The Effects of 5-Fluorodeoxycytidine, 5-Fluorodeoxyuridine, and Related Compounds on Transplanted Mouse Leukemias*

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The anti-tumor activity of the fluorinated uracil derivatives, 5-fluorouracil (FU), 5-fluorouridine (FUR), 5-fluorodeoxyuridine (FUDR), and 5-fluorouronic acid (FO), prepared by Duschinsky, Pleven, Malbica, and Heidelberger (9) 1 have been demonstrated in mice and rats by Heidelberger et al. (11-13). These results have been confirmed and extended by Law (14), Liebling and Humphreys (15), and our own group (3). McIver et al. (16) and Curreri et al. (7) have reported objective evidence of regression of carcinomas in patients following intravenous injection of 5-fluorouracil. Since these results were usually accomplished only with considerable toxic manifestations, it was hoped that other fluorinated pyrimidines might be less toxic for the same degree of therapeutic activity. Heidelberger et al. (18) reported that 5-fluorocytosine (FC) at doses of 25 or 35 mg/kg/day was without effect against the Flexner-Jobling carcinoma in rats, the Ehrlich ascites tumor, and Sarcoma 180 in mice, and Law (14) reported it ineffective on mouse leukemia. Schnitzer and Grunberg 2 have noted no anti-tumor effects in Sarcoma 180, even at doses as high as 500 mg/kg daily. Fox, Wempen, and Duschinsky (10) have recently synthesized 5-fluorocytidine (FCDR) and 5-fluorodeoxycytidine (FCDR) (Chart 1). These compounds have been studied for their effect against a spectrum of transplanted mouse leukemias, and the results are herewith reported.

MATERIALS AND METHODS

The technic for evaluation of the chemotherapeutic activity of a given drug by means of its capacity to prolong the survival time of mice with transplanted leukemia has been described previously (4). In a typical experiment approximately 100 mice were given injections intraperitoneally of 0.1 cc. of a saline suspension of leukemic cells so diluted that 0.1 cc. contained 1,000,000 cells. Twenty-four hours after inoculation, the mice were divided into comparable groups of ten mice each, with one set of controls, and the remaining nine groups treated intraperitoneally daily or 3 times weekly for a 20-day period with the compounds under study. The mice were observed for the development of leukemia and autopsied at death. If gross evidence of leukemia was not conclusive, microscopic sections were taken. The mean survival times of treated and control mice were compared. The rationale behind the various steps of this technic has been discussed in detail in prior publications (4, 5).

Many of these studies were done on the 50th to 90th transplant generations of leukemia B82, which originated as a spontaneous leukemia in a C58 mouse in October, 1953. This transplanted acute lymphatic leukemia kills in 10–15 days, with an elevation of the white blood count to the 50,000–100,000 level and marked enlargement of liver and spleen, and some enlargement of lymph nodes. This leukemia in many experiments was injected subcutaneously to give local tumors; in such case it is designated B82T. In this case, the mice were sacrificed at 14 days and the tumors weighed. In this form Leukemia B82T lends itself particularly well to quantitative measurement of effect and was particularly useful in quantitating the molecular equivalents of the various fluorinated pyrimidines. Leukemias B82 and B8174 were carried in F1 hybrids of the BALB female × C58 male cross. Leukemia L1210, originally supplied by Dr. Lloyd Law, and L1210/A, L1210/MP, L1210/AG, L1210/ADMP (8), and a line of L1210 made resistant to Actizoholin were carried in DBA mice or F1 hybrids thereof. In addition to some of the fluorinated pyrimidines were tested in the same mice against the following leukemias, kindly supplied by Dr. Michael Potter: Chloroleukemia P1081; mast-cell leukemia P815; reticulum-cell leukemia P320; and acute lymphocytic leukemia P388. 3

RESULTS AND DISCUSSION

As can be seen from a scatter diagram (Chart 2), FU caused a definite increase in the survival


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time of mice inoculated with leukemia L1210. The median survival time of the controls was 10.3 days, as against 18.6 days for those treated with FU, 25 mg/kg 3 times weekly. Table 1 shows the relative effectiveness of FU, FUR, and FUDR against leukemia B82T. It can be seen that, on a molecular basis, FUR was 5–10 times as effective as FU, whereas FUDR was about as effective as FU. Charts 3 and 4 demonstrate that FU was active against an amethopterin-resistant strain of L1210 leukemia (L1210/A) and a strain made resistant to both amethopterin and the combination of mercaptopurine and DON spectrum. FCDR was in very short supply and could not be tested adequately against the whole spectrum; there was no significant effect, however, against L1210 and L1210/A, at doses which were half of the maximum tolerated dose.

