Effects of A-Methopterin on Formate Incorporation into the Nucleic Acids of Susceptible and Resistant Leukemic Cells

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The lack of cytotoxic specificity of the known anticancer agents and the development of drug resistance by originally inhibited neoplastic tissues have been causes of repeated disappointment in the field of cancer chemotherapy.

It seems likely that the usefulness of the presently known anticancer agents might be considerably increased if knowledge of the mechanisms of drug resistance in cancer could be obtained and successfully applied in its prevention. The development of sublines of mouse leukemia that are resistant to (4) or partially dependent on (4, 5, 7) antileukemic agents has provided a most useful tool for investigation of these mechanisms.

Drug resistance in micro-organisms has been known for many years and has been rather conclusively ascribed to chemical selection of existing drug-resistant mutants in a bacterial population. Evidence has been obtained which strongly suggests that the development of resistance in mouse leukemia is the result of a similar series of events (6).

It appears that the 4-aminopteroylglutamic acids prevent conversion of folic acid to citrovorum factor (8), a coenzyme essential to nucleic acid synthesis (1@). The fact that desoxyribonucleic acid (DNA) guanine and adenine was measured at 6 hours. It is well known that formate is a precursor of the 2- and 8-carbon atoms of the nucleic acid purines and of the methyl carbon atom of thymine (14).

Previous studies have shown that 4-aminopteroylglutamic acids profoundly inhibit incorporation of formate-C\(^{14}\) into nucleic acid purines of mouse viscera (12).

In order to compare the action of A-methopterin on nucleic acid synthesis in the susceptible leukemic strain and the dependent subline of L 1210 leukemia the following experiments were carried out: Groups of DBA mice received subcutaneous inoculations of susceptible or dependent leukemic cells and in certain instances were treated with A-methopterin and in others were left untreated. On the seventh day following leukemic inoculations, all mice were injected with 2.0 mc. each of formate-C\(^{14}\). After 6 hours the animals were sacrificed, and leukemic tumors and viscera of the various groups were pooled and homogenized in a refrigerated Waring Blender. Aliquots of the leukemic tumor homogenates and the viscera homogenates were then subjected to isolation procedures which provided small quantities of DNA and RNA (18). These nucleic acids were then hydrolyzed with perchloric acid, and the DNA guanine, adenine and thymine, and RNA guanine and adenine were isolated with ion-exchange columns (Dowex-S0). The actual amounts of the purines and thymine obtained were determined spectrophotometrically, and carrier was added in known amounts to facilitate isolation from ion-exchange column eluates. Corrections were then applied in calculations of specific activities based on the known dilution factors.

The details of the treatment of animals and the results ob-
The column "initial homogenate" refers to activity data on the whole tissue indicated.

DISCUSSION

Results presented in Table 1 show that formate incorporation into DNA guanine, adenine and thymine, and into RNA guanine and adenine of the sensitive L 1210 leukemia and viscera is profoundly inhibited by A-methopterin. This was not unexpected in view of previous observations (19). The most interesting result was that A-methopterin treatment caused an increase in formate incorporation by the A-methopterin-dependent leukemia and at the same time inhibited formate incorporation into the visceral nucleic acids of animals bearing these leukemic tumor masses. These data clearly indicate a different metabolic response of the dependent leukemia and the viscera to A-methopterin.

The questions whether nucleic acid metabolism is the primary site of the anti-leukemic action of A-methopterin and whether mutations which result in a failure of A-methopterin to inhibit formate utilization in resistant cells are largely responsible for A-methopterin resistance are still lacking direct evidence. In support of these interpretations are observations that (a) thymidine (a moiety of DNA) will prevent the toxicity of 4-aminopteroylglutamic acids in bacteria (1); (b) DNA will partially prevent the antileukemic action of A-methopterin (10), while serine, glycine, methionine, and formate are inactive in this respect; (c) A-methopterin causes a build-up of 4-amino-5-carboxamidomimidazole in E. coli (15). However, it should be pointed out that the inhibition of formate incorporation into the whole tissue homogenates caused by A-methopterin in these experiments cannot be accounted for on the basis of inhibition of nucleic acid synthesis, unless one assumes that inhibition of nucleic acid synthesis is followed by failure of the cells to incorporate formate into protein. Only about 10 per cent of the formate carbon in the initial homogenates can be accounted for in the nucleic acid bases.

