Chemotherapy of Advanced Mouse Leukemia L1210: Comparison of Methotrexate Alone and in Sequential Therapy*

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SUMMARY

The sequential treatment of mice with advanced leukemia L1210 employing a single injection of 2-chloro-4',4''-di-2-imidazolin-2-ylyterephthalanilide, dihydrochloride (NSC-38280) or cyclophosphamide followed by multiple treatment with methotrexate (MTX) was examined. Mice that received a single injection of NSC-38280 or cyclophosphamide followed by MTX given daily or every 4 days displayed more extensive increases in median survival time than did mice receiving MTX alone on a daily or every-4-day treatment schedule. An initial treatment with either NSC-38280 or cyclophosphamide followed by daily MTX was approximately twice as effective as daily MTX alone, whereas NSC-38280 or cyclophosphamide followed by MTX every 4 days was 3-4 times more effective than MTX alone every 4 days.

In a previous study (4) it was shown that methotrexate (MTX) was more effective against mouse leukemia L1210 when treatment was initiated early in the course of the disease than when it was initiated after the disease had become systemic. The reduced efficacy of MTX therapy against the advanced disease appeared to be attributable to a diminution in the effectiveness of the drug against an increased cell population (3, 8). A study of the influence of the schedule of treatment on the efficacy of MTX against leukemia L1210 (4) showed that the optimal interval between treatments depended on the extent of systemic leukemic infiltration at the time of treatment initiation. When treatment was begun early in the course of the disease, MTX was considerably more effective when given every 4 days than when given daily or as a single treatment. When treatment initiation was delayed until the disease was more advanced, daily treatment was generally more effective than treatment every 2d or 3d day, and the every-4-day schedule or a single treatment was relatively ineffective. The failure of the every-4-day schedule against advanced leukemia L1210 was attributed to the inability to administer a sufficient number of widely spaced treatments to produce a therapeutic effect. This was, in turn, related to the relative ineffectiveness of MTX when given as a single treatment (4). These observations suggested that an initial treatment with an agent which is effective as a single treatment might reduce or attenuate the leukemic cell population and render it more susceptible to subsequent MTX therapy. An enhancement of the effectiveness of both daily MTX and widely spaced MTX treatments would result in a broadening of the range of treatment schedules over which the drug is effective against advanced leukemia.

Extensive studies of the influence of the schedule of treatment on anti-leukemic (L1210) activity (5, 7, 10, 11, 13) showed that a single injection of 2-chloro-4',4''-di-2-imidazolin-2-ylyterephthalanilide, dihydrochloride (NSC-38280) or cyclophosphamide provided important increases in the survival time of mice with advanced disease. The current experiments were, therefore, conducted to investigate the effectiveness of a single treatment with NSC-38280 or cyclophosphamide followed by MTX, administered daily or every 4 days, in the treatment of advanced leukemia L1210.

MATERIALS AND METHODS

A saline suspension of leukemia (L1210)-infiltrated splenic tissue from stock tumor mice was inoculated subcutaneously into the right hind leg of 20- to 26-gm. CD2F1-(BALB/cAn X DBA/2)F1-hybrid mice. Female mice were employed in the experiment summarized in Chart 1, and male mice were used in the experiment shown in Table 1. Inoculation of serially diluted cell suspensions indicated that, in each experiment, the experimental inoculum (approximately 2 × 10^4 cells per mouse) was at least 100 times the inoculum level required to produce death from tumor in 100 per cent of untreated mice.

Methotrexate (MTX) and cyclophosphamide were dis-
Chart 1.—Sequential treatment of advanced leukemia L1210; single treatment with 2-chloro-4',4''-di-2-imidazolin-2-ylterephthalanilide, dihydrochloride (NSC-38280) followed by multiple treatment with methotrexate (MTX). The mice were given either MTX daily or every 4 days from day 7 to death; or a single NSC-38280 treatment on day 7 followed by MTX daily or every 4 days from day 8.

RESULTS

Chart 1 illustrates the capacity of a single treatment with NSC-38280 to increase the effectiveness of subsequent multiple MTX therapy in mice with advanced leukemia L1210. Untreated mice exhibited a median survival time of 9 days. Sequential therapy employing 250 mg/kg of NSC-38280 on day 7, followed by 1.0 mg/kg/day of MTX daily from day 8, was approximately twice as effective as daily treatment from day 7 with the optimal level (1.0 mg/kg/day) of MTX. Treatment with 250 mg/kg of NSC-38280 on day 7, followed by 10 mg/kg of MTX every 4th day from day 8, was approximately 4 times more effective than the optimal level of MTX alone (80 mg/kg) when given every 4th day from day 7. Sequential therapy employing MTX on either of the treatment schedules was more effective than optimal treatment with MTX alone or the optimal single dose (500 mg/kg) of NSC-38280 alone.

The experiment summarized in Table 1 shows that a single cyclophosphamide treatment followed by daily or intermittent MTX therapy was more effective against advanced leukemia L1210 than either daily MTX alone or a single cyclophosphamide treatment. In this case, the sequential treatment was punctuated by a 4-day interval between the injection of cyclophosphamide and the initiation of MTX therapy. Comparison of the optimal treatment levels for each of the therapies employed shows that the single initial cyclophosphamide treatment (222 mg/kg) resulted in an approximately twofold increase in the activity of daily MTX and a 34-fold increase in the activity of MTX given every 4 days.

