Studies on the Mechanism of Action of 5-Iododeoxyuridine, an Analog of Thymidine*

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SUMMARY

5-Iododeoxyuridine inhibited the utilization of radioactive orotic acid, formate, and thymidine for the biosynthesis of DNA-thymine, but not of orotic acid for the biosynthesis of DNA-cytosine or of RNA-pyrimidines, by mouse Ehrlich ascites tumor cells in vitro. The analog was a competitive antagonist of thymidine in this system; however, in view of the incorporation of the iodo-compound into DNA in place of thymidine, the site of inhibition probably occurs at either the monophosphate or triphosphate level.

5-Iododeoxyuridine has been prepared (11) by a modification of a previously published procedure (18), and reports of its biological activities have appeared (1-3, 8, 11, 12, 19). This analog inhibited competitively the utilization of radioactive thymidine for the biosynthesis of deoxyribonucleic acid (DNA)-thymine in mammalian (8, 11, 12) as well as microbial1 systems and, under these conditions, is utilized in lieu of thymidine for the formation of the DNA-polymer.

The present report describes studies of the mechanism of action of 5-iododeoxyuridine and measurements of its inhibitory effects on the utilization of radioactive formate, orotic acid, and thymidine for the biosynthesis of nucleic acid pyrimidines of mouse Ehrlich ascites carcinoma cells in vitro.

MATERIALS AND METHODS

Preparation of cell suspension.—The Ehrlich ascites tumor2 was maintained in DBA/2 mice purchased from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Five or 6 days following the intraperitoneal inoculation of mice with tumor cells, ascitic fluid was collected from a suitable number of mice and pooled in a graduated centrifuge tube (40 ml.) which contained 5 ml. of the modification of Chamber’s solution described by Totter (23); after centrifugation, the residual packed cells were resuspended in 3 volumes of this solution.

Preparation of solutions.—Sodium formate-C¹⁴, sodium orotate-4-C¹⁴, and thymidine-C¹⁴H₄ had specific activities of about 1 μc/μmole. Iododeoxyuridine was prepared by a method previously described (11).

Isolation of DNA bases.—After incubation of the cellular suspension at 37° C. for 3-4 hours, the contents of identical beakers (Table 1) were combined, and the reaction was stopped by the addition of 5 volumes of ethanol (95 per cent). The DNA was separated by the Schneider modification (22) of the Schmidt-Thannhauser procedure (21). After hydrolysis with perchloric acid, the bases were separated by paper chromatography with a system composed of isopropanol-HCl (2 N) (24). The individual bases were eluted with water and again subjected to paper chromatography in a butanol-water system (7). The concentrations of the individual bases were determined in the Beckman UV-spectrophotometer; their radioactivity was measured in a windowless flow counter following the plating of appropriate aliquots in the center of a stainless steel planchet.

RESULTS

The effect of 5-iododeoxyuridine on the utilization of various precursors of nucleic acid pyrimidines was investigated to establish the site of inhibition. The results in Table 1 indicate that there was little or no inhibition of the utilization of radioactive orotic acid for the biosynthesis of the
pyrimidines of ribonucleic acid (RNA) or of cytosine of DNA. However, there was a marked inhibition of the utilization of orotic acid for the biosynthesis of DNA-thymine. As could be anticipated, the utilization of formate for the formation of the methyl group of DNA-thymine was similarly depressed. The incorporation of thymidine into DNA-thymine was inhibited and, as the ratio of 5-iododeoxyuridine to thymidine was increased, the degree of inhibition increased. With a molar ratio of inhibitor to metabolite of 1:1, 5-iododeoxyuridine was more than 4 times as potent an inhibitor of the utilization of orotic acid than of thymidine for the biosynthesis of DNA.

**TABLE 1**

<table>
<thead>
<tr>
<th>metabolite</th>
<th>Amount of metabolite (μmol)</th>
<th>Ratio of 5-iododeoxyuridine to metabolite</th>
<th>per cent inhibition</th>
<th>RNA</th>
<th>DNA</th>
</tr>
</thead>
</table>
| Orotic acid-
4-C14   | 1.0                        | 5:1                                       | None               | 30  | 50  |
| Formate-C14 | 0.5                       | 1:1                                       | 20                 | 35  | 70  |
| Thymidine-
C14H3 | 0.01                       | 1:5                                       | 0.5 μmoles         | 95  | 95  |

