Modulation of 5-Fluorouracil-induced Toxicity in Mice with Interferon or with the Interferon Inducer, Polyinosinic-Polyctidylic Acid

Robert L. Stolfi, Daniel S. Martin, Robert C. Sawyer, and Sol Spiegelman


ABSTRACT

Partially purified preparations of mouse interferon, administered during the 2-day period following the administration of a toxic dose of 5-fluorouracil (FUra), yielded significant protection from mortality in BALB/c x DBA/2 F1 mice. Protection against FUra-induced toxicity was also observed when the interferon inducer polyinosinic-polycytidylic acid (poly I-poly C) was administered with FUra. The temporal relationship between the administration of poly I-poly C and FUra was found to be a critical determinant of the intensity of toxic manifestations. In relation to FUra alone, poly I-poly C could enhance (when administered 48 hr before FUra), diminish (when administered together with FUra), or not affect (when administered 48 hr after FUra) the degree of resultant toxicity. Cytofluorometric analysis of the DNA content of bone marrow cells indicated a transient period (about 42 hr) of inhibition of cell cycling following the administration of poly I-poly C, followed by reentry into cycle (between 42 and 66 hr) and a return to normal cycle phase distribution by 90 hr. This disturbance of the kinetic pattern of cell cycling in bone marrow would explain the administration time-dependent variability of the effect of poly I-poly C on FUra toxicity, since FUra is known to be a cell cycle-specific cytotoxic drug. Potential practical application of this observation to the clinical use of FUra in cancer therapy is discussed.

INTRODUCTION

As early as 1962, an "anticellular effect" was ascribed to interferon and was recognized as distinct from its antiviral activity (27). Recent studies with more highly purified preparations have confirmed the antiproliferative activity of interferon (28). In fact, it is now generally believed that all cell strains and lines, normal as well as malignant, have a certain although widely varying degree of sensitivity to the growth-inhibitory effect of interferon (2, 12, 14).

Among the cell types studied, murine and human granulocyte progenitor cells have been found to be susceptible to the antiproliferative action of interferon (11, 21, 22, 25, 32, 39). Although the molecular basis for the antiproliferative effect remains unknown, Gidali et al. (10), utilizing the thymidine suicide technique, have demonstrated that reaction with mouse serum interferon in vitro or administration of an interferon inducer in vivo caused a reduction in the proportion of S-phase colony-forming cells in regenerating murine bone marrow. Further, it has been demonstrated that interferon was not cytotoxic for bone marrow cells and that inhibition of colony development could be demonstrated only when interferon remained in contact with the cells during a prolonged period of the culture time (9, 10).

It occurred to us that the antiproliferative action of interferon might affect the activity of certain drugs used in cancer chemotherapy. With many cytotoxic drugs, the lethal cellular event does not occur until some time after the interaction of the drug and the target site when the cell must proceed through some critical stage of its cycle in which the affected site (or a product from the affected site) is required (37). Further, it is known that lethality can be circumvented if the cell can repair or replace the damaged function before that critical phase of the cycle is reached (38). Therefore, the interferon-induced inhibition of cell cycle progression might extend the time period available for natural repair processes and result in decreased lethality from a given drug treatment. Since we have worked extensively in the area of metabolic modulation with FUra, we chose this antimetabolite as a representative of cytotoxic chemotherapy for the initial evaluation of this concept.

FUra is considered to be a cell cycle-specific agent in that proliferating cells exhibit greatly enhanced susceptibility in comparison to nonproliferating cells (4). FUra appears capable of exerting cytotoxicity through 2 distinct mechanisms: (a) the pyrimidine analogue can be incorporated into and interfere with critical functions of RNA leading indirectly to inhibition of DNA synthesis (5, 18, 19, 31, 33, 34, 42, 43); and (b) it can combine with and inhibit the action of the enzyme thymidylate synthetase resulting in direct inhibition of DNA synthesis (6, 7, 13, 36). Therefore, we hypothesized that a transient inhibition of cell cycle progression of sufficient duration, effected through administration of interferon after treatment with FUra, might permit substantial replacement of FUra-containing RNA, as well as the synthesis of new molecules of thymidylate synthetase, so that DNA synthesis then could proceed normally and the cell would be effectively rescued. From previous successful in vivo studies in which uridine was administered for varying periods of time to prevent toxicity following the incorporation of lethal amounts of FUra into RNA (20), it was estimated that a 48-hr delay in DNA synthesis would be required.

