Metabolism and Therapeutic Efficacy of 9-β-D-Arabinofuranosyl-2-fluoroadenine against Murine Leukemia P388

Vassilios I. Avramis and William Plunkett

Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

ABSTRACT

The biochemical basis for the differential therapeutic activity of equally toxic doses of 9-β-D-arabinofuranosyl-2-fluoroadenine (F-ara-A) administered on two schedules to tumor-bearing mice has been studied. A single dose (234 mg/kg) of F-ara-A in solution decreased the number of P388 leukemia cells by >10³, whereas a multiple-dose regimen (41 mg/kg every day for 5 days) of equal toxicity to the host was ineffective at reducing the tumor burden. No antitumor activity was seen when an equal dose of the relatively insoluble F-ara-A was injected as a suspension. The 5'-triphosphate of F-ara-A accumulated in P388 cells in levels proportional to the dose of the nucleoside and disappeared from these cells at an exponential rate with a half-life of 2.9 hr, which was independent of the cellular concentration of the nucleotide. The extent and duration of the inhibition of DNA synthesis of P388 cells was dependent on the dose of F-ara-A, but the rates of recovery were similar and in proportion to the cellular concentration of the analog triphosphate. The extent of the inhibition of DNA synthesis in host bone marrow and intestinal mucosa was also related to the dose of F-ara-A, but the recovery of these tissues proceeded to similar, incomplete levels (<60% of initial) 24 hr after F-ara-A injection of either 41 or 234 mg/kg. These results suggest that the equal toxicity of the two regimens of F-ara-A may be attributed to the similar extent of inhibition of host-tissue DNA synthesis evoked by each. In contrast, the greater extent and longer duration of inhibition of P388 cell DNA synthesis caused by the single dose of F-ara-A was responsible for its superior therapeutic activity. Measurements of F-ara-A triphosphate concentrations and the DNA-synthetic capacity of tumor and host tissues are determinants of the action of F-ara-A and may be used to predict optimal therapeutic dose schedules.

INTRODUCTION

The susceptibility of ara-A to inactivation by deamination as a major limitation to its therapeutic effectiveness as an antimetabolite is a major limitation to its therapeutic activity in tumor-bearing mice (4, 8, 9, 15, 17). However, the activity of these compounds may not be confined to adenosine deamination, and inhibition of the deaminase does cause a variety of undesirable side effects (7, 20, 21, 23). In addition, we have demonstrated recently the accumulation of dATP concentrations in the leukemic cells of patients receiving therapy with ara-A in combination with the deaminase inhibitor, deoxycoformycin, that exceeded the concentration of the active metabolite, ara-ATP (13). These findings suggested that such combination chemotherapy may result in a cellular milieu that is antagonistic to the inhibitory action of ara-ATP on DNA synthesis.

F-ara-A is a congener of ara-A that does not serve as a substrate for adenosine deaminase (2) and therefore does not require a deaminase inhibitor for optimal activity (5, 14). F-ara-A is phosphorylated to the triphosphate F-ara-ATP, which like ara-ATP is thought to be the active metabolite responsible for the observed cytotoxicity and therapeutic activity in tumor-bearing mice (1, 2, 14). Studies with mice have demonstrated that F-ara-A and its metabolites are distributed in many tissues; the small intestine ranks among the tissues with the highest concentrations of F-ara-A metabolites (6). Consistent with determinations in whole cells in culture (14), recent studies have demonstrated that F-ara-ATP may be slightly more inhibitory to DNA polymerase α than is ara-ATP (22).

Our studies were directed at determining the biochemical basis for the therapeutic activity of F-ara-A in tumor-bearing mice. The results indicate that the cellular F-ara-ATP concentrations and the DSC of tumor and host tissues are determinants of the action of F-ara-A and may be used to predict optimal dose schedules.

MATERIALS AND METHODS

Materials. Initial samples of F-ara-A were generously provided by Dr. J. A. Montgomery, Southern Research Institute, Birmingham, Ala. (10). More recently, the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, supplied us with this compound. [methyl-3H]Thymidine (40 Ci/mmol) was obtained from ICN Pharmaceuticals, Inc. (Irvine, Calif.).

