × B^2)/2, where A and B are the tumor length and width (in mm), respectively; (b) tumor growth inhibition (T/C) is calculated by using the median tumor weight in the treated group (T) when the median tumor weight in the control group (C) reached approximately 900 mg. Tumor growth delay (T − C) is the difference between the median time (in days) required for the treatment group tumors (T) to reach 700 mg and the median time (in days) for the control group tumors (C) to reach the same weight; and (c) tumor cell kill net (log_{10} = (T − C) − (duration of treatment in days)/(3.32)(Td)). In this study, the antitumor activity is considered highly active (++++) when the log_{10} kill (net) is >2.0. Activity rating scores of +++++ or ++++ are needed for translocation to clinical activity and equate with complete and partial tumor regression, respectively. A score of either + or ++ is not considered active by usual clinical criteria (18).

Western Blot Analysis. WSU-DLCL2 cells from bryostatin 1-treated SCID mice (75 µg/kg, i.p., for days 1, 2, and 3) and cultures (10 nM, for days 1, 2, 3, and 4) or controls were washed twice with 1× PBS, resuspended in Triton X-100 lysis buffer [300 mM sodium chloride, 50 mM Tris-HCl (pH 7.6), 5% Triton X-100, and protease inhibitors], and kept at 4°C for at least 45 min. Cells were centrifuged at 14,000 g for 10 min, and the supernatant containing the cytosolic extract was saved. The protein concentration was determined using the Micro BCA protein estimation kit (Pierce, Rockford, IL). For Western analysis, 20 µg from each sample were separated on a 12% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were blocked for 1 h in PBST (5% nonfat dry milk solution in PBS with 0.1% Tween 20). Membranes were then incubated in a 1:1000 concentration of Bax, Bcl-2 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and/or PARP (Trawigen, Gaithersburg, MD). The membranes were washed in PBST twice and exposed to horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Inc.). The proteins were visualized using the enhanced chemiluminescence reagents (Amersham International Ltd., Buckinghamshire, United Kingdom).

7AAD Staining and Flow Cytometry. 7AAD (Calbiochem-Novabiochem, La Jolla, CA) was dissolved in acetone, diluted in PBS to a concentration of 200 µg/ml, and kept at −20°C protected from light as described previously (15). Briefly, 100 µl of 7AAD solution were added to 1 × 10^6 cells, suspended in 1 ml of PBS, and mixed well. WSU-DLCL2 cells protected from light were stained for 20 min on ice. Cells were pelleted, the supernatant was removed, and the pellet was washed twice with PBS. Samples from bryostatin 1-treated cells or untreated (control) cells were analyzed by flow cytometry (FACScan; Becton Dickinson, Mountain View, CA). Data were acquired on 20,000 cells and processed using Lysys II software (Becton Dickinson). Scattergrams were generated by combining forward light scatter with 7AAD fluorescence.

RESULTS

Effect on the Cell Growth of WSU-DLCL2. WSU-DLCL2 cells were exposed to bryostatin 1 (10 nM), CHO, or B+CHO. The drug effect was observed over 3 days. Bryostatin 1 showed little growth inhibition, whereas CHO inhibited the growth more than 50% compared with control (Fig. 1A). However, the effect of adding bryostatin 1 to CHO proved to inhibit the cell growth by greater than 100% (Fig. 1A). These results highlight the superiority of B+CHO over bryostatin 1 or CHO alone. It is noteworthy that prednisone (P) was used only in the in vivo studies.

Apoptosis. Tetrachrome slides made at 72 h showed that B+CHO exhibited 40% cell death compared with only 14% in CHO treatment alone (Fig. 1B). Again, these results confirm the advantage of adding bryostatin 1 to CHO chemotherapy.

