Synergistic Inhibitory Action of A-Methopterin and a Diaminopyrimidine upon Leukemia L 1210 in Mice

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INTRODUCTION

Certain 2,4-diaminopyrimidines, synthesized for possible use as metabolic antagonists, were found to have antimalarial activity (3, 5). These pyrimidines were also able to inhibit the growth of L. casei and L. citrovorum, and this effect could be reversed by folic acid or by folic acid (4–8). For this reason it has been proposed that these pyrimidines probably inhibited the folic acid-folinic acid conversion (1, 3, 5), as has been proposed for the folic acid analogs (14, 19). This hypothesis has received support from the reports that Sarcoma 180 (2) and leukemia Ak4 (1), tumors inhibited by folic acid analogs, were also inhibited by these pyrimidines.

Data are herewith presented on the effect of one of these drugs, 2,4-diamino-5-(3',4'-dichlorophenyl)-6-methylpyrimidine (W-50-197)1 on the survival time and tumor size in mice inoculated with the transplantable leukemia L 1210 (11).

MATERIALS AND METHODS

An L 1210 tumor brei, diluted 1:4 with Locke’s solution, was inoculated in the axillary spaces of 751 DBA2 mice. Drug injections, when given, were withheld until 72 hours after transplantation. Drugs were administered intraperitoneally every other day for three doses, unless otherwise specified. Aseptic techniques were used. Animals were weighed at the beginning of each experiment, once weekly thereafter, and at autopsy. A-methopterin was dissolved in neutral distilled water, while W-50-197 was dissolved in acidulated water.2 Drugs were diluted so as to deliver the required dosage in mg/kg of body weight in a volume of 0.2 cc. Control mice given inoculations of L 1210 received injections of the diluting fluid alone or were not given injections. Twenty additional mice not inoculated with leukemia L 1210 were used to determine the maximum tolerated dose of W-50-197 for normal DBA2 mice.

RESULTS

Dose response graphs of W-50-197 (1.25 mg/kg to 20 mg/kg) and of A-methopterin (0.75 mg/kg to 6 mg/kg) appear in Chart 1. One hundred forty-five mice were used in the study on W-50-197 and 122 mice in the study on A-methopterin, with 66 animals used as leukemic controls for both drugs. When W-50-197 was given at a level of 5 mg/kg or less, it was ineffective in increasing the survival time, while doses of 10 mg/kg and 20 mg/kg significantly increased the survival time. A-methopterin was ineffective on this regimen at a level of less than 3 mg/kg but was effective at 3 mg/kg or 6 mg/kg. The higher dose, however, was only slightly more effective in increasing the survival.

The results of combination therapy with the two drugs at effective doses are shown in Table 1. In three series utilizing 99 mice, the combination of A-methopterin (3 mg/kg) and W-50-197 (10 mg/kg) increased the survival time significantly over that of either drug given alone (P less than 0.01).

It was noted, however, that the leukemic animals receiving W-50-197 alone at 10 mg/kg for three doses lost weight. Also, normal animals did not survive the administration of six doses of W-50-197 at this level given every other day. However, normal animals did tolerate without weight loss twelve doses of W-50-197 at 5 mg/kg

1 A stock solution of W-50-197 was prepared as follows, as suggested by Dr. George Hitchings of the Wellcome Research Laboratories: 200 mg were added to 20 cc. distilled water containing 0.1 cc. of glacial acetic acid. The mixture was warmed, cooled, and diluted to 25 cc.
given every other day, and subsequent experiments utilized only the lower dosages of W-50-197 (0.625 to 5 mg/kg).

Further exploration of combination therapy with the two drugs was undertaken in five series of experiments with 214 mice; the drug was given every other day beginning 3 days after transplantation of leukemia L 1210.

**TABLE 1**

**Combination Therapy of A-Methopterin and W-50-197 When Drugs Are Given at Individually Effective Doses (Three Series)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>No. mice</th>
<th>Mean survival (days)</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td>8.4</td>
<td>0.38</td>
</tr>
<tr>
<td>A-methopterin</td>
<td>3</td>
<td>22</td>
<td>12.3</td>
<td>0.49</td>
</tr>
<tr>
<td>W-50-197</td>
<td>10</td>
<td>55</td>
<td>11.7</td>
<td>0.47</td>
</tr>
<tr>
<td>A-methopterin</td>
<td>5</td>
<td>20</td>
<td>14.7</td>
<td>0.61</td>
</tr>
<tr>
<td>W-50-197</td>
<td>10</td>
<td>55</td>
<td>14.7</td>
<td>0.61</td>
</tr>
</tbody>
</table>

every other day beginning 3 days after transplantation. When A-methopterin (3 mg/kg) was given with graded doses of W-50-197 (0.625–5 mg/kg), the most effective dose of W-50-197 was 2.5 mg/kg. When W-50-197 (2.5 mg/kg) was then given with graded doses of A-methopterin (0.75–3 mg/kg), the most effective dose of A-methopterin was 1.5 mg/kg. From the survival data in the dose response graphs (Chart 1) it can be seen that W-50-197 at 2.5 mg/kg and A-methopterin at 1.5 mg/kg, given singly, are ineffective against leukemia L 1210. However, when the drugs were given in combination at these levels, they exhibited synergism and potentiated the antileukemic action of each other as seen in Table 2. In three series with 105 mice, the combination of A-methopterin at 1.5 mg/kg and W-50-197 at 2.5 mg/kg for six doses increased the duration of survival of leukemic mice from a mean of 8.3 days to a mean of 15.9 days (P less than 0.01).