Selective inhibition of proliferation of normal cells as a means of reducing their susceptibility to chemotherapeutic drugs has been demonstrated in several cell culture systems (3, 26, 29, 30, 40, 41). However, most of the agents used to modify cellular proliferation possess too great an inherent toxicity for in vivo applicability (1, 16, 24). Results of the present study

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2 To whom requests for reprints should be addressed, at St. Anthony’s Cancer Research Center, 89-15 Woodhaven Boulevard, New York, New York 11421. Received March 26, 1982; accepted November 5, 1982.
3 The abbreviations used are: FUra, 5-fluorouracil; poly I-poly C, synthetic double-stranded polyinosinic acid-polycytidylic acid; LD50, 10% lethal dose; LD90, 50% lethal dose.
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indicate that purified mouse interferon, or the interferon inducer, poly I-poly C (8, 15, 44), administered at a critical period of time in relation to the administration of lethal doses of FUra ameliorates bone marrow toxicity as reflected in peripheral leukocyte levels and prevents mortality in normal BALB/c × DBA/2 F1 (hereafter called CDBF1) mice.

MATERIALS AND METHODS

Mice. Normal, healthy female CDBF1 mice, 2 to 3 months old, were used for all experiments. These mice were obtained from our murine breeding colony which has been described previously (35). The average body weight of these mice was 21.5 g. The mean WBC of all 400 mice used in these studies before treatment was 14,797/cu ram; however, there was a fair degree of variation in this value from one experiment to another; therefore, the mean pretreatment WBC is indicated for each experiment.

Interferon. Two different preparations of partially purified mouse interferon were kindly supplied by Dr. William E. Stewart. A third preparation of mouse interferon was obtained from Dr. Ion Gresser.

Drugs and Chemicals. FUra was purchased from Sigma Chemical Co., St. Louis, Mo. The copolymer poly I-poly C was purchased from Calbiochem-Behring Corp., Los Angeles, Calif. Each agent was dissolved in 0.85% NaCl solution so that the desired dose was administered in 0.1 ml/10 g of body weight.

Toxicity Measurements. Mice were weighed at least twice per week for measurement of body weight changes. In preliminary experiments (not shown) where body weight measurements (and WBC) were performed more frequently, it was determined that the administration of poly I-poly C together with FUra did not alter the kinetics of weight loss or WBC depression seen with FUra alone (although, as will be described here, it did alter the magnitude of these changes). The Student's t test was used to determine the statistical significance of differences in body weight loss between groups. Peripheral WBC levels were measured electronically in tail vein blood with a Fisher cell counter. Mice were distributed into experimental groups according to their pretreatment WBC so that mice with approximately equal WBC were represented in each group. WBC then were measured at timed intervals after initiation of treatment. Statistical significance of differences in mean WBC levels between groups was determined with the unequal variance F-test.

Bone Marrow Cell Cycle Analysis. A single femur was dissected free from each mouse immediately after sacrifice by cervical dislocation. Six ml of ice-cold Roswell Park Memorial Institute Tissue Culture Medium 1640 were forced through the lumen of the femur with the aid of a 25-gauge needle mounted on a 10-ml syringe. The marrow was dispersed by pipetting, the cell suspension was counted microscopically, and 5 × 106 cells were pelleted and resuspended in 5 ml of ice-cold propidium iodide (0.05 mg/ml in phosphate-buffered saline, pH 7.2, containing 0.6% Nonidet P-40). Staining with the fluorescein dye was allowed to proceed for 15 min at 4°C before counting. Thirty thousand cells were counted using an argon-ion laser with the Ortho Model 30 cytofluorograph. Fluorescent and light-scattering signals were displayed on an oscilloscope as a histogram of fluorescence intensity versus incidence. Using the multichannel distribution analyzer, the limits of the curve corresponding to the G1, S, and G2 regions were selected visually, and the area of the selected region of the curve was integrated electronically. The average coefficient of variation for the G1 peak was 3.46%.

