Combination Chemotherapy in Sequence for the Treatment of L1210 Leukemia

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ABSTRACT

In the studies presented L1210 leukemia was used to show that various equipotent combinations of two drugs can be freely substituted for each other during the course of sequential treatment with other drugs and that the order of administration will influence the therapeutic response to sequential treatment with various drug combinations. Mice inoculated i.p. with L1210 ascites tumor cells were treated with 4'-demethylepipodophyllotoxin-9-(4,6-O-2-thienylidene-β-D-glucopyranoside), 1-β-α-arabinofuranosylcytosine plus methotrexate, or Adriamycin plus cyclophosphamide in various dosages and sequences. Three ratios of dosages for each two-drug combination were selected to produce equivalent prolongation of life span when administered on Days 1, 4, and 7. When these two-drug combinations were used for sequential treatment with 4'-demethylepipodophyllotoxin-9-(4,6-O-2-thienylidene-β-D-glucopyranoside) on Day 1, 1-β-α-arabinofuranosylcytosine plus methotrexate on Day 4, and Adriamycin plus cyclophosphamide on Day 7, each of the three ratios of dosages for the two two-drug combinations was equivalent in prolonging the lives of mice inoculated with L1210 tumor cells. When the sequence of administration was varied, marked differences were observed in the effectiveness of treatment. These observations indicate that a variety of dosages of oncolytic drugs can be used to produce equivalent therapeutic response and that the order of administration may determine the overall effectiveness of sequential multiple-drug therapy.

INTRODUCTION

In general, guidelines for selecting drugs for use in combinations are ill defined. Schedules for the administration of drugs in combination are selected either randomly from previous clinical experience with the individual agents or by extrapolation from pharmacological or cell kinetic data. The selection of dosages of drugs for use in combination is generally by consensus with some consideration given to the expected persistence of toxicity, and in addition clinical protocols generally contain an arbitrary recommendation to reduce the dosages of all oncolytic drugs by one-half if intolerable toxicity develops. The number of drugs to be used in combination is generally determined by the number of available agents that individually produce therapeutic responses among patients with similar diagnoses. Although increasing emphasis is placed on the use of certain drugs for inducing remission and on others for continuation or maintenance therapy, there is very little information available to favor a specific sequence of treatment with 2 or more drug combinations.

In these studies with L1210 leukemia as a model system, specific limitations were imposed to approximate more closely the clinical situation. Foremost among these considerations was the expectation that limitations on time and on the number of available patients would prevent a thorough clinical investigation of more than 2 drugs in combination. Therefore, the studies were designed to treat L1210 ascites tumors with a sequence of therapy in which a drug was used only once and in which two 2-drug combinations were administered with VM-26. Our earlier studies provided the necessary preliminary data for treatment with VM-26, ara-C plus MTX, and Adriamycin plus cyclophosphamide (1, 2, 12).

The 2 concepts tested in these studies are that various equipotent combinations of dosages of 2 drugs may be freely substituted for each other during the course of sequential treatment with other drugs and that the order of administration influences the therapeutic response to sequential treatment with various drug combinations.

MATERIALS AND METHODS

Mice. One million L1210 ascites cells were injected i.p. on Day 0 into female C57BL/6J × DBA/2J F1 (hereafter called BDF1) mice from The Jackson Laboratory, Bar Harbor, Maine. Treatment was initiated 24 hr later on Day 1, and all drugs were individually administered i.p. in a volume of 0.01 ml/g body weight.

Preparation of Drugs. VM-26, 10 mg, was dissolved in 0.1 ml of dimethyl sulfoxide to which 1 ml of Tween 80 was added, and this was followed by 8.9 ml of 0.9% NaCl solution. Final dilutions of VM-26 were made with 0.9% NaCl solution. The vehicle for VM-26 was prepared and diluted appropriately with omission of the drug. VM-26 was provided gratuitously by Sandoz Pharmaceuticals, Hanover, N. J. ara-C, MTX, and cyclophosphamide, formulated for clinical use, were obtained from the National Cancer Institute. Adriamycin was purchased from Adria Laboratories, Inc., Wilmington, Del. Each of these latter drugs was dissolved freshly in sterile 0.9% NaCl solution immediately before administration. Either the vehicle for VM-26 or 0.9% NaCl solution was administered to other groups of leukemic mice.