Although W-50-197 was effective in increasing the survival time of mice only at high doses (10–20 mg/kg) when given singly, it was effective in inhibiting local tumor growth even at lower doses. In Table 3 are shown the caliper measurements of the tumor masses in the right axilla 6 days after transplantation and 1 day after the second drug dose was administered. When W-50-197 was given in combination with A-methopterin, retardation of local tumor growth was considerably greater than had been found after therapy with either single drug. However, when a tumor brei, prepared from the spleens of animals with tumors too small to measure, was transplanted into normal mice, the usual manifestations of the growth of leukemia L 1210 appeared within the usual time.

**DISCUSSION**

Evidence has been presented that A-methopterin and W-50-197 are more effective as inhibitors of leukemia L 1210 when given in combination.
than when given singly. At higher doses the effectiveness of the combination is less than additive, while at lower doses the combination is more than additive. The synergism at the lower doses is particularly remarkable because it occurs at dose levels at which either drug given alone is ineffective in increasing survival time.

Results of related studies with the diaminopyrimidines, of which W-50-197 is one example, are perplexing and do not adequately delineate the real nature of the mode of action of these drugs. (a) In our own experience and in that of others (1, 2), the monochlorophenyl and dichlorophenyl derivatives are both antimalarial drugs and microbial growth inhibitors, but only the dichlorophenyl compounds are tumor inhibitors. (b) While the toxic effects of both the monochlorophenyl and the dichlorophenyl compounds, when used as tumor inhibitors, are said to be reversed by folic acid, in much the same way as the action of the folic acid analogs is reversed by folic acid (1), the antimalarial activity of a monochlorophenyl derivative was not reversed by folic acid but was reversed by folic acid (4). (c) Folic acid antagonists such as A-methopterin inhibit microbial or tumor growth but are not antimalarials. (d) Toxicity studies on W-50-63, a monochlorophenyl derivative, indicate that the rat, a species as sensitive as the mouse to A-methopterin, is relatively insensitive to W-50-63, while the monkey is very sensitive (16, 17). (e) Studies at the National Cancer Institute on the guinea pig, a species relatively insensitive to A-methopterin (9, 19), indicate that this species is also very sensitive to W-50-197. Such evidence would suggest that the interpretation of the mode of action of these drugs must take into consideration the physiologic response of the species in which they are tested. If A-methopterin and W-50-197 act along the same metabolic pathway as suggested (1, 8), the synergistic action of the two drugs at otherwise subinhibitory single doses supports the viewpoint that the drugs probably act at different points.

It should be emphasized that the injection

1 Guinea pigs did not survive when injected with W-50-197 (10 mg/kg) for three doses given every other day. Death was preceded by leukopenia. At autopsy there was a decrease in the size of the spleen, enlargement of the adrenal glands, severe depletion of the marrow, and, in two of the six animals examined at this dose level, there were nonpenetrating gastric erosions. All six animals contained kidneys in which there was a peculiar lesion of the epithelium of the calyces and anterior portion of the ureters. This lesion can best be described as a pseudodyskeratosis of the transitional epithelium and probably is similar to the lesion recently described by Schmidt et al. (17) in the kidneys of monkeys treated with a related drug, W-50-63 (Daraprim). A report on the effect of diaminopyrimidines in guinea pigs is in preparation (E. M. Nadel).

Effective chemotherapy of leukemia has been hampered by the onset of drug resistance (10) which is developed by the host or by the tumor or by both (15). More effective chemotherapy logically should reside in the combined use of drugs (18). Synergism of 6-mercaptopurine4 and that of 8-aza-2'-deoxyguanosine (10) with A-methopterin are examples of combinations following which more effective chemotherapy has been achieved from the use of drugs with different modes of action. Further benefits of combined therapy can be expected in the utilization of A-methopterin in combination with one or more antimetabolites differing from it in mode of action.

SUMMARY

A-methopterin, a folic acid analog, and 2,4-diamino-5-((S',4'-dichlorophenyl)-6-methylpyrimidine (W-50-197), an antimalarial drug, were given alone and in combination to test the effect on tumor size and survival of mice with the transmissible leukemia L 1210. Survival data on 751 mice were based on a drug regimen which began 72 hours after subcutaneous transplantation of the tumor with treatment every other day. W-50-197 at 10 mg/kg increased survival time and retarded the local tumor growth, but was toxic. At doses of 5 mg/kg or less there was no increase in survival time, and the local tumor growth was retarded by doses as low as 2.5 mg/kg. A-methopterin at 3 mg/kg increased the survival time and retarded local tumor growth. At 1.5 mg/kg A-methopterin neither increased the survival nor retarded local tumor growth. Combined therapy was more effective than either drug given singly. At high doses the combined effect of A-methopterin and W-50-197 was less than additive, while at low doses the two drugs, given in combination, were synergistic and potentiated each other's antileukemic action more than additively. Thus, combined therapy with A-methopterin at 1.5 mg/kg and W-50-197 at 2.5 mg/kg, doses which were ineffective when given alone, was associated with a significant retardation in local tumor growth and an increase in survival time of 92 per cent.

4 Dr. H. E. Skipper (personal communication).
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4. FALCO, E. A.; RUSSELL, P. B.; VANDERWERFF, H.; and HITCHINGS, G. H. Antagonism of a 2,4-Diaminopyrimidine and Citrovorum Factor in the Rat, p. 34C. Abstr., 121st meeting of Am. Chem. Soc., 1952.


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