Table 3 shows the relative activity of the 5-fluorinated cytosine derivatives against leukemia B82T. Only a slight effect was seen with FC at 500 mg/kg daily for 5 days, whereas FCR at 1.2 mg/kg/day and FCDR at 24.5 mg/kg/day were extremely active against this particular form of leukemia.

Table 4 shows the dose-response curve with (L1210/ADMP) (2). This strain is completely resistant to mercaptopurine but not to high doses of 6-diazo-5-oxo-norleucine (DON).

Table 2 shows the relative activity of the various fluorinated pyrimidines studied against a spectrum of transplanted leukemias in C58 or DBA mice or F1 hybrids thereof. The first column gives the numerical designation, type, resistance status, and strain of origin of each leukemia. It can be seen that FU has a wide spectrum of activity but that FO appears considerably less effective against P388, P1081, and L1210/A. FUR and FCR were not tested against the complete spectrum. FUDR was particularly active against P1081 (as previously noted by Law) and B82, and showed some significant effect against the remainder of the L. W. Law, personal communication, 1958.
one of these pathways in common, it would appear
that the methylation of DUMP to TMP is an
important reaction for the leukemic cell. Prelimi-
nary studies by Eidinoff and Rich have suggested
experience of other investigators who, in bacteria
(11) and in developing marine embryos, have shown that thymidine will completely prevent
the toxicity of FU and FUDR. Studies are under
way at the present time in an attempt to explain
this apparent paradox.

The fact that the fluorinated pyrimidines are
effective against L1210 leukemias made resistant
both to amethopterin and to mercaptopurine sug-
gests that, whatever the mechanism of action
of these compounds, it must be somewhat different
from that of amethopterin, even though one of
the important sites of amethopterin activity is
the prevention of a donation of a one-carbon
fragment to DUMP to form TMP. The fact
that these compounds are effective in leukemias
resistant to mercaptopurine and amethopterin sug-
gests also to the clinician that they might be of

![Chart 2](http://cancerres.aacrjournals.org)

**Chart 2.**—The effect of 5-fluorouracil on the survival
time of mice with Leukemia L1210.

### TABLE 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mmol/kg)</th>
<th>Wt. change* (gm.)</th>
<th>Tumor wt. (mg.)</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (controls)</td>
<td>0.006</td>
<td>+0.5</td>
<td>29</td>
<td>93</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>0.006</td>
<td>+0.1</td>
<td>34</td>
<td>93</td>
</tr>
<tr>
<td>5-Fluorouridine</td>
<td>0.008</td>
<td>+0.1</td>
<td>34</td>
<td>93</td>
</tr>
</tbody>
</table>

* Weight change, 12th to 14th days.
† Tumors were dissected and weighed on the 14th day following inoculation. There
were 10 mice in each group.

that growth inhibition by FCDR involves this
methylation step as an important site of its anti-
tumor action.

On the other hand, in chemotherapeutic studies
in vivo, thymidine at 1/4th the maximum tolerated
dose increased markedly the toxicity of otherwise
tolerated doses of FUDR, FCDR, and FU (Table
5). There was no prevention of the anti-leukemic
effect at any dose level tried (25–100 mg/kg
daily of thymidine), and, if anything, there was
some suggestion that the anti-leukemic effect was
increased. This was in direct contrast to the ex-