In Vivo Efficacy of Bryostatin 1. When SCID mice received s.c. injection in each flank of equal numbers of WSU-DLCL2 cells (1 × 10^7), 80% of the animals developed tumors. The tumors in each flank were palpable by the third week. Dt in
Table 1. Antitumor activity of CHOP versus B+CHOP in the WSU-DLCL2-SCID xenograft model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>T/C (%)</th>
<th>T − C (days)</th>
<th>log_{10} kill (net)</th>
<th>Activity score</th>
<th>Mean (mg)</th>
<th>Range (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>100</td>
<td>0.0</td>
<td>0.0</td>
<td>--</td>
<td>722</td>
<td>626–1068</td>
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<tr>
<td>B(1×)</td>
<td>5</td>
<td>49.0</td>
<td>6</td>
<td>0.6</td>
<td>+</td>
<td>759</td>
<td>366–1108</td>
</tr>
<tr>
<td>B(2×)</td>
<td>5</td>
<td>39.0</td>
<td>7</td>
<td>0.5</td>
<td>+</td>
<td>624</td>
<td>430–730</td>
</tr>
<tr>
<td>CHOP</td>
<td>4</td>
<td>25.8</td>
<td>16</td>
<td>2.2</td>
<td>+++</td>
<td>114</td>
<td>22–196</td>
</tr>
<tr>
<td>B+CHOP</td>
<td>4</td>
<td>15.1</td>
<td>25</td>
<td>3.6</td>
<td>++++</td>
<td>54</td>
<td>0.0–171</td>
</tr>
<tr>
<td>B(1×)-CHOP</td>
<td>7</td>
<td>14.6</td>
<td>12</td>
<td>1.7</td>
<td>+++</td>
<td>171</td>
<td>95–140</td>
</tr>
<tr>
<td>B(2×)-CHOP</td>
<td>7</td>
<td>12.0</td>
<td>15</td>
<td>2.0</td>
<td>++++</td>
<td>123</td>
<td>64–196</td>
</tr>
</tbody>
</table>

* B(1×), bryostatin 1 given at 75 μg/kg, i.p., one injection. B(2×), bryostatin 1 given at 75 μg/kg, i.p., two injections. CHOP was given at MTD ×1 injection, i.e., cyclophosphamide (100 mg/kg, i.v.), doxorubicin (3.3 mg/kg), vincristine (0.5 mg/kg), and prednisone (0.2 mg, orally) daily for 5 days. B+CHOP, bryostatin 1 given concurrently with CHOP. B(1×)-CHOP, bryostatin 1 given on day one then followed by CHOP on day 2. B(2×)-CHOP, bryostatin 1 given on day 1 and 2 then followed by CHOP on day 3. Rating score of +++ (active) or ++++ (highly active) is needed to effect partial or complete tumor regression; + or ++ is not considered active by usual clinical criteria (18).

SCID mice was 2.0 days. When WSU-DLCL2 was passaged in SCID mice, the take rate was 100%. Drug efficacy trials were conducted on animals with palpable tumors (tumors of approximately 200 mg).

Table 1 shows the antitumor activity of bryostatin 1, when given at 75 μg/kg i.p., as one and two injections, CHOP alone, or B+CHOP given at MTDs, against WSU-DLCL2-bearing SCID mice. Tumor growth inhibition (T/C), tumor growth delay (T − C), and log_{10} kill were 49%, 39%, 25.8%, 15.1%, 14.6%, and 12%; 6, 7, 16, 25, 12, and 15 days; and 0.6, 0.5, 2.2, 3.6, 1.7, and 2.0, respectively. T/C values are used to determine tumor response. Bryostatin 1 is considered inactive when given as one injection (T/C = 49%), all other treatments are considered active against this type of human tumor (T/C values of ≤42% are indicative of antitumor activity). However, if log_{10} kill net values are added as a criterion, only B+CHOP is clinically considered highly active. All others were considered active by usual clinical criteria. It should be noted that an activity rating score of +++ (active) or ++++ (highly active) was needed to effect partial or complete tumor regression. Thus, a score of + or ++ is not considered active by the usual clinical criteria (18). The mean and range of tumor weights varied dramatically among various treatments. Animals treated with CHOP or B+CHOP showed the best tumor weight and range. The smallest tumor (54 mg) was seen in the B+CHOP treatment group, with a range of 0.0–171 mg. Tumor growth patterns of control, bryostatin 1, and B+CHOP are shown in Fig. 2. B+CHOP showed a significant (P = 0.028) tumor growth delay compared with all other treatments and demonstrated a much better survival rate.

