Factors Influencing the Specificity of Action of an Antileukemic Agent (Aminopterin). Multiple Treatment Schedules plus Delayed Administration of Citrovorum Factor

ABRAHAM GOLDIN, JOHN M. VENDITTI, STEWART R. HUMPHREYS, DON DENNIS, NATHAN MANTEL, AND SAMUEL W. GREENHOUSE

(Laboratory of Chemical Pharmacology and Biometry Section, National Cancer Institute, Bethesda, Md.*)

It has been shown that the relative effect of aminopterin on tumor and host, expressed quantitatively as the specificity of action of the drug, may be altered by the manner in which the drug is employed (2-4). Two of the procedures reported increased the antileukemic specificity of action of aminopterin. (a) Delayed treatment with citrovorum factor (CF): At equal cost in drug dose mortality, a single dose of aminopterin, when followed 12 or 24 hours later by a single massive dose of CF, was more effective than a single dose of aminopterin alone in increasing the survival time of leukemic mice. (b) Appropriate multiple treatment schedules with aminopterin: At equal cost in drug dose mortality, three treatments with aminopterin, spaced 2 days apart, resulted in more extensive survival time of leukemic mice than a single treatment with aminopterin. A similar increase in survival time was apparent following two treatments with aminopterin spaced 4 days apart.

The current experiments were conducted to investigate whether these two methods for increasing the antileukemic specificity of action of aminopterin would supplement each other. For this purpose, a preliminary experiment was conducted to determine the optimal time for administration of delayed CF following treatment with aminopterin.

METHODS

The experimental and statistical methods have been previously described (2-4). The experiments were conducted with Leukemia L1210, in 8-10-week-old male mice ([A/LNDBA/2J]F1). Stock tumor was taken from DBA/2J male mice. The animals were given inoculations in the right thigh of 0.1 ml. of a saline suspension of tumor cells.

Aminopterin was prepared in 2 per cent sodium bicarbonate. CF was administered in distilled water. The drugs were freshly prepared at each injection interval. Each dose was administered subcutaneously in the axillary region in the constant volume of 0.01 ml/gm. The mice were randomized individually into the designated experimental groups prior to administration of drug. The order of injection was randomized by experimental group. When multiple aminopterin treatment was employed, the randomization of order of injection was repeated at each injection interval.

RESULTS

Chart 1 presents the results of the preliminary experiment (data not shown) intended to determine the optimal delay in administration of CF following treatment with aminopterin. The results of this preliminary experiment suggest that the optimal delay is in the neighborhood of 12 hours. With this delay interval, there is a greater extension in survival time for mice succumbing to tumor for fixed cost in toxic mortality from aminopterin than for any of the other delay periods considered.

The increased antileukemic action resulting from delayed administration of CF may be attributable to a relatively lower endogenous reserve of protection against aminopterin toxicity in the tumor as compared with that in the host (2, 3). By delaying administration of CF, the aminopterin is permitted to damage the tumor irreversibly, while the host is maintained by its endogenous protection. The delayed CF is thus still able to afford the host protection, but it cannot reverse the damage to the tumor (3). However, too early administration of delayed CF will not permit taking full advantage of the irreversible damage.
which aminopterin can inflict on the tumor, and, if the delayed CF is administered too late, it may permit extensive irreversible damage to the host by the aminopterin.

In experiments previously reported (3) and the preliminary experiment reported here, it is suggested that the irreversible damage to the tumor by aminopterin is virtually completed in the first 24 hours following treatment with aminopterin. However, even with this 12-hour delay, the CF still affords the host substantial protection against aminopterin toxicity. In the current preliminary experiment, administration of 400 mg/kg of CF delayed 12 hours still resulted in a better than tenfold increase in the aminopterin LD50 as against that for mice receiving aminopterin alone.