DISCUSSION

The current study shows that it is possible, with the use of sequential treatment, to improve the therapeutic response of advanced leukemia L1210 beyond what can be achieved with MTX alone. This was accomplished by employing a "priming" dose of NSC-38280 or cyclophosphamide, followed by treatment with daily or intermittent MTX.

A previous comparison of the optimal dosage levels for a number of NSC-38280 treatment schedules had shown that this agent was equally effective when given daily, every 2d day, every 4th day, or as a single treatment to mice with advanced leukemia L1210 (11). Thus, although a direct comparison was not made in this study, the previous data (11) suggest that the sequential therapy employed for NSC-38280 and MTX may be more effective against advanced leukemia L1210 than either drug alone under optimal conditions of dosage and treatment schedule.
TABLE 1

<table>
<thead>
<tr>
<th>Treatment and schedule</th>
<th>Optimal treatment level (mg/kg)</th>
<th>Median survival time (days)</th>
<th>Increase in median survival time over controls (per cent)</th>
<th>Relative increase in median survival time (daily MTX = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTX alone, daily from day 7</td>
<td>0.80</td>
<td>25.5</td>
<td>183</td>
<td>100</td>
</tr>
<tr>
<td>MTX alone, every 4 days from day 7</td>
<td>19.0</td>
<td>15.5</td>
<td>72</td>
<td>39</td>
</tr>
<tr>
<td>Cyclophosphamide, day 7 only</td>
<td>333</td>
<td>19.5</td>
<td>116</td>
<td>64</td>
</tr>
<tr>
<td>Cyclophosphamide, day 7 only + MTX, daily from day 11</td>
<td>222</td>
<td>41</td>
<td>356</td>
<td>194</td>
</tr>
<tr>
<td></td>
<td>1.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide, day 7 only + MTX, every 4 days from day 11</td>
<td>222</td>
<td>32</td>
<td>256</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>5.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* When used alone each drug was employed over a wide range of doses. For sequential therapy, three levels of cyclophosphamide were used, and each was followed by a wide range of MTX dosage levels. The optimal treatment level for each of the therapies employed is defined as the treatment level providing the maximum increase in the median survival time of the mice.

† In the advanced leukemia L1210 assay system, daily MTX treatment is employed as a standard for anti-leukemic effectiveness. The effectiveness of any therapy at the optimal treatment level may be related to the effectiveness of the optimal daily dose of MTX.

Since treatment every 7 days has been found to be the optimal schedule for cyclophosphamide alone against advanced leukemia L1210 (10), no judgment can be made from this experiment regarding the relative therapeutic effectiveness of cyclophosphamide alone under optimal conditions of treatment and the sequential therapy employed.

It is of interest that the remarkable effectiveness of the sequential therapies shown in Chart 1 and Table 1 was obtained with drug pairs which are capable of providing therapeutic synergism against advanced leukemic disease when both agents are given on a multiple treatment schedule. When both drugs were given daily to death, the combination of NSC-38280 and MTX was more effective in increasing the survival time of mice with advanced L1210 than was daily treatment with NSC-38280 alone or MTX alone (12). Optimal combination levels of daily MTX and daily cyclophosphamide were only slightly more effective than was optimal daily treatment with each of the drugs separately (9). However, cyclophosphamide, when used alone, exerted its maximum degree of effectiveness on a weekly treatment schedule (5, 7, 10), and the combination of daily MTX plus weekly cyclophosphamide provided marked therapeutic synergism (9).

The underlying basis for the improved response with sequential therapy has not been clarified. It is possible that the therapeutic advantage is attributable to a decrease or attenuation of the systemic leukemic cell population by the initial treatment with NSC-38280 or cyclophosphamide. A single injection of cyclophosphamide has been shown to result in the destruction of leukemic cells, as reflected in its capacity to reduce the percentage of tumor "takes" among mice with early leukemia L1210 (13). The capacity of a single NSC-38280 treatment to elicit a fairly extensive increase in the survival time of leukemic mice is also strongly suggestive of cytotoxicity or stasis of leukemic growth.

In the assay systems employed, the maximum achievable effect of an individual drug or a drug combination is expressed at the optimal dose. At higher doses the toxicity of the treatment becomes limiting for the leukemic mice. Thus, with the individual drugs and with sequential therapy, toxicity for the leukemic host was limiting. In previous studies (12, 13) it was shown that MTX and also cyclophosphamide were more toxic for mice with advanced leukemia than for normal mice. With MTX, for example, the optimal daily anti-leukemic dose (0.65 mg/kg) increased the median survival time from 9 days (controls) to 24 days (eighteen daily treatments). In normal mice the same dose was given for 31 days without causing any weight loss or death (12). A comparison of therapy in leukemic and normal mice has provided evidence that an appropriate drug combination, by virtue of its increased anti-leukemic effectiveness, may actually decrease the sensitivity of the leukemic host to toxicity, and in such case the toxicity of the combination therapy in leukemic animals approaches the toxicity of the combination in normal animals (9, 12). It would be of interest to make a similar comparison for the current sequential therapy in normal and leukemic animals. The importance of this type of study has been stressed by Griswold et al. (6).

There has been considerable interest in the employment of drug combinations, both concomitantly and sequentially, in clinical leukemia (1, 2, 9). The current experiments in animals show that sequential therapy can be evaluated for its relative therapeutic effectiveness. This
type of model may provide an experimental tool for helping to evaluate compounds of clinical interest.

REFERENCES


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