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2 The abbreviations used are: VM-26, 4'-demethylepipodophyllotoxin-9-(4,6-O-2-thienylidene-β-D-glucopyranoside); ara-C, 1-β-α-arabinofuranosylcytosine; MTX, methotrexate.
Treatment Schedules. Two schedules of treatment were used in these studies. Various groups of mice were treated either on Days 1, 4, and 7 or on Day 1 only. In the first study VM-26, MTX plus ara-C, or Adriamycin plus cyclophosphamide was administered 3 times. In the second study VM-26 was administered on Day 1; MTX plus ara-C, in 1 of 3 combinations, was subsequently administered on Day 4 and was followed by 1 of 3 combinations of Adriamycin plus cyclophosphamide on Day 7. In the third study treatment was administered on Days 1, 4, and 7 in 1 of the 6 possible sequences. In the fourth study treatment with VM-26 or 1 of the drug combinations was administered on Day 1 to various groups of mice.

Evaluation of Response. For mice dying with evidence of leukemia, ascites, hepatomegaly, or splenomegaly, the response was determined by the life span in days of the host from the time of inoculation of the tumor cells. Mice surviving for 60 days from inoculation and lacking evidence of leukemia at autopsy were recorded as survivors. The number of cells remaining after treatment on Day 1 was estimated from the regression equation derived for the life span of various groups of mice inoculated i.p. with 10 to 1 million L1210 cells from the same suspension that was used in the chemotherapy study.

RESULTS

BD2F1 mice were inoculated i.p. with 1 million L1210 ascites tumor cells on Day 0 and were treated with VM-26, ara-C plus MTX, or Adriamycin plus cyclophosphamide on Days 1, 4, and 7 (Table 1). The lower dosage of VM-26, 2.5 mg/kg, was slightly less effective than expected; however, this dosage was considered more nearly optimal for the second study. VM-26 at 5 mg/kg more closely approximated the response produced by the drug combinations. Mice treated with 0.9% NaCl solution lived approximately 7.5 days, while the mean life span for mice dying with leukemia after treatment on Days 1, 4, and 7 ranged from 16 to 21 days in the first study and from 16 to 19 days in the replicate study, without considering the response to 2.5 mg of VM-26 per kg. Five of the 30 animals treated with Adriamycin plus cyclophosphamide survived for more than 60 days and by gross examination were apparently free of tumor at autopsy.

The dosages of drugs in Table 1 were considered to be as close to therapeutic equivalence as we could expect to obtain within the limits of experimental variation. In addition, the slight variation between different treatments should be reduced further in the remaining experiments since no course of therapy would be repeated.

Sequential Treatment with 3 Ratios of Dosages for the 2-Drug Combinations. The data in Table 2 show that the 9 possible combinations at 3 ratios of dosages for each 2-drug combination were equivalent. These are the results from 2 separate studies in which VM-26, 2.5 mg/kg, was administered on Day 1. One of the 3 combinations of ara-C plus MTX was administered on Day 4. Adriamycin plus cyclophosphamide was subsequently administered at 1 of the 3 ratios of dosages on Day 7. One animal apparently free of tumor after 60 days was omitted from the study. Mice treated with the appropriate vehicle had a mean life span of 7.5 ± 1.4 (S.D.) days. These data demonstrate that various therapeutically equivalent dosages of drugs in combined sequence can produce the same response in mice with leukemia.

Table 1

The response of L1210 leukemia to various dosages of drugs administered in sequence

On Day 1 after i.p. inoculation of 1 million L1210 ascites tumor cells (Day 0), the mice were treated i.p. with VM-26, 2.5 mg/kg. On Day 4 they were given i.p. injections of 1 of the 3 combinations of MTX plus ara-C. On Day 7 one of the 3 combinations of Adriamycin plus cyclophosphamide was administered. The results from 2 separate studies are combined to provide 10 animals/group with 1 exception; 1 mouse treated on Day 4 with 4 mg of MTX plus 720 mg of ara-C per kg and on Day 7 with 5 mg of Adriamycin plus 22 mg of cyclophosphamide per kg survived for 60 days and was apparently free of tumor at autopsy.

Table 2

Response of L1210 leukemia to various dosages of drugs administered in sequence

Dosage of Adriamycin + cyclophosphamide (mg/kg) Life span (days) for mice treated with MTX + ara-C at dosages of:

<table>
<thead>
<tr>
<th>Dosage of Adriamycin + cyclophosphamide (mg/kg)</th>
<th>Life span (days) for mice treated with MTX + ara-C at dosages of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.3 + 1200 mg/kg</td>
<td>4 + 720 mg/kg</td>
</tr>
<tr>
<td>5 + 22</td>
<td>19.2 ± 3.3</td>
</tr>
<tr>
<td>1.8 + 36</td>
<td>17.2 ± 1.7</td>
</tr>
<tr>
<td>0.7 + 60</td>
<td>19.4 ± 3.2</td>
</tr>
</tbody>
</table>

a Mean ± S.D.