Accordingly, for the principal experiment reported here, whenever delayed CF was used, it was administered 12 hours subsequent to treatment with aminopterin. In this experiment (Table 1, Charts 2 and 3) eight different treatment schedules, with varying doses of aminopterin, were given to mice which had been previously inoculated with tumor. These were:

1. Aminopterin alone (0.78-46.3 mg/kg) on day 4 following inoculation.
2. Aminopterin (6.00-214 mg/kg) and delayed CF on day 2 following inoculation.
3. Aminopterin (6.00-214 mg/kg) and delayed CF on day 4 following inoculation.
4. Aminopterin (6.00-214 mg/kg) and delayed CF on day 6 following inoculation.
5. Aminopterin (0.78-46.3 mg/kg total) on days 2 plus 6 following inoculation.
6. Aminopterin (3.60-214 mg/kg total) and delayed CF on days 2 plus 6 following inoculation.
7. Aminopterin (0.47-27.8 mg/kg total) on days 2 plus 4 plus 6 following inoculation.
8. Aminopterin (1.30-129 mg/kg total) and delayed CF on days 2 plus 4 plus 6 following inoculation.

The delayed CF was always 200 mg/kg at each administration 12 hours subsequent to each aminopterin treatment.

The results for each of these schedules are expressed as a line in Chart 2, relating average survival time for mice succumbing to tumor to the probit of toxic mortality due to aminopterin. For ease of presentation, these eight lines are represented as parallel, and, in fact, as a group they did not show significant departure from parallelism.

The most obvious result (Chart 2) is that the greatest extension in survival time occurred when delayed CF was used in conjunction with multiple aminopterin treatment on days 2 plus 6. Thus, the two methods for increasing the antileukemic specificity of aminopterin, when used in conjunction, did supplement each other.

The supplementation of these two effects was not so evident when delayed CF was used in conjunction with multiple aminopterin treatment on days 2 plus 4 plus 6. As indicated below (discussion of Chart 3) this may have resulted from the presence of residual administered CF, which would afford the tumor protection against subsequent aminopterin treatment. This apparently may occur when the interval between administration of CF and the following treatment with aminopterin is too short.

Other results (Chart 2) serve to verify and extend results previously reported. Thus:

a) For single treatment (on day 4) with aminopterin, delayed CF increased the antileukemic specificity of action of aminopterin. A similar result has been reported (3).

b) For single treatment with aminopterin plus delayed CF, the antileukemic specificity of action was greater for treatment on day 4 than on day 2.
or day 6. A similar result has been reported for treatment with aminopterin alone (4).

c) Multiple treatment with aminopterin alone (days 2 plus 6, or days 2 plus 4 plus 6) served to increase its antileukemic specificity of action as opposed to single treatment. A similar result was previously reported (4).

In Chart 3 are shown, for the four schedules involving multiple treatment, the lines relating average survival time for mice succumbing to tumor to the total dosage of aminopterin.

The relationships for aminopterin alone should not be altered by administration of delayed CF if the delay is sufficiently great to permit the irreversible damage to the tumor to have gone to completion (3). In fact, it may be observed that this occurred for multiple treatment on days 2 plus 6. The lines for aminopterin alone and aminopterin plus delayed CF were substantially the same.

However, for multiple treatment on days 2 plus 4 plus 6, this did not occur. The line for aminopterin with delayed CF was substantially lower and shallower than the line for aminopterin alone. Apparently, the tumor was afforded protection by the CF, and the assumption is that such protection results from the incomplete elimination of the CF between treatments when the interval between treatments is too short.

It may also be noted in Chart 3 that the line for multiple treatment with aminopterin alone on days 2 plus 4 plus 6 is higher and steeper than that for treatment on days 2 plus 6. This occurred, since the toxicity of the aminopterin for the tumor increases with the number of treatments given (4).