RESULTS

Protection of CDBF1 Mice from FUra-induced Toxicity with Mouse Interferon. Three different preparations of mouse interferon were tested for potential amelioration of FUra toxicity in vivo. In each experiment, 2 groups of 9 or 10 CDBF1 mice received a toxic 3-course treatment with FUra at 130 mg/kg/week. In addition to the FUra alone, one of the groups received 5 × 104 units of mouse interferon i.p. simultaneously with each dose of FUra, and again at 3.5, 7, 24, 27.5, and 31 hr after each dose of FUra. Results of 4 separate experiments are presented in Table 1. Body weight loss associated with this FUra regimen was significantly reduced (p = 0.05, or less) by interferon in 3 of the 4 experiments (and reduced by 10% in the fourth experiment which was not statistically significant). The kinetic pattern of weight loss was similar in both groups with nadirs occurring at 3 or 4 days after the third course of FUra. Mortality associated with this FUra regimen was significantly reduced (p = 0.05, or less) by interferon in each of the 4 experiments. In total, 32 of the 39 mice treated with FUra alone (i.e., 82%) died within 30 days after the last dose of drug, whereas in mice receiving FUra plus interferon, only 8 of 39 (i.e., 21%) died within this observation period (x2 = 27.146; p < 0.001).

Effect of the Interferon Inducer Poly I-Poly C on FUra-induced Toxicity. The synthetic polynucleotide poly I-

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>% of body wt change at nadir</th>
<th>Mortality (dead/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>346</td>
<td>FUra30 qw x 3</td>
<td>-31.0 ± 7.2</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>FUra30 + interferon qw x 3</td>
<td>-21.3 ± 2.8</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>352</td>
<td>FUra30 qw x 3</td>
<td>-23.8 ± 2.3</td>
<td>p &gt; 0.02</td>
</tr>
<tr>
<td></td>
<td>FUra30 + interferon qw x 3</td>
<td>-15.3 ± 1.5</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>367</td>
<td>FUra30 qw x 3</td>
<td>-33.0 ± 1.7</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>FUra30 + interferon qw x 3</td>
<td>-21.7 ± 2.1</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>369</td>
<td>FUra30 qw x 3</td>
<td>-37.3 ± 1.1</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>FUra30 + interferon qw x 3</td>
<td>-26.5 ± 2.9</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

a Mice were observed for 30 days following the last treatment.
b FUra subscript, dose in mg/kg; qw x 3, once a week for 3 weeks.
c Mean ± S.E.
d Interferon, 5 × 104 units, administered i.p. simultaneously with each dose of FUra, and again at 3.5, 7, 24, 27.5, and 31 hr after each dose of FUra. Mouse interferon Lot 24S (used for Experiments 346 and 352) and Lot 118 (used for Experiment 369) were provided by W. E. Stewart. Mouse interferon for Experiment 367 was obtained from I. Gresser.
poly I, a known inducer of interferon in mice (23), also was tested for potential to ameliorate FUra toxicity in vivo. Poly I-poly C was used at a dose of 4 mg/kg, since it had been shown to produce elevated serum interferon and transient inhibition of the S phase of bone marrow colony-forming units at that dose (10). Groups of 9 mice each received 3 weekly courses of treatment with a toxic dose of FUra (130 mg/kg/week), or with poly I-poly C (4 mg/kg) simultaneously with FUra, or at 48 hr before or after FUra. A strict timing requirement for the manifestation of protection against FUra toxicity in vivo was demonstrated. Data from one of 3 experiments with similar results are presented in Table 2. In this experiment, mice treated with FUra alone (Group 1) showed a body weight loss of 18% and a significantly depressed (p < 0.001) WBC of 3,878/cu mm on Day 14 (i.e., the day of the third course of treatment) in comparison with a pretreatment WBC of 15,530/cu mm. The differences in body weight loss and WBC levels between Groups 1 and 3 on Day 14 were statistically significant (p < 0.001 and < 0.05, respectively). Thirty days later, 8 of the 9 mice treated with FUra alone were dead while all of the mice treated with poly I-poly C and FUra simultaneously still were alive (p < 0.001). When poly I-poly C was given 48 hr before FUra (Group 2), instead of protecting, toxicity was enhanced. On Day 14, body weight loss (32%, Group 2) was significantly greater (p < 0.01) than that in mice treated with FUra alone (18%, Group 1), and WBC depression (1,767/cu mm, Group 2) was significantly more profound (p < 0.05) in Group 2 than in Group 1 (the FUra control). In addition, the death rate was accelerated in Group 2 as evidenced in Table 2 by 33% mortality on Day 14, a time at which mortality had not yet begun to develop in Group 1. When poly I-poly C was administered with a 48-hr delay after FUra (Group 4), there was no discernable effect on the parameters of toxicity observed (compare Groups 1 and 4 in Table 2). Mice treated with poly I-poly C alone (Group 5) did not show any weight loss, or mortality and WBC levels on Day 14 (13,889/cu mm) did not differ significantly from the pretreatment level in this experiment.

