Effects of A-Methopterin on the Purine Biosynthesis of Susceptible and Resistant TA3 Ascites Cells*

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Biochemical studies have shown that ascites tumor cells possess the ability to utilize two alternate pathways for the synthesis of nucleic acid purines: one in which the purine structure is built up from small precursors and the other which involves the utilization of preformed purines presumably obtained from the diet or from other cells (7). Resistance to azaserine, an agent which interferes with the de novo synthesis of purines, has been found to involve a shift in the synthesis of nucleic acid purines from one pathway to the other (9). To determine whether resistance to other agents may occur in a similar manner, a study was initiated on the mechanisms of resistance to A-methopterin, another compound which inhibits purine synthesis de novo. This agent is used extensively in the treatment of acute leukemia in human beings. A major limitation to this therapy is that patients whose disease is originally sensitive to this agent ultimately relapse despite continuance of therapy. This relapse is presumably due to the development of drug resistance (2). Folic acid antagonists have been shown to prevent the conversion of folic acid to citrovorum factor (5, 8). This coenzyme is required for the de novo biosynthesis of purines (5). Experimental neoplasms exhibiting resistance to the antifolic acids have been described (1, 4, 5). One of these was used by Skipper et al. (10) to study the effects of A-methopterin on the incorporation of formate-C14 into nucleic acid purines of two sublines of the L1210 leukemia, one of which is susceptible to inhibition by this compound. The other, on repeated transplantation, has become dependent on A-methopterin for optimum growth. A-methopterin caused a significant increase in formate incorporation into the nucleic acid purines of the dependent cells while inhibiting formate utilization by sensitive leukemic cells.

In the present study, the two alternate metabolic pathways of nucleic acid purine synthesis were investigated in A-methopterin-treated TA3 ascites carcinoma cells which are susceptible and resistant to this antagonist. Both cell lines were capable of purine synthesis de novo and preformed purine utilization. Purine formation de novo, as measured by the uptake of labeled glycine into both nucleic acid and acid-soluble purines, was inhibited in the sensitive population for at least 6 hours. No inhibition of glycine-C14 incorporation occurred in the resistant tumor. Inhibition of the formation of purines de novo in sensitive ascites cells is compensated for in part by an increased utilization of preformed adenine.

MATERIALS AND METHODS

Experiments were conducted with the TA3 ascites carcinoma in CAF1 male mice 8–11 weeks of age. The selection of a resistant population was achieved by consecutive passages, with inocula of 107 ascites cells per mouse, in animals given injections intraperitoneally of one dose of 1.0 mg of A-methopterin/kg administered 24 hours after tumor implantation. After suitable time was allowed for the cell population to accumulate, tumor cells from one such mouse were used for transplantation. Maximum growth of A-methopterin-treated ascites tumor cells appeared to take place at transfer generation 53. From this time the resistant tumor was maintained on the dose of 1.0 mg of A-methopterin/kg injected once daily 24 hours after tumor transplantation.

Tumors were used for biochemical studies 6 days after the inoculation of 107 ascites cells per mouse. Glycine-2-C14 and adenine-8-C14 were injected intraperitoneally in 0.25–0.50 ml. of saline.1 1CAF1 mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

1 Glycine-2-C14 was obtained from Tracerlab, Inc., and adenine-8-C14 from California Research Corporation, both on allocation by the United States Atomic Energy Commission.

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A-methopterin was administered by intraperitoneal injection in 0.20–0.50 ml. of a 2 per cent solution of sodium bicarbonate. At the prescribed time, the ascites cells were withdrawn under light ether anesthesia with a #18 needle and a hypodermic syringe. The cells were isolated by centrifugation, the acid-soluble fraction was extracted with 0.2 M perchloric acid (PCA), and the nucleic acids were hydrolyzed and extracted by being heated with 0.4 M perchloric acid (PCA) in a boiling water bath for 30 minutes. The acid-soluble fraction was hydrolyzed by being heated for 45 minutes in an autoclave at 120° C. The purines in each fraction were isolated for the determination of specific radioactivity by chromatography on Dowex-50, then on paper, followed by direct counting on paper disks. The details of this procedure have been described earlier (6).