In Vitro and In Vivo Effect of Bryostatin 1 on Bcl-2, Bax, and PARP Protein Expression. Fig. 3A shows Bcl-2, Bax, and PARP expression in WSU-DLCL2 before and after treatment with bryostatin 1. Bryostatin 1 alone was unable to induce changes in the expression of Bcl-2 protein, which is highly expressed in WSU-DLCL2 cells. The proapoptotic Bax protein, which is not highly expressed in control cells, increased in a time-dependent manner after exposure of WSU-DLCL2 cells to bryostatin 1 (10 nM) for 24, 48, and 72 h. This modulation in the Bax:Bcl-2 protein ratio is important for potentiating cells to undergo apoptosis. Cleavage of the PARP is also an important indicator of apoptosis induction. PARP, which is found as a M_r 116,000 band, was dramatically cleaved 24 h after bryostatin 1 treatment to its M_r 85,000 catalytic form. The parent M_r 116,000 form is conserved over the course of this study, as is the M_r 85,000 form. These data indicate that exposure to bryostatin 1 induced PARP cleavage, the molecular marker for apoptosis.

Fig. 3B shows Bcl-2, Bax, and PARP expression in WSU-DLCL2 tumors excised from WSU-DLCL2-SCID xenograft mice. High levels of Bcl-2 and Bax protein expression are found in tumors from control mice, and PARP is significantly cleaved from its M_r 116,000 form to the catalytic M_r 85,000 form. Tumors taken from mice after 1, 2, and 3 days of bryostatin 1 show reduced Bax protein at days 1, 2, and 3, whereas the Bcl-2 protein level decreases at days 1 and 2 but increases to control level on day 3. A significant increase in the M_r 116,000 PARP protein is recorded over days 1, 2, and 3 with a concomitant decrease in the M_r 85,000 form (Fig. 3B).

Induction of Apoptosis in WSU-DLCL2 Cells by Bryostatin 1. Typical fluorescence-activated cell-sorting scattergrams of control and bryostatin 1-treated WSU-DLCL2 cells are shown in Fig. 4. The figure shows three regions defined by 7AAD staining: (a) 7AAD-negative (viable cells, bottom); (b) 7AAD-dim (early to mid-apoptotic cells, middle); and (c) 7AAD-bright (late-apoptotic or dead cells, top). The percentage of apoptotic cells in the bryostatin 1-treated cells was insignificant compared with the control. However, extending the incubation period of the WSU-DLCL2 cells to 96 h did result in an 8% increase in the percentage of apoptotic cells.
DISCUSSION

In this study, we show that administering the four-drug regimen CHOP with bryostatin 1 to SCID mice bearing human DLCL tumors resulted in significantly higher antitumor activity compared with CHOP or bryostatin 1 alone.

Two decades ago, using the CHOP combination of agents, 30–40% of advanced-stage DLCL was found to be curable (19). Although numerous attempts were made to improve the cure rate, no further progress has been made (3, 6). The WSU-DLCL2 cell line was established from a relapsed DLCL patient who was clinically resistant to therapy (10). The disease relapsed after high-dose chemotherapy and radiation, followed by bone marrow transplantation. In the in vitro study, the WSU-DLCL2 cell line was exposed to bryostatin 1 alone, CHO alone, and B1CHO (Fig. 1A). The results highlighted the obvious superiority of B1CHO over bryostatin 1 or CHO alone. B1CHO induced more in vitro cell death compared with bryostatin 1 or CHO alone (Fig. 1B).

The ability of these cells to grow as xenografts in SCID mice makes it a useful preclinical model to search for effective drugs against resistant lymphoma and to explore not only mechanisms of cell killing but mechanisms of resistance. Among the large number of marine invertebrate-derived anticancer agents, bryostatin 1 was selected for clinical development against hematopoietic disease. To further extend our work using bryostatin 1 as an antilymphoma and chemotherapy-modulating agent, we combine it with CHOP in the WSU-DLCL2-SCID model. In this study, we used the model of advanced disease, i.e., therapy was started when palpable tumors have developed (2–3 weeks after transplantation). CHOP was given at its one-time injection MTD as described previously, and all animals survived treatment without death or undue toxicity (<10% weight loss).