Tumors and Mice. P388 lymphocytic leukemia was kindly provided by Dr. J. Mayo, National Cancer Institute, Fort Detrick, Md. The tumor was maintained i.p. in female DBA/2 mice (Simonsen Laboratories, Gilroy, Calif.) by weekly transfer inoculations of 10⁶ cells. All determinations of toxicity, therapeutic efficacy, and metabolism of F-ara-A were conducted in male C57BL/6 × DBA/2 (hereafter called BD2F₁) mice from the same source, averaging 20 g.

Toxicity of F-ara-A. Healthy male BD2F₁ mice were assigned randomly into groups of 8. The toxicity of F-ara-A was determined for a single dose (range, 75 to 1000 mg/kg) and a multiple dose schedule (every day for 5 days; range, 20 to 150 mg/kg/dose). F-ara-A was administered in solution by i.p. injection except as indicated in Table 1. The projected toxicity values of F-ara-A (LD₅₀) on each of the 2 dosage regimens were calculated from the toxicity data by the log dose-probit method described by Skipper et al. (19).

Therapeutic Efficacy of F-ara-A. The principles described by Schabel et al. (16) were used for the quantitative evaluation of antitumor...
activity. To provide standards for determining the number of P388 cells surviving treatment in studies of the therapeutic efficacy of F-ara-A, 7 groups of 16 mice were given i.p. inoculations of log10 dilution of P388 cells, ranging from 10^1 to 10^7 cells/mouse. Both weight loss and spleen size were considered in the evaluation of deaths due to toxicity of tumor-bearing mice. The tumor population-doubling time, calculated from plots of the median survival time of each group against the number of cells inoculated, was 0.78 day, a value that compared favorably with that reported for similar determinations in much larger numbers of mice (16).

**Determination of DSC.** The procedures used to determine DSC of P388 cells, host gastrointestinal mucosa, and bone marrow have been described (11). Briefly, groups of 4 BD2F1 mice were given injections of the indicated amounts of F-ara-A 7 days after i.p. inoculation of 10^6 P388 cells. Thirty min prior to the specified time, the mice were given s.c. injections of 50 µCi [3H]thymidine on the back. After a 30-min incorporation time, the mice were killed by cervical dislocation, P388 cells, host gastrointestinal mucosa, and bone marrow and intestinal mucosa, rapidly proliferating tissues on which dose-limiting toxicity might be exerted

**Effect of F-ara-A on DSC of P388 Cells and Host Tissues.** Mammalian cells metabolize F-ara-A to the triphosphate, F-ara-ATP, which is thought to exert its inhibitory effect by inhibition of DNA synthesis (2, 14). The effect of LD10 doses of F-ara-A given on the 2 different schedules on the DSC of P388 cells and host bone marrow and intestinal mucosa, rapidly proliferating tissues on which dose-limiting toxicity might be exerted by a DNA-active agent, have been compared to determine the

**RESULTS**

**Toxicity of F-ara-A.** Two dosage regimens of F-ara-A were chosen, single dose and daily for 5 days, to establish the therapeutic range of F-ara-A and to assess both acute and cumulative toxicity to tumor-free mice (Chart 1). The maximum tolerated dose (LD10) of F-ara-A administered as a single dose was 234 mg/kg, whereas the same toxicity was evoked by 5 consecutive daily doses of 41 mg/kg. The 50% lethal doses were calculated to be 375 and 52 mg/kg for the single- and multiple-dose regimens, respectively. Deaths due to toxicity under the single-dose regimen all occurred by 7 days after injection; no delayed toxicity (2 to 3 weeks) was observed. Similarly, weight loss in survivors of toxic single doses was maximal after 7 to 10 days. This was followed by an increase of body weight that paralleled control mice. In contrast, the steeper slope generated by the multiple-dose regimen is indicative of the cumulative toxicity of this schedule (Chart 1).

**Therapeutic Efficacy of F-ara-A.** The therapeutic efficacy of equally toxic doses of F-ara-A administered on the 2-dose schedules was evaluated in mice bearing P388 leukemia (Table 1). The projected toxicity of each dose was calculated from the data in Chart 1, and the number of cells estimated to have survived treatment with F-ara-A was determined from parallel observations of the survival of mice inoculated with serial dilutions of P388 cells (not shown). The therapeutic activity of F-ara-A administered as a single dose was clearly greater than a treatment of the same toxicity given on the 5-day schedule. The greater percentage of increase in life span of the mice treated with single doses of F-ara-A is reflected by the estimated fewer number of cells surviving therapy.