*The reaction mixtures consisted of packed cells (0.25 ml.), pig serum (0.1 ml.), iododeoxyuridine and the indicated radioactive metabolite in the amounts listed in the table, and Trotter's modified Chamber's solution (27) to 2.5 ml. The incubations were conducted in duplicate in 20-ml. beakers in a Dubnoff metabolic shaker at 37° C. (air); agitation was at 90 cycles per minute for 4 hours.

logical assumption is made that 5-iododeoxyuridine or one of its phosphorylated derivatives profoundly inhibits the utilization of a common metabolite derived from orotic acid or thymidine (e.g., thymidylic acid or a more highly phosphorylated derivative), then the observed difference is not as much a reflection of the inhibition potential as it is of the differences in the rates of conversion of the precursors to the common metabolite, as well as of the amounts of dilution encountered.

The results shown in Table 2 indicate that the inhibition by 5-iododeoxyuridine of the utilization of thymidine for the biosynthesis of DNA-thymine is competitive. Thus, when the concentration of 5-iododeoxyuridine was increased, the degree of inhibition increased; also, when the amount of 5-iododeoxyuridine in the reaction mixture was held constant, the inhibition could be abolished by an appropriate increase in the concentration of thymidine. The specific activity of the isolated DNA-thymine was essentially unaltered when the ratio of 5-iododeoxyuridine to thymidine was 50:1, although the amount of 5-iododeoxyuridine in the incubation vessels varied from 0.01 to 0.10 μmol.

**TABLE 2**

| Concentration of thymidine-
C14H3 (μmol) | Specific activity of isolated DNA-thymine in presence of varying concentrations of 5-iododeoxyuridine† |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>2,200</td>
</tr>
<tr>
<td>0.10</td>
<td>4,400</td>
</tr>
<tr>
<td>5.0 μmol</td>
<td>2,007</td>
</tr>
<tr>
<td>5.0 μmol</td>
<td>650</td>
</tr>
</tbody>
</table>

*Details of the incubation conditions are described in Table 1.

† Specific activity of isolated DNA-thymine in counts/min/μmol.

Approximately the same amount of 5-iododeoxyuridine was required to reduce the specific activity of DNA thymine by 50 per cent, whether the radioactive precursor possessed a preformed methyl group, i.e., thymidine, or the metabolite, i.e., formate, was utilized for the formation of the methyl group (Chart 1). Hence, it would appear that 5-iododeoxyuridine exerts its inhibitory effect, presumably after anabolic alteration, on the utilization of the methylated precursor of DNA.
thymine, rather than on its formation. The “50 per cent molar inhibition index” for the utilization of thymidine was approximately 10. This is in marked contrast to the “index” of 1,000 previously observed with 6-azathymidine, another analog of thymidine (4, 18). Possible reasons for this striking difference between the two analogs of thymidine have been discussed (13).

Comparison with 5-bromodeoxyuridine.—5-Bromodeoxyuridine also has been shown (6) to inhibit the utilization but not the synthesis of thymidine. Accordingly, a comparison was made of the inhibitory effects of these two analogs on the utilization of radioactive thymidine for the biosynthesis of DNA-thymine (Table 3). 5-Bromodeoxyuridine exerted approximately a five-fold greater inhibitory effect than 5-iododeoxyuridine. However, a comparison of the antitumor activities of the two halogenated compounds in mice indicated that, under conditions in which marked inhibitory activity was demonstrated with 5-iododeoxyuridine, the brominated derivative was inactive (19).

Effect of potassium iodide.—To eliminate the possibility that the inhibitory effect of 5-iododeoxyuridine was a result of the release of iodide, which was a factor of importance in the study of the biological activities of 5-iodoorotic acid (14, 18), an excess of up to 100 molar equivalents of potassium iodide was included in the reaction mixture, in addition to radioactive thymidine. This excess of iodide exerted no inhibitory effect on the utilization of radioactive thymidine for the biosynthesis of DNA-thymine.

Effect of some halogenated pyrimidine derivatives on DNA-biosynthesis.—5-Iodouridine and 5-bromouridine exerted no effect on the utilization of radioactive formate for the biosynthesis of DNA-thymine by Ehrlich ascites cells in vitro (Table 4). In contrast, 5-iodoorotic acid had a stimulatory effect similar in magnitude to that observed previously with orotic acid, uridine, and deoxyuridine (16, 20). In earlier studies, the growth of a strain of Lactobacillus bulgaricus 09 which requires orotic acid as a growth factor was shown to be supported by 5-iodoorotic acid; however, this result is attributable to the dehalogenation of the iodo-compound with the formation of orotic acid (14). It is suggested that the Ehrlich ascites cells also dehalogenate 5-iodoorotic acid with the formation of orotic acid.