Tumor growth patterns of control, bryostatin 1, CHOP, and B1CHO are shown in Fig. 2. B1CHO showed significant (P = 0.028) tumor growth delay compared with all other treatments and demonstrated a much better survival rate. We concluded from this study that adding one dose of bryostatin 1 (at MTD) to the CHOP regimen (at the MTD for SCID mice) is well tolerated and improved the single-agent antitumor activity. Animals were observed for toxicity, and their s.c. tumors were measured. Animals were euthanized when their total tumor...
burden reached 1500 mg (10% of body weight) to avoid discomfort. The end points of study were tumor growth inhibition (T/C), tumor growth delay (T – C), and log<sub>10</sub> kill. Table 1 shows the antitumor activity of CHOP or B+CHOP given at the MTD against WSU-DLCL<sub>2</sub>-bearing SCID mice.

When tumor responses are determined by the T/C value, bryostatin 1 is considered inactive when given as one injection (T/C = 49%). All other treatments are considered active against this type of human tumor (a T/C value of ≤42% is indicative of antitumor activity). However, when log<sub>10</sub> kill net values are added as criterion, only B+CHOP is clinically considered highly active, whereas the others were considered active by usual clinical criteria. It should be noted that an activity rating score of ++ (active) or +++ (highly active) was needed to effect partial or complete tumor regressions. Thus, a score of + or ++ is not considered active by usual clinical criteria (18). The mean and range values of tumor weights varied dramatically among various treatments. CHOP or its combination with bryostatin 1 showed the best mean tumor weight and range. The smallest tumor (54 mg) was seen in the B+CHOP treatment group (range, 0.0–171 mg). Previously, we have shown that the highest activity in this model was demonstrated by vincristine given sequentially after bryostatin 1 (10). Bryostatin 1 potentiates the antitumor activity of vincristine in WSU-DLCL<sub>2</sub>-SCID xenograft mice, with bryostatin 1 sensitizing WSU-DLCL<sub>2</sub> cells to the effect of vincristine because the reverse sequence of vincristine followed by bryostatin 1 did not increase apoptosis (10). In a separate experiment, our data showed that treatment giving bryostatin 1 two days after CHOP did not show better antitumor activity compared with either CHOP alone or B+CHOP (data not shown).

It has been shown in some types of NHLs and in acute myeloid leukemia that Bcl-2 down-regulation augmented sensitivity to chemotherapeutic agents (20–22). However, other studies have demonstrated that in pediatric acute lymphoblastic leukemia, high levels of Bcl-2 are not correlated with resistance to cytotoxic drugs (23, 24). Clearly, expression of other Bcl-2-related genes, presumably Bax, may modulate the effect of Bcl-2 itself and influence whether or not cells will undergo apoptosis.

The Bax gene encodes a M<sub>s</sub> 21,000 protein that shares considerable amino acid homology with Bcl-2 and is capable of binding to the latter protein (25, 26). Thus, Bax can form Bcl-2:Bax heterodimers as well as Bax:Bax homodimers, the latter being preferentially formed when the level of Bax exceeds that of Bcl-2. Formation of Bcl-2:Bax heterodimers appears to inhibit the antiapoptotic function of Bcl-2, whereas the Bax:Bax homodimers contribute to initiation of apoptosis (27). Thus, higher levels of Bax relative to Bcl-2 after a death signal may increase the cell susceptibility to apoptosis.

WSU-DLCL<sub>2</sub> cells taken from in vitro (Fig. 3A) or from excised tumor (Fig. 3B) express high levels of Bcl-2 protein and a low level of Bax. Bryostatin 1 caused a gradual increase in Bax expression but did not change the expression of Bcl-2. Bryostatin 1 induced marginal cleavage of PARP from its M<sub>s</sub> 116,000 form to its M<sub>s</sub> 85,000 form, which has been shown to result in apoptosis (28). Marginal apoptosis induction was also recorded (~8%) using a flow cytometry method that takes advantage of DNA strand breaks being recognized and bound by 7AAD (Fig. 4).

Collectively, the results obtained from this work highlight the obvious superiority of B+CHOP over CHOP or bryostatin 1 alone and begin to elucidate some of the mechanisms behind the potentiating effects of bryostatin 1. The results from this study should prove useful in guiding the clinical application of these novel agents in the treatment of lymphomas.

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REFERENCES


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