Table 1

The response of L1210 leukemia to 3 treatments with various dosages of VM-26, MTX plus ara-C, or Adriamycin plus cyclophosphamide

On Days 1, 4, and 7 after the i.p. inoculation of 1 million ascites tumor cells, mice were treated i.p. with (a) VM-26, (b) MTX plus ara-C, (c) Adriamycin plus cyclophosphamide, or (d) 0.9% NaCl solution. The mean day of death ± S.D. from the day of inoculation is reported for animals dying with evidence of leukemia. Mice surviving for 60 days were free of any gross evidence of leukemia at autopsy.

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td>**Dosage (mg/kg)</td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>7.8 ± 0.52</td>
</tr>
<tr>
<td>VM-26</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>MTX + ara-C</td>
<td>1.3 + 1200</td>
</tr>
<tr>
<td></td>
<td>4.0 + 720</td>
</tr>
<tr>
<td></td>
<td>12 + 260</td>
</tr>
<tr>
<td>Adriamycin + cyclophosphamide</td>
<td>5 + 22</td>
</tr>
<tr>
<td></td>
<td>1.8 + 36</td>
</tr>
<tr>
<td></td>
<td>0.7 + 60</td>
</tr>
</tbody>
</table>
Sequential Treatment with Drug Combinations

Response to Variation in Sequence of Treatment. The order of treatment was varied for the 2 studies presented in Table 3. Drugs were administered in each of the 6 possible sequences. For this study the dosage of VM-26 was 5 mg/kg, and the middle ratio of dosages was selected from Table 2: 720 mg of ara-C plus 4.0 mg of MTX and 1.8 mg of Adriamycin plus 36 mg of cyclophosphamide per kg of body weight. As shown in Line 1, doubling the dosage of VM-26 cured 4 of 10 mice, whereas in the prior experiment with this sequence but with 2.5 mg of VM-26 per kg only 1 mouse was cured (Table 2). Although the mice that died with L1210 leukemia had approximately the same mean life span, there was a marked variation in the number of 60-day survivors. These do not appear to be a distinct advantage associated with the initial administration of any 1 course of treatment. Two schedules of treatment, Lines 2 and 4, failed to provide any 60-day survivors. By the chi-squared test variation in the incidence of 60-day survivors between Lines 2, 3, and 4 was significant ($p < 0.01$). With the appropriate sequence of drugs, L1210 tumor cells can apparently be eradicated by dosages of drugs that fail to cure mice in alternate sequences.

Response of L1210 Leukemia to a Single Treatment. The dosages for the preceding studies were selected on the basis of a summation of responses by L1210 leukemia to 3 treatments. To test for a variation in response to each course of treatment, we treated mice on Day 1 only with VM-26, ara-C plus MTX, or Adriamycin plus cyclophosphamide after i.p. injection of 1 million L1210 cells on Day 0 (Table 4). In Experiment 1 treatment with Adriamycin plus cyclophosphamide was more effective than was treatment with ara-C plus MTX ($0.02 > p > 0.01$ by the t test). In Experiment 2 a single treatment with ara-C plus MTX was less effective than was treatment with Adriamycin plus cyclophosphamide or with VM-26. A single treatment with either Adriamycin plus cyclophosphamide or VM-26 was equivalently effective in both studies.

In parallel with both of these studies (Table 4), 6 other groups of mice were given injections of different inocula varying from 10 to 1 million tumor cells/mouse. The size of the residual population of tumor cells after treatment on Day 1 was estimated from the regression equation for life

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**Table 3**

Response of L1210 leukemia to various sequences of multiple-drug treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Life span (days)</th>
<th>Survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>VM-26</td>
<td>ara-C + MTX</td>
<td>Adriamycin + cyclophosphamide</td>
<td>19.0 ± 4.1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>VM-26</td>
<td>Adriamycin +</td>
<td>ara-C + MTX</td>
<td>18.4 ± 1.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Adriamycin + cyclophosphamide</td>
<td>VM-26</td>
<td>Adriamycin + cyclophosphamide</td>
<td>17.3 ± 0.6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Adriamycin + cyclophosphamide</td>
<td>ara-C + MTX</td>
<td>Adriamycin + cyclophosphamide</td>
<td>18.0 ± 2.1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Adriamycin + cyclophosphamide</td>
<td>ara-C + MTX</td>
<td>VM-26</td>
<td>21.5 ± 3.9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Adriamycin + cyclophosphamide</td>
<td>VM-26</td>
<td>23.8 ± 5.4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Table 4**