Because of the relative insolubility of F-ara-A (0.62 mg/ml at 37º), stock solutions for injection of toxic and therapeutic doses were prepared by dissolving the nucleoside by heating and subsequently injecting a cooled, supersaturated solution. To gain a better perspective on the importance of solution, the therapeutic activity of a suspension of F-ara-A equal to the single-dose LD10 of the drug administered in solution was investigated (Table 1). Delivered in solution, this dose had marked therapeutic activity; however, when injected i.p. as a suspension (only 0.28 mg/ml at 25º was in solution) the ability to prolong the life span of tumor-bearing mice was insignificant.

**Effect of F-ara-A on DSC of P388 Cells and Host Tissues.** Mammalian cells metabolize F-ara-A to the triphosphate, F-ara-ATP, which is thought to exert its inhibitory effect by inhibition of DNA synthesis (2, 14). The effect of LD10 doses of F-ara-A given on the 2 different schedules on the DSC of P388 cells and host bone marrow and intestinal mucosa, rapidly proliferating tissues on which dose-limiting toxicity might be exerted by a DNA-active agent, have been compared to determine the

**Table 1**

<table>
<thead>
<tr>
<th>Projected</th>
<th>Dose (mg/kg)</th>
<th>No. of mice</th>
<th>MST&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% of ILS&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Estimated cells surviving therapy</th>
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<td>toxicity&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>10</td>
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<td></td>
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<tr>
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<td>159</td>
<td>qd, 1 day</td>
<td>8</td>
<td>21</td>
<td>110</td>
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<tr>
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<td>8</td>
<td>21</td>
<td>110</td>
</tr>
<tr>
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<td>234</td>
<td>qd, 1 day</td>
<td>8</td>
<td>21</td>
<td>110</td>
</tr>
<tr>
<td>LD&lt;sub&gt;10&lt;/sub&gt;</td>
<td>256</td>
<td>qd, 1 day</td>
<td>8</td>
<td>21</td>
<td>110</td>
</tr>
<tr>
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<td>qd, 5 days</td>
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<td>18</td>
<td>80</td>
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<td>75</td>
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<tr>
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<td>qd, 1 day</td>
<td>40</td>
<td>21.5</td>
<td>108</td>
</tr>
<tr>
<td>234 (suspension&lt;sup&gt;c&lt;/sup&gt;)</td>
<td>qd, 1 day</td>
<td>24</td>
<td>13.5</td>
<td>25</td>
<td>3.2 x 10&lt;sup&gt;10&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup> Calculated from the data in Chart 1.
<sup>b</sup> MST, median survival time; ILS, increased life span; LD<sub>1</sub>, 1% lethal dose (other subscript numbers indicate corresponding percentage of deaths); qd, every day.
<sup>c</sup> Administered as a saturated, well-mixed suspension.
basis for the observed equal toxicity but differential therapeutic activity. After a single dose of 41 mg/kg (Chart 2), the DSC of P388 cells was maximally inhibited to 14% of controls by 4 hr but recovered between 12 and 18 hr and subsequently doubled the control values by 24 hr. In contrast, host bone marrow and mucosa were each maximally inhibited to 35 to 40% of control values by 2 hr, but neither showed a consistent progression toward recovery in the following 22 hr.

As expected, the extent of inhibition of P388 DSC was substantially greater after injection of a single-dose LD10 amount of F-ara-A (Chart 3) than after the lesser dose just discussed. The tumor DSC was reduced to less than 2% of control values by 6 hr but recovered to control levels by 24 hr. Interestingly, the rate of recovery was essentially identical to that seen after the lower dose of F-ara-A (Chart 2). The DSCs of the bone marrow and mucosa of the host were each inhibited to less than 10% of controls at 2 hr but recovered to 40% of control values by 8 hr at a rate similar to the P388 recovery. Thereafter, recovery progressed only to 55% of controls by 24 hr.

