The Nucleic Acid Inhibiting Action of 4-Amino-N¹⁰-Methylpteroylglutamic Acid in Mice with a Sensitive and Resistant Strain of Leukemia*

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It is well established that folic acid antagonists provide temporary palliation in certain cases of acute leukemia in children (7) and that several compounds of this class increase the survival time of mice with transplanted leukemia Ak 4 (4, 10, 11). However, eventual failure in the treatment of leukemia by chemotherapy with such compounds occurs consistently.

The mechanism involved in the development of drug resistance in leukemia is at present poorly understood. The lack of fundamental knowledge on this point makes it difficult to utilize the known anti-leukemic agents to the fullest advantage.

It has been recently demonstrated that a hitherto sensitive strain of leukemia may be rendered resistant to 4-amino-N¹⁰-methylpteroylglutamic acid (A-methopterin) by repeated passage through treated mice (5). This strain shows a cross resistance to five other 4-amino antagonists of pteroylglutamic acid (9). These observations may parallel the ultimate failure of folic acid antagonists in the treatment of patients with acute leukemia.

Law and Boyle (9) have reported the development of resistance in three separate sublines of a transplantable lymphoid leukemia in dba mice following successive transplants in mice treated with three different folic acid antagonists. These refractory strains carried over resistance from one antagonist to another. One of us has demonstrated that a strain of mice made resistant to A-methopterin retained its sensitivity to the anti-leukemic effects of a crude antagonist of pteroylglutamic acid (3) and 2,6-diaminopurine (6).

Recent findings concerning the effect of folic acid antagonists on nucleic acid synthesis suggest that folic acid or some metabolite of this compound may be acting as the prosthetic group of an enzyme necessary for purine synthesis (6, 18). Certainly, A-methopterin or 4-aminopteroylglutamic acid (aminopterin) exerts a profound inhibitory effect on nucleic acid synthesis in vivo (18).

The following experiments were conducted with the thought in mind that the failure of the resistant strain of leukemia to respond to A-methopterin might be due to a failure of this compound to inhibit sufficiently nucleic acid synthesis in the refractory leukemic cells.

EXPERIMENTAL

The 4-amino-N¹⁰-methylpteroylglutamic acid resistant subline of mouse leukemia (Ak 4-R) used in these studies has already been described (5). This drug-fast strain showed no significant response to treatment with A-methopterin, while the original strain (Ak 4) responds to the extent that one observes a consistent increase in life span, in treated compared to untreated mice, of greater than 100 per cent.

The method used to determine the rate of synthesis of nucleic acids and nucleic acid purines has also been reported (18). This technic consisted of injection of carbon 14-labeled sodium formate (a rather specific precursor of the 2- and 8-carbon atoms of the nucleic acid purine skeleton [11]) and isolation of the viscera nucleic acids and nucleic acid purines after a period of 6 hours. Activity assays on these fractions provide data which can be used to estimate the rate of biosynthesis of nucleic acid purines and, in turn, the larger nucleic acid polymers. As has been mentioned, strong folic acid antagonists profoundly affect incorporation of formate carbon into these important cell fractions (18). We have also observed that x-radiation, 2,6-diaminopurine, cortisone,
and a combination of urethan plus nitrogen mustard significantly affect purine synthesis under conditions where over-all formate fixation into tissue is not reduced (14).

To compare the action of a given dose of A-methopterin on nucleic acid purine synthesis in the nonresistant (Ak 4) and the resistant (Ak 4-R) leukemic strains, experiments were carried out as follows: Groups of Akm mice were inoculated with Ak 4 and Ak 4-R leukemia, and, after the leukemia was well advanced (see blood counts, Table 1), the mice were in certain instances treated on the sixth and seventh, or sixth, seventh, and eighth days with A-methopterin. The level of injection was in all cases 3.0 mg/kg. On the seventh or eighth days after inoculation, the various groups were injected with 1.4 μc. of HC14OONa per mouse, and after 6 hours in a metabolism chamber the mice were sacrificed and the livers and spleens extirpated, pooled, and homogenized in a refrigerated Waring Blender. Aliquots of this liver and spleen homogenate were then subjected to isolation procedures (14) which provided small quantities of combined nucleic acids and combined nucleic acid purines. These fractions were assayed for carbon 14 content by a gas phase procedure (19). The data obtained are summarized in Table 1.