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>% of body wt change</th>
<th>WBC/cu mm</th>
<th>Dead/total</th>
<th>p</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FUra</td>
<td>-17.7 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,875 ± 576</td>
<td>0/9</td>
<td>8/9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Poly I-poly C 48 hr</td>
<td>-32.3 ± 2.6&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1,767 ± 180&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3/9</td>
<td>9/9 (NS)&lt;sup&gt;j&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>FUra + poly I-poly C</td>
<td>+1.4 ± 1.6&lt;sup&gt;j&lt;/sup&gt;</td>
<td>9,533 ± 1,802&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0/9</td>
<td>0/9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>FUra 48 hr</td>
<td>-20.1 ± 2.5 (NS)</td>
<td>4,967 ± 740 (NS)</td>
<td>0/9</td>
<td>8/9 (NS)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Poly I-poly C</td>
<td>+7.4 ± 1.4&lt;sup&gt;j&lt;/sup&gt;</td>
<td>13,889 ± 889&lt;sup&gt;j&lt;/sup&gt;</td>
<td>0/9</td>
<td>0/9</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Three courses of the indicated treatment were administered with a 1-week interval between courses. FUra was administered i.p. at a dose of 130 mg/kg, and poly I-poly C was administered i.p. at a dose of 4 mg/kg.

<sup>b</sup> The day of the third course of treatment.

<sup>c</sup> Mean WBC before initiation of treatment was 15,530 ± 627.

<sup>d</sup> Mice were observed for 30 days following the last course of treatment.

<sup>e</sup> Mean ± S.E.

<sup>f</sup> p < 0.01 compared to Group 1.

<sup>g</sup> p < 0.05 compared to Group 1.

<sup>h</sup> NS, not statistically significant.

<sup>i</sup> p < 0.001 compared to Group 1.

**Table 3**

<table>
<thead>
<tr>
<th>Posttreatment time (hr)</th>
<th>Treatment</th>
<th>G&lt;sub&gt;1&lt;/sub&gt;</th>
<th>S</th>
<th>G&lt;sub&gt;2&lt;/sub&gt;</th>
<th>Mean %&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Control</td>
<td>74.8 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.6 ± 0.4</td>
<td>11.6 ± 0.6</td>
<td>NS&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Poly I-poly C</td>
<td>78.0 ± 0.03</td>
<td>10.7 ± 0.4</td>
<td>11.4 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Control</td>
<td>72.3 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.7 ± 0.9</td>
<td>14.0 ± 0.4</td>
<td>NS&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Poly I-poly C</td>
<td>75.6 ± 0.2</td>
<td>9.2 ± 0.4</td>
<td>15.2 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Control</td>
<td>74.0 ± 0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.5 ± 0.3</td>
<td>12.5 ± 0.5</td>
<td>NS&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Poly I-poly C</td>
<td>70.2 ± 1.0</td>
<td>16.6 ± 0.9</td>
<td>13.2 ± 0.6</td>
<td>NS&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>90</td>
<td>Control</td>
<td>74.4 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.1 ± 1.0</td>
<td>12.5 ± 0.6</td>
<td>NS&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Poly I-poly C</td>
<td>73.5 ± 0.6</td>
<td>12.2 ± 0.8</td>
<td>14.3 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined from counts of 30,000 femur marrow cells from each of 4 mice. The statistical probability of the difference between mean values of control versus poly I-poly C-treated cells in G<sub>1</sub>, S, and G<sub>2</sub> phases of the cell cycle is presented.

<sup>b</sup> Mean ± S.E.

<sup>c</sup> NS, not statistically significant; i.e., p > 0.05.
visually, and the areas corresponding to these regions were integrated electronically. Results are presented in Table 3. At 18 hr after administration of poly I-poly C, there was a small but significant shift in the proportion of cells in cycle. The G1 population was significantly increased and the S population was significantly decreased in whole bone marrow suspensions. The same pattern was observed at 42 hr after administration of poly I-poly C. However, the inhibition of DNA synthesis was obviously over by 66 hr. At this time, there were significantly fewer cells in G1, and a larger proportion in S (although the difference between control and poly I-poly C treated is not quite significant, at \( p = 0.08 \)). By 90 hr, bone marrow from the poly I-poly C-treated mice could not be distinguished from controls by DNA content.