F-ara-ATP Kinetics in P388 Cells. The HCIO4-soluble material from the experiments presented in Charts 2 and 3 was analyzed for F-ara-ATP by high-pressure liquid chromatography (Chart 4). The concentration of F-ara-ATP in P388 cells reached maximal values of 128 and 560 μM 4 hr after F-ara-A injection of 41 and 234 mg/kg, respectively. Analysis of the F-ara-ATP accumulated after injection of doses intermediate to those shown in Chart 4 also indicated maximal cellular concentrations of F-ara-ATP had been achieved by 4 hr. These determinations indicated that the amount of F-ara-ATP accumulated was proportional to the dose of F-ara-A. The rate of elimination of F-ara-ATP from P388 cells was essentially identical after each dose of F-ara-A, the half-lives of which were 2.8 and 2.9 hr for the 41- and 234-mg/kg doses, respectively. Attempts to detect F-ara-ATP in extracts from the bone marrow and gastrointestinal mucosa samples from this experiment failed due to the lack of adequate quantities of starting materials. However, in a separate but otherwise identical experiment, single-point determinations in tumor-bearing mice 4 hr after being given injections of [3H]F-ara-A indicated that the nmol of F-ara-ATP per μmol deoxyribose values were 47.9, 0.038, and 0.192 in P388, intestinal mucosa, and bone marrow, respectively.

When the data describing the effect of each dose of F-ara-A on the inhibition of DNA-synthetic capacity of P388 cells (Charts 2 and 3) were plotted against the cellular concentrations of F-ara-ATP detected at the same time (Chart 4), the relationship between the 2 parameters was apparent (Chart 5). The cellular concentrations of F-ara-ATP at 10 and 50% of control DSC were 250 and 20 μM, respectively. The relationship between cellular F-ara-ATP concentrations and DSC of P388 cells, depicted in Chart 5, indicates that the inhibition of DSC in P388 cells was dependent on the concentration of F-ara-ATP in the cells, a correlation that was independent of the initial dose of F-ara-A.

DISCUSSION

These studies were directed at a comparison of the therapeutic activity of equitoxic doses of F-ara-A administered on different schedules to tumor-bearing mice. As suggested by the results of studies of F-ara-A toxicity in vitro (1, 14) and, by
observed after a single dose of 234 mg/kg. This finding is consistent with the observed equal toxicity of the treatment regimens. In previous studies with ara-A, consecutive doses administered after recovery of host DSC but before recovery of the tumor increased the therapeutic activity of the drug (11). To evaluate the importance for host-tissue recovery, a second injection of the maximum tolerated single dose (LD_{50}) of F-ara-A was administered daily for 1 to 7 days after the first dose (data not shown). Although long-term survivors were obtained when the second dose was given after a 4-day interval, deaths due to drug toxicity were evident even at the longest times between doses. This suggests that host tissues were affected by the cumulative toxicity of this drug. Studies of the concentrations of F-ara-ATP and the effects on the DSC of these tissues will be useful for a better understanding of these phenomena.

Our earlier experiments with cells in culture demonstrated that both the extent and the duration of inhibition of DNA synthesis may be predicted from a knowledge of the cellular concentration of F-ara-ATP and the rate at which it decayed in the absence of exogenous F-ara-A. It appears that these principles may be applied to the in vivo situation as well. As was demonstrated in L1210 (2), the accumulation of F-ara-ATP by P388 cells was proportional to the dose of F-ara-A injected (Chart 4). It is probable that, once injected, F-ara-A was rapidly cleared from the peritoneal cavity, as was found for ara-A (12); thus, insignificant amounts remained to be taken up by cells after 4 hr. Thereafter, the F-ara-A concentration in the tumor cells decreased with a half-life of about 3 hr; this decrease was independent of the initial concentration of the nucleotide (Chart 4). In addition, as cellular F-ara-ATP concentrations decreased, the rates of recovery of DNA synthesis were nearly identical in the tumor cells of mice given injections of either dose of F-ara-A (Charts 2 and 3). The relationship between these parameters, illustrated in Chart 5, suggests that cellular F-ara-ATP concentrations of 200 to 300 μM were required for a 90% inhibition of DNA synthesis. This degree of inhibition is probably requisite for significant antitumor activity. Knowledge of the concentrations of F-ara-ATP in both tumor and host tissues and their effects on the DSC of these tissues may be useful determinants in the rational design of F-ara-A dose levels and schedules.

REFERENCES

Biochemistry of F-ara-A Therapy

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