### TABLE 1

<table>
<thead>
<tr>
<th>EXP. NO.</th>
<th>STRAINS OF LEUKEMIA</th>
<th>NO. OF MICE</th>
<th>DAYS OF TREATMENT</th>
<th>DAY OF HC14OONOA INJECTION</th>
<th>SPECIFIC ACTIVITY (μc/mole carbon)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WBC</td>
<td>Combined nucleic acids</td>
</tr>
<tr>
<td>1</td>
<td>Ak 4</td>
<td>7</td>
<td>none</td>
<td>7</td>
<td>42,500</td>
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<tr>
<td>2</td>
<td>Ak 4-R</td>
<td>10</td>
<td>none</td>
<td>7</td>
<td>38,500</td>
</tr>
<tr>
<td>3</td>
<td>Ak 4</td>
<td>10</td>
<td>6,7</td>
<td>7</td>
<td>7,250</td>
</tr>
<tr>
<td>4</td>
<td>Ak 4-R</td>
<td>10</td>
<td>6,7</td>
<td>7</td>
<td>21,500</td>
</tr>
<tr>
<td>5</td>
<td>Ak 4</td>
<td>10</td>
<td>6,7,8</td>
<td>8</td>
<td>8,800</td>
</tr>
<tr>
<td>6</td>
<td>Ak 4-R</td>
<td>9</td>
<td>6,7,8</td>
<td>8</td>
<td>30,000</td>
</tr>
</tbody>
</table>

**Note:** All experiments were of 6 hours' duration after intraperitoneal injection of 1.4 μc. of sodium formate.

A) The refractory strain of leukemia has a limited ability to detoxify A-methopterin. Since the anti-leukemic activity of this drug is based on preferential toxicity to the leukemic cell, a slight loss in this specificity would nullify the temporary effectiveness of this compound.

B) The Ak 4-R strain employs a different scheme for introduction of the single ureide carbon atoms into nucleic acid purines which is not so effectively blocked by A-methopterin. This possibility seems less likely, since it appears from the present data that the difference in formate fixation in the sensitive and resistant strains is but a relative one.

c) The resistant leukemia has acquired the ability to synthesize folic acid (or the citrovorum factor) and thus can reverse the anti-leukemic action of A-methopterin. A failure of A-methopterin plus sulfonamides (PABA antagonists) to increase the life span of mice with the resistant leukemia suggests that this mechanism is unlikely. Also, microbiological assays of folic acid and the citrovorum factor have failed to show any significant difference between Ak 4 and Ak 4-R spleens or or livers.

All the liver and spleen cells from which nucleic acids were isolated in this study were not, of course, leukemic. From previous studies, it can be estimated very roughly that at 8 days after inoculation of Ak 4 leukemia into Akm mice the liver almost doubles and the spleen more than triples in weight. This suggests that more than half the cells of the organs used in this study were made up of the respective leukemic strains. Had we been dealing...
ing with wholly neoplastic tissue, it seems likely that greater differences might have been observed.

Data presented in Table 1 on formate fixation in the nucleic acid purines from livers and spleens of leukemic mice, when compared to previously reported results obtained on viscera from nonleukemic animals (14), suggests that this disease is significantly accelerating nucleic acid synthesis.

Further studies on the effects of A-methopterin on formate fixation in deoxyribonucleic acid guanine, adenine, and thymine and ribonucleic acid guanine and adenine in the Ak 4 and Ak 4-R leukemias are planned.

SUMMARY

Nucleic acid synthesis in the livers and spleens of mice with a refractory strain of leukemia was not inhibited to the same extent by A-methopterin as was observed in mice with a leukemic strain which responds to treatment with this compound.

REFERENCES

The Nucleic Acid Inhibiting Action of 4-Amino-N\textsuperscript{10}-Methylpteroylglutamic Acid in Mice with a Sensitive and Resistant Strain of Leukemia

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