In survival experiments, mice weighing 18–25 gm. were distributed into groups of equal weight, and each mouse was given inoculations intraperitoneally of 10^7 ascites cells. Therapy was initiated 24 hr. later and continued for 6 consecutive days. Mice were maintained during the experiment on Purina Laboratory Chow pellets and water ad libitum. The average survival time of groups of animals was used as a measure of tumor inhibition. Saline-treated controls were routinely included in all survival experiments.

RESULTS AND DISCUSSION

An A-methopterin-resistant subline of the TA3 ascites carcinoma was developed by treating CAFj mice bearing TA3 ascites cells with A-methopterin. Table 1 gives the results of experiments, performed at intervals, which indicate the degree of resistance to A-methopterin. The survival time of groups of tumor-bearing animals treated with A-methopterin was used as a test for the presence of a resistant population. Biochemical studies were initiated at the 60th transfer generation, and the survival data are presented from this time on. The A-methopterin-resistant subline was found to be completely resistant to a dose of 0.25 mg/kg once daily for 6 days starting 24 hours after tumor implantation. Controls were concurrent saline-treated tumor-bearing animals.

### Table 1

<table>
<thead>
<tr>
<th>Transfer generation</th>
<th>Control</th>
<th>A-methopterin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>14.4±2.6* (22)†</td>
<td>24.0±4.1 (23)</td>
</tr>
<tr>
<td>60</td>
<td>15.0±4.5 (5)</td>
<td>12.2±6.6 (5)</td>
</tr>
<tr>
<td>69</td>
<td>11.0±2.7 (7)</td>
<td>6.6±0.8 (7)</td>
</tr>
<tr>
<td>79</td>
<td>7.8±1.3 (8)</td>
<td>8.9±1.8 (8)</td>
</tr>
<tr>
<td>88</td>
<td>14.8±5.8 (5)</td>
<td>9.8±3.8 (5)</td>
</tr>
</tbody>
</table>

* Average deviation from mean.
† Figures in parentheses indicate numbers of mice per group.

### Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg/day)</th>
<th>No. of daily injections</th>
<th>Av. survival time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-Thioguanine</td>
<td>1.0</td>
<td>2</td>
<td>15.5±2.2* (15)†</td>
</tr>
<tr>
<td>Azaserine</td>
<td>0.4</td>
<td>2</td>
<td>21.1±3.7 (15)</td>
</tr>
<tr>
<td>2,4-Diamino-5-(3',4'-dichlorophenyl)-6-ethylpyrimidine</td>
<td>5.0</td>
<td>1</td>
<td>15.0±1.6 (10)</td>
</tr>
<tr>
<td>5-Fluouracil</td>
<td>15.0</td>
<td>1</td>
<td>35.1+10.5§ (15)</td>
</tr>
</tbody>
</table>

* Average deviation from mean.
† Figures in parentheses indicate numbers of mice per group.
§ Four mice survived over 50 days; calculated as 50-day survivors.

We wish to acknowledge the assistance of Mrs. Dorothy McManus and Mrs. Myrtle Gilboe in measurements of radioactivity.

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methopterin-resistant tumor to thioguanine. On the other hand, the inhibitor of de novo purine synthesis, azaserine, produced a greater prolongation of life in mice bearing the resistant ascites cells. 2,4-Diamino-5-(3',4'-dichlorophenyl)6-ethyl-

Chart 1.—Effect of A-methopterin on the incorporation of glycine-2-C\textsuperscript{14} into nucleic acid and acid-soluble purines of A-methopterin-sensitive and -resistant TA3 ascites cells. Tumor-bearing mice were given injections of a single intraperitoneal dose of 0.5 mg of A-methopterin/kg. At the times indicated on the chart, 100 \( \mu \)g of glycine-2-C\textsuperscript{14} (25 \( \times \) 10\textsuperscript{4} counts/min/mg) was injected, and metabolic utilization for 1 hour was permitted. Results at S-time were obtained with tumor-bearing animals receiving simultaneous injections of A-methopterin and glycine-C\textsuperscript{14}; those at zero time were obtained with control tumor-bearing mice receiving labeled glycine only. Each bar represents the average of results obtained from the separate analyses of three to six mice; “AS” signifies “acid-soluble,” and “NA” refers to “nucleic acid.”