<table>
<thead>
<tr>
<th>Leukemic Strain</th>
<th>Type</th>
<th>Mouse Strain</th>
<th>5-Fluorouracil</th>
<th>5-Fluorouracil</th>
<th>5-Fluorouracil</th>
<th>5-Fluorouracil</th>
<th>5-Fluorouracil</th>
<th>5-Fluorouracil</th>
<th>5-Fluorouracil</th>
<th>5-Fluorouracil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose (mg/kg)</td>
<td>No. doses</td>
<td>T/C*</td>
<td>Percent response</td>
<td>Dose (mg/kg)</td>
<td>No. doses</td>
<td>T/C*</td>
<td>Percent response</td>
</tr>
<tr>
<td>B22</td>
<td>Lymphoid</td>
<td>C58</td>
<td>3 × wk</td>
<td>3 × wk</td>
<td>3 × wk</td>
<td>3 × wk</td>
<td>3 × wk</td>
<td>3 × wk</td>
<td>3 × wk</td>
<td>3 × wk</td>
</tr>
<tr>
<td>L1210/A</td>
<td>Resist, asceptin</td>
<td>25</td>
<td>3 × wk</td>
<td>16.6/9.5</td>
<td>70</td>
<td>25</td>
<td>3 × wk</td>
<td>12.2/10.1</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>L1210/MP</td>
<td>Resist, 6-mercaptozurine</td>
<td>12.5</td>
<td>daily</td>
<td>17.3/7.7</td>
<td>122</td>
<td>25</td>
<td>3 × wk</td>
<td>15.4/7.3</td>
<td>110</td>
<td>75</td>
</tr>
<tr>
<td>L1210/Acellbabeledin</td>
<td>Resist, 6-mercaptozurine</td>
<td>25</td>
<td>3 × wk</td>
<td>16.1/10.1</td>
<td>59</td>
<td>25</td>
<td>3 × wk</td>
<td>24.2/13.6</td>
<td>77</td>
<td>5</td>
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<tr>
<td>P388</td>
<td>Lymphoid</td>
<td>DBA</td>
<td>25 daily</td>
<td>21.1/11.6</td>
<td>82</td>
<td>25</td>
<td>3 × wk</td>
<td>15.5/12.9</td>
<td>20</td>
<td>75</td>
</tr>
<tr>
<td>P1081</td>
<td>Chloro-leukemia</td>
<td>DBA</td>
<td>12.5 daily</td>
<td>84.3/27.6</td>
<td>205</td>
<td>12.5 daily</td>
<td>37.8/27.6</td>
<td>36</td>
<td>5</td>
<td>3 × wk</td>
</tr>
<tr>
<td>P815</td>
<td>Mast cell</td>
<td>DBA</td>
<td>12.5 daily</td>
<td>16.0/8.1</td>
<td>98</td>
<td>82</td>
<td>3 × wk</td>
<td>19/11.3</td>
<td>68</td>
<td>75</td>
</tr>
<tr>
<td>P239</td>
<td>Reticulum cell</td>
<td>DBA</td>
<td>12.5 daily</td>
<td>31.4/17.3</td>
<td>82</td>
<td>25</td>
<td>3 × wk</td>
<td>15/11.3</td>
<td>68</td>
<td>75</td>
</tr>
<tr>
<td>B8174T</td>
<td>Lymphoid</td>
<td>C58</td>
<td>25</td>
<td>3 × wk</td>
<td>169/919</td>
<td>81</td>
<td>12.5</td>
<td>3 × wk</td>
<td>308/919</td>
<td>66</td>
</tr>
</tbody>
</table>

* Survival time, in days, of treated/controls, except for leukemia B22 and B8174T, for which tumor weight (in mg) is given. In the latter two cases tumors were dissected and weighed on the 14th day following inoculation with leukemia. There were ten mice in each group.

+ Response is given as a per cent increase in survival time, except for leukemias B22 and B8174T, for which the value represents tumor inhibition.
Table 3

Relative Anti-Leukemic Activity of 5-Fluorinated Cytosine Derivatives Against Leukemia B16

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mmoles/kg)</th>
<th>Weight change* (mg/kg daily)</th>
<th>Tumor weight† (mg.)</th>
<th>Per cent tumor inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (controls)</td>
<td>0.1</td>
<td>+2.6</td>
<td>903</td>
<td></td>
</tr>
<tr>
<td>5-Fluorocytosine</td>
<td>0.005</td>
<td>+0.4</td>
<td>62</td>
<td>93</td>
</tr>
<tr>
<td>5-Fluorocytidine</td>
<td>0.1</td>
<td>+0.4</td>
<td>23.3</td>
<td>97</td>
</tr>
<tr>
<td>5-Fluoroxyctidine</td>
<td>0.05</td>
<td>+1.1</td>
<td>181</td>
<td>80</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>0.1</td>
<td>+1.5</td>
<td>417</td>
<td>54</td>
</tr>
<tr>
<td>5-Fluoroxyuridine</td>
<td>0.05</td>
<td>+0.4</td>
<td>61</td>
<td>98</td>
</tr>
</tbody>
</table>

* Weight change, 12th–14th day.
† Tumors were dissected and weighed on the 14th day following inoculation. There were ten mice in each group.
value in patients with leukemias which have become resistant to those conventional agents.

SUMMARY

The 5-fluorinated pyrimidine derivatives, 5-fluorouracil (FU), 5-fluoroorotic acid (FO), 5-fluorouridine (FUR), 5-fluorodeoxyuridine (FUDR), 5-fluorocytidine (FCR), and 5-fluorodeoxycytidine (FCDR), showed activity against various transplanted mouse leukemias. Because of shortness of supply, 5-fluorocytosine (FC) was not tested adequately but had relatively little effect even at the massive doses employed.

FU and FUDR were active against amethopterin- and mercaptopurine-resistant lines of leukemia L1210.

In leukemia B82, the ribonucleosides, FUR and FCR, were approximately 10-20 times as active on a molecular basis as their corresponding deoxyribonucleosides (FUDR and FCDR) or than fluorouracil.

Dose-response curves on Leukemia B82 would suggest that FUDR and FCDR have a relatively high chemotherapeutic index against Leukemia B82 and for this reason merit clinical trial.

REFERENCES


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