Response of L1210 leukemia to a single course of treatment on Day 1

Twenty-four hr after i.p. inoculation of 1 million ascites tumor cells, various groups of mice were treated with drugs or the vehicles for the drugs. Mean life span (days ± S.D.) is reported for 10 animals in each group with 1 exception; in Experiment 2 one animal survived after treatment with Adriamycin plus cyclophosphamide. Drugs were administered in the following dosages: (a) 5 mg of VM-26; (b) 720 mg of ara-C plus 4 mg of MTX; and (c) 1.8 mg of Adriamycin plus 36 mg of cyclophosphamide per kg of body weight. Sterile 0.9% NaCl solution or the vehicle (dimethyl sulfoxide, Tween 80 plus 0.9% NaCl solution) for VM-26 was administered to other groups of mice inoculated with L1210 ascites cells. Additional groups of 5 mice were inoculated with 10, 100, 1,000, 10,000, or 100,000 tumor cells for estimation of the average number of residual tumor cells (17).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Life span (days)</th>
<th>No. of animals</th>
<th>Residual cells</th>
<th>Life span (days)</th>
<th>No. of animals</th>
<th>Residual cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>VM-26</td>
<td>12.3 ± 1.16</td>
<td>10</td>
<td>314</td>
<td>14.8 ± 0.92</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>ara-C + MTX</td>
<td>11.9 ± 0.99</td>
<td>10</td>
<td>643</td>
<td>12.0 ± 0.47</td>
<td>10</td>
<td>1172</td>
</tr>
<tr>
<td>Adriamycin + cyclophosphamide</td>
<td>13.0 ± 0.82</td>
<td>10</td>
<td>89</td>
<td>15.2 ± 1.79</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7.7 ± 0.48</td>
<td>10</td>
<td>89</td>
<td>7.9 ± 0.32</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>0.9% NaCl solution</td>
<td>7.9 ± 0.32</td>
<td>10</td>
<td>89</td>
<td>7.8 ± 0.42</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

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Various therapeutically equivalent combinations of 2 drugs could be substituted freely for each other in the treatment of L1210 leukemia, and by inference we suggest that, when standard dosages of drugs are not tolerated in combination by certain patients, alternate ratios of dosages might provide equivalent therapeutic efficacy and avoid toxicity. In addition, by using drugs in synergistic combinations we attempted to attain the greatest possible benefit from each drug at the dosage used. As an indication of the effectiveness of this approach, the incidence of 60-day survivors after treatment in the optimal sequence exceeded the responses obtained after treatment on Days 1, 4, and 7 with the optimal dosages for each 2-drug combination alone (1,2).

REFERENCES


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span versus the size of the inoculum (17) and is shown in Table 4. Although significant variation was observed after a single course of treatment with the 2-drug combinations and with VM-26, we are presently unable to correlate this variation with the dependence of response on the schedule of treatment shown in Table 3.

DISCUSSION

In approaching these studies we proposed to use the optimal dosage for treatment with VM-26 and each drug combination on the selected schedule. Two technical problems prevented this approach: first, animals could not tolerate sequential treatment with the optimal dosages for the 2-drug combinations plus VM-26 and, second, since optimal dosages of the drugs produced different responses early therapy of varying potency could interfere markedly with the interpretation of response to sequential treatment with different drugs.

Although by some classifications there is overlap in the general oncolytic action of some of the 5 drugs, cross-resistance between the agents has not been reported, and both 2-drug combinations are therapeutically synergistic for the treatment of L1210 leukemia (1, 2). VM-26 is a very effective drug for the treatment of L1210 leukemia, although its i.p. use is limited by the occurrence of delayed toxicity in mice (6, 12). This agent blocks the division of cells at the G2 phase of the cell cycle (11), inhibits oxidative phosphorylation and NADPH transhydrogenase (7, 8), and produces single-strand breaks in DNA (10). MTX inhibits dihydrofolate reductase and, as a consequence, the synthesis of purines and thymidine as well as of amino acids (4, 14). This S-phase-active agent is very effective for the treatment of L1210 leukemia in combination with ara-C (1), an inhibitor of the synthesis of DNA (5). Adriamycin blocks the division of cells at G2 phase and may produce its oncolytic action in S phase by inhibiting the synthesis of DNA or causing single-strand breaks (3, 9, 15). Cyclophosphamide is an alkylating agent and is considered to be most cytocidal to cells that are actively progressing through the division cycle (6, 16).

The data in Table 1 confirm (1, 2) that an equivalent increase in life span could be induced by varying the ratios of dosages for 2 agents and that the selected treatments are similarly effective. The data in Table 2 indicate that equivalent response to various ratios of dosage also occurred when drugs were administered in sequence. Table 3 shows that the sequence of treatment altered the effectiveness of that treatment. The data in Table 4 indicate that, although the summation of response by L1210 leukemia to treatment on Days 1, 4, and 7 was equivalent, the response to treatment on Day 1 only and, by inference, also on Days 4 and 7 varied for treatment with Adriamycin plus cyclophosphamide, ara-C plus MTX, and VM-26.
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