Pyrimidine did not alter the growth rate of the A-methopterin-susceptible population but appeared to increase the growth rate of resistant tumor cells. 5-Fluorouracil was equally effective in prolonging the survival time of mice bearing either cell line.

Coenzymes related to tetrahydrofolic acid are required for the maintenance of effective de novo purine synthesis (8); for this reason the effect of A-methopterin on this pathway in cells of the A-methopterin-resistant and -sensitive tumors was investigated. The incorporation of glycine-2-C\textsuperscript{14} into nucleic acid and acid-soluble purines was used as a measure of the capacity of the ascites cells to form purines de novo. A marked inhibition of the incorporation of labeled glycine into purines of sensitive cells was found; the results are illustrated in Chart 1. More than 80 per cent inhibition of the sensitive population occurred when the drug was administered simultaneously with the glycine-C\textsuperscript{14}. Ninety per cent or greater inhibition of this subline was found when de novo synthesis was measured 1 hour after the injection of the folic acid antagonist. Inhibition of this pathway in the susceptible line was sustained for at least 6 hours. In contrast, A-methopterin did not affect the de novo biosynthesis of purines in the resistant subline. Stimulation of this pathway was observed when the glycine-C\textsuperscript{14} was injected 2-3 hours after administration of the antifolic agent.

A concurrent measurement of the utilization of adenine-8-C\textsuperscript{14} by these two cell lines was made. A-methopterin did not inhibit adenine utilization by either tumor subline. A stimulation of the uptake of preformed adenine into cells of the susceptible population took place when the adenine-C\textsuperscript{14} was administered 2\( \frac{1}{2} \) hours after the injection of the
This is apparently owing to a compensatory mechanism, in which the cells might be regarded as attempting to overcome the inhibition of nucleic acid biosynthesis de novo by an increased utilization of preformed purines. In the resistant tumor population, a decreased incorporation of adenine-8-C14 was found when the labeled precursor was administered simultaneously or 1 hour after the injection of the antitumor. This decrease is correlated with the observed increase in de novo synthesis in this tumor line. Measurements of adenine-8-C14 utilization of the A-methopterin-sensitive and -resistant tumor cell lines are presented in Chart 2.

These results indicate that resistance to A-methopterin in the TA3 ascites cell tumor employed in these experiments is associated in part with the inability of the antagonist to prevent the de novo biosynthesis of nucleic acid and acid-soluble purines.

SUMMARY

Resistance to A-methopterin in a TA3 ascites cell carcinoma was shown to be stable and inheritable. A decrease in sensitivity to the guanine antagonist, thiouguanine, and an increase in sensitivity to the inhibitor of purine synthesis de novo, azaserine, appeared to be associated with the development of resistance to A-methopterin in this tumor. Sensitivity to 5-fluorouracil was retained. Biochemical studies indicated that both A-methopterin-resistant and -sensitive cells were capable of de novo synthesis and of utilization of preformed purines. Inhibition of the de novo pathway, as measured by glycine-2-C14 incorporation into nucleic acid and acid-soluble purines, occurred in cells of the sensitive subline for at least 6 hours. No inhibition of glycine utilization took place in the resistant tumor.

Metabolic shifts in the alternate pathways to purine biosynthesis appeared to take place during A-methopterin treatment in both tumor lines.

REFERENCES

Effects of A-Methopterin on the Purine Biosynthesis of Susceptible and Resistant TA3 Ascites Cells

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