One of the hypotheses which is sometimes offered to explain drug resistance is the existence of alternative pathways of making inhibited products. The present results show that formate is utilized by the A-methopterin-dependent strain of leukemia in de novo synthesis of nucleic acid purines and thymine. Also, it is clear that A-

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. OF MICE</th>
<th>TISSUE</th>
<th>TREATMENT</th>
<th>INITIAL HOMOGENATE</th>
<th>SPECIFIC ACTIVITIES (µC/MOLE C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DNA Guanine</td>
<td>Adenine</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>Leuk. L 1210-S</td>
<td>None</td>
<td>4.9  906</td>
<td>287</td>
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<tr>
<td></td>
<td></td>
<td>Viscera</td>
<td>None</td>
<td>6.9  171</td>
<td>280</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>Leuk. L 1210-S</td>
<td>A-meth.*</td>
<td>1.8  54</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viscera</td>
<td>A-meth.*</td>
<td>5.6  90</td>
<td>16</td>
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<tr>
<td>3</td>
<td>15</td>
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<td>None</td>
<td>1.8  55</td>
<td>51</td>
</tr>
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<td></td>
<td></td>
<td>Viscera</td>
<td>None</td>
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<td>99</td>
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<tr>
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<td>15</td>
<td>Leuk. L 1210-D</td>
<td>A-meth.*</td>
<td>4.8  127</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Viscera</td>
<td>A-meth.*</td>
<td>4.9  15</td>
<td>10</td>
</tr>
</tbody>
</table>

* A-methopterin (5 mg/kg) was administered intraperitoneally immediately before injection of formate-C14 on the 7th post-inoculation day.
† A-methopterin (5 mg/kg) was administered intraperitoneally on the 1st, 3rd, 5th, and 7th post-inoculation days. Formate-C14 was injected immediately after the last A-methopterin injection on the 7th day.

Note: 5 µc of formate-C14 was injected/80-gm mouse. All experiments were terminated at 6 hours after formate injection. Viscera include livers, spleens, small intestines, kidneys, and testes. Average leukemic tumor weights for groups 1, 2, 3, and 4 were: 670, 670, 700, and 800 mg., respectively.

Table 1

The Effect of A-Methopterin on Formate Incorporation into Nucleic Acid Moieties of Susceptible and Dependent Leukemic Cells

1 H. E. Skipper, M. Bell, and J. Chapman, unpublished data.
reactions involve inhibitions by normal metabolic products of the organism. These inhibitions are not unrelated in character to those obtained with synthetic analogs of metabolites” (9).

A second possible explanation of dependence is that A-methopterin inhibits the excessive production of a normal metabolite. If one assumed that CF was not the final formylating coenzyme but was intermediate en route to this coenzyme (CF'), then the following might be suggested as a means of fitting the present facts together: The dependent strain might be deficient in the enzyme necessary for conversion of CF → CF'. Thus, there would be a build-up of CF in the untreated dependent cell which might compete with CF', thus inhibiting growth. If A-methopterin inhibited the conversion of folic acid to CF in the dependent strain, this would depress the competing CF pool and thus promote growth of the dependent leukemic cells. Likewise, administration of CF would be expected to prevent the growth promotion of A-methopterin in the dependent leukemia.

The present data are compatible with the thesis that A-methopterin promotes activation of the enzyme systems involved in formate transfer or prevents some natural inhibition of these enzymes. Further work on these questions is in progress.

SUMMARY

It has been demonstrated that A-methopterin inhibits formate incorporation into nucleic acid purines and thymine in leukemic cells which are sensitive to this compound and also in viscera of mice bearing these susceptible leukemic cells. A-methopterin, however, causes a significant increase in formate incorporation into the nucleic acids of leukemic cells which have become dependent on A-methopterin for optimal growth while inhibiting formate incorporation in the visceral nucleic acids of mice bearing the dependent strain of leukemia.

Possible mechanisms involved are discussed.

REFERENCES

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