**DISCUSSION**

These experiments afford evidence that two demonstrated methods for increasing the anti-

---

**TABLE 1**

HOST AND TUMOR RESPONSE TO AMINOPTERIN WITH AND WITHOUT DELAYED ADMINISTRATION OF CITROVOKUM FACTOR. SINGLE AND MULTIPLE TREATMENT*

<table>
<thead>
<tr>
<th>CF dose (mg/kg)</th>
<th>Aminopterin day 4</th>
<th>Aminopterin day 4</th>
<th>Aminopterin day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.78</td>
<td>0.30</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>1.56</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>3.60</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>10.0</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>16.7</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>27.8</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
</tr>
<tr>
<td>46.3</td>
<td>7.00</td>
<td>7.00</td>
<td>7.00</td>
</tr>
<tr>
<td>77.2</td>
<td>5.60</td>
<td>5.60</td>
<td>5.60</td>
</tr>
<tr>
<td>214</td>
<td>6.50</td>
<td>6.50</td>
<td>6.50</td>
</tr>
</tbody>
</table>

* The lines are shown nonparallel here, since they show significant departures from parallelism. Effectively, however, in Chart 2 they have been shown as parallel.

---

Control: 88 mice in various control groups (2 per cent NaHCO₃; untreated) all died of tumor between the 13th and 18th day following tumor inoculation; average = 15.3 days.

Inoculum = 135,000 cells per mouse.

* Calculated probits obtained from the fitted maximum likelihood dose response curves. A 4-way common probit slope was fitted for treatment groups receiving a single treatment with aminopterin with and without CF. A 2-way common probit slope was fitted for aminopterin treatment groups days 2 + 4 + 6 with and without CF. In each case the Cornfield-Mantel procedure (1) was used, with one or two cycles calculated, as necessary.
leukemic specificity of action of aminopterin may be employed to supplement each other.

The first of these two methods is the use of delayed CF. The evidence suggests that this method takes advantage of a relatively low endogenous reserve of protection in the tumor as compared with the host (2, 3). CF is withheld until the tumor has received maximal irreversible damage by the aminopterin, but it is administered early enough to afford protection to the host.

The second of these two methods is the use of multiple treatment with aminopterin. Here, too, it is suggested that advantage is taken of a relatively lower endogenous reserve of protection against aminopterin toxicity in the tumor (2-4). When multiple doses are employed, there is a long interval of exposure of the tumor to damaging doses of aminopterin. Thus, even relatively small doses of aminopterin on multiple treatment may result in extensive irreversible damage to the tumor. The endogenous protection of the host, however, is apparently great enough to protect it against these small doses of aminopterin, and in the interval between treatments the host has opportunity to restore its endogenous protection. The host, actually, may be quite vulnerable to multiple treatment with aminopterin, but the increase in vulnerability is relatively greater for the tumor (4).

The demonstration of the supplementation of the antileukemic specificity of action of aminopterin provides further indication of the importance of a knowledge of factors influencing the specificity of action of antitumor agents. With aminopterin, for example, it may be possible to inflict sufficient damage on the tumor employing extended multiple treatment schedules to achieve frequent complete regressions. At the same time, it may be possible, through the use of delayed CF and proper spacing of treatment, to keep toxic effects to the host at a minimum. Such studies are in progress.

**SUMMARY**

Multiple treatment with aminopterin and the administration of CF subsequent to treatment with aminopterin both increase the antileukemic specificity of action of aminopterin in mice. A combination of these treatments was shown to be more effective in this respect than either one alone.

**REFERENCES**

1. CORNFIELD, J., and MANTEL, N. Some New Aspects of the Application of Maximum Likelihood to the Calculation of
GOLDIN et al.—Antileukemic Action of Aminopterin plus C.F.


4. ———. Factors Influencing the Specificity of Action of an Antileukemic Agent (Aminopterin). Time of Treatment and Dosage Schedule. Ibid., pp. 311-14.
Factors Influencing the Specificity of Action of an Antileukemic Agent (Aminopterin). Multiple Treatment Schedules plus Delayed Administration of Citrovorum Factor