**TABLE 3**

COMPARISON OF THE EFFECTS OF 5-BROMODEOXYURIDINE AND 5-IODODEOXYURIDINE ON THE UTILIZATION OF RADIOACTIVE THYMIDINE FOR THE BIOSYNTHESIS OF DNA-THYMINE OF MOUSE EHRLICH ASCITES TUMOR CELLS IN VITRO*

<table>
<thead>
<tr>
<th>Concentration of Analog (µmoles/ml)</th>
<th>Specific activity of isolated DNA-thymine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iododeoxyuridine (counts/min/µmole)</td>
</tr>
<tr>
<td>None</td>
<td>6,200</td>
</tr>
<tr>
<td>0.08</td>
<td>6,200</td>
</tr>
<tr>
<td>0.40</td>
<td>4,100</td>
</tr>
<tr>
<td>2.00</td>
<td>570</td>
</tr>
</tbody>
</table>

* Details of the incubation conditions are described in Table 1. Thymidine-C<sup>14</sup>H<sub>3</sub>O: 0.02 µmoles/ml; specific activity: 1.5 µc/µmole.

**TABLE 4**

EFFECT OF 5-IODOOROTIC ACID, 5-IODOURIDINE, AND 5-BROMOURIDINE ON THE BIOSYNTHESIS OF DNA THYMINE OF MOUSE EHRLICH ASCITES TUMOR IN VITRO*

<table>
<thead>
<tr>
<th>Analog (10 µmoles)</th>
<th>Relative specific activity of DNA-thymine†</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>1.00</td>
</tr>
<tr>
<td>Idoorotic acid</td>
<td>2.94</td>
</tr>
<tr>
<td>Idoouridine</td>
<td>0.87</td>
</tr>
<tr>
<td>Bromouridine</td>
<td>1.04</td>
</tr>
</tbody>
</table>

* Details of incubation mixtures are given in Table 1.
† Specific activity of DNA-thymine in the control vessel equated to 1.00.

**DISCUSSION**

Halogenation of uracil or its derivatives in the 5-position of the pyrimidine ring has resulted in the formation of compounds which possess biological interest as well as potential chemotherapeutic utility. One would normally regard the size of the halogen substituent as the determinant of the primary function of the halogenated uracil derivative as an antagonist either of uracil or of thymine. Whereas iodouracil, bromouracil, and chlorouracil have been shown to be incorporated into DNA, chlorouracil as well as fluorouracil may be utilized for RNA biosynthesis (9). On the basis of van der Waal’s radii alone, the chloro derivative should be designated as an analog of thymine; however, other factors must be of importance as well, in view of its behavior with respect to RNA. Of pertinence also is the excretion of a relatively large
amount of uracil in the urine of the mouse upon the administration of 6-azathymine, a thymine analog (17), and the subsequent demonstration of the inhibition by 6-azathymine, but not by 6-azauracil, of the cell-free system which catabolizes uracil (15). In this connection, it should be emphasized that the primary antimetabolic activity of azauracil, of the cell-free system which catabolizes the inhibition by 6-azathymine, but not by 6-
mation of thymidine could be obtained from ex
selective inhibitor of the enzymic decarboxylation of orotcholine-5'-phosphate to form uridine-5'-phosphate (5, 10).

Although iododeoxyuridine inhibited the utilization of formate for the biosynthesis of DNA-thymine, no evidence for an inhibition of the formation of thymidine could be obtained from examinations of the acid-soluble fraction of inhibited Ehrlich ascites cells in vitro (16). Since 5-iododeoxyuridine is a competitive antagonist of the utilization of thymidine for the biosynthesis of DNA-thymine of mouse Ehrlich ascites cells, it would appear that 5-iododeoxyuridine prevents the utilization rather than the formation of thymidine (or its derivatives).

Mantsavinos has demonstrated that 5-iododeoxyuridine inhibits the utilization of radioactive thymidylid acid for the synthesis of DNA in a cell-free system. Since kinases were present in this system and since 5-iododeoxyuridine is incorporated into DNA (2, 3, 8, 12), it would appear that the site of the competitive inhibition probably occurs at either the monophosphate or the triphosphate level. Additional investigation will be required to determine which of these sites is of primary importance.

REFERENCES

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