**Single-Dose Titration of FUra Toxicity in Mice Treated with Poly I-poly C.** Groups of 9 mice each were treated with one of varying doses of FUra or with FUra plus poly I-poly C at 4 mg/kg. Animals were weighed daily, WBC levels were measured on Day 9, and the mice were observed for mortality for 30 days. Six days after treatment, substantial dose-related weight loss was observed in FUra-treated mice with a maximum of 25% weight loss recorded in mice that received FUra at 350 mg/kg (Table 4). Although weight loss was observed in mice treated with FUra plus poly I-poly C, the amount of weight loss at any given dose of FUra, at any given time, was less in the group that received FUra plus poly I-poly C. For example, with FUra at 350 mg/kg, there was 25% weight loss on Day 6; but in the group in which poly I-poly C was added, FUra-induced weight loss was reduced to 14% (\( p < 0.01 \)). The same pattern of poly I-poly C-associated protection of peripheral WBC levels at 9 days after treatment, and ultimately of mortality, is evident in Table 4. Using the method of Litchfield and Wilcoxon (17), it was calculated from these mortality data that the LD10 of FUra (236 mg/kg; 95% confidence limits, 218 to 256) was increased approximately 50% (to 360 mg/kg; 95% confidence limits, 339 to 392) and that the LD50 of FUra (260 mg/kg; 95% confidence limits, 230 to 271) also was increased approximately 50% (to 400 mg/kg; 95% confidence limits, 367 to 436) when poly I-poly C was administered simultaneously with the FUra. This experiment was repeated with essentially identical results.

**Route of Administration and Dose Dependency.** Poly I-poly C was administered at varying dosages, 4, 1, and 0.25 mg/kg, either i.p. or i.v. simultaneously with FUra at 130 mg/kg weekly for 3 weeks. FUra at 130 mg/kg alone in this experiment resulted in depression of the WBC to 3,757/cu mm on Day 14 (compared to a pretreatment level of 16,644/cu mm in these mice, \( p < 0.001 \)), profound weight loss to 38%, and a final mortality of 100%. As may be seen from the results in Table 5, the protective effect of poly I-poly C was dose dependent, and similar protection was observed at any given dose with both the i.p. and i.v. route of administration. Statistically significant protection was manifest in terms of body weight, WBC, and mortality at poly I-poly C doses of 4 and 1 mg/kg, but not 0.25 mg/kg.

**DISCUSSION**

As indicated by data presented in Table 1, partially purified preparations of mouse interferon, administered at timed intervals during the 2-day period following the administration of a toxic dose of FUra, yielded significant protection from mortality in CDBF mice. Since it is known that nonproliferating cells exhibit diminished sensitivity to FUra as compared to proliferating cells (4), it appears likely that the decreased toxicity seen in these experiments can be ascribed to the antiproliferative action of interferon on normal target tissues of the host.
Protection against FUra-induced toxicity was also observed here when the interferon inducer poly I-poly C was administered with FUra. Protection was found to be dose dependent and was obtained when poly I-poly C was administered i.p. or i.v. A dose of 4 mg/kg produced almost total protection from a 3-course treatment with FUra at 130 mg/kg/week (Table 5). One week after the second course of treatment, WBC levels, although slightly depressed in mice treated with poly I-poly C plus FUra (11,244/cu mm), were significantly higher than in mice treated with FUra alone (3,757/cu mm). Body weight loss was only 5% compared to 38% in mice treated with FUra alone, and the 100% mortality of FUra was completely circumvented. A poly I-poly C dose of 1 mg/kg was only slightly less protective than was 4 mg/kg/week; however, substantial toxicity was obtained in mice treated with 0.25 mg/kg/week. Therefore, for all of the experiments with poly I-poly C, a dose of 4 mg/kg was used.

A titration of the toxicity of a single course of FUra, administered with and without poly I-poly C, revealed an increase of 50% in the LDR and in the LDC50 of FUra when it was administered in conjunction with poly I-poly C at 4 mg/kg. This represents a significant degree of protection for a drug with as steep a dose-toxicity response as that of FUra. At any one dose of FUra, poly I-poly C protection was noted in all 3 parameters of toxicity measured here, i.e., body weight change, WBC, and ultimate mortality.

Cytofluorograph measurements of propidium iodide-stained bone marrow cells obtained from mice at timed intervals after the administration of poly I-poly C indicate that poly I-poly C leads to inhibition of entry into the DNA-synthetic phase of the cell cycle with accumulation of cells in the G1 phase in one or more of the cell types comprising mouse bone marrow. From this result, it is not possible to determine if the cells accumulating in G1 were killed by poly I-poly C or represent viable cells in which DNA synthesis was merely inhibited. However, since earlier studies demonstrated the noncytotoxic nature of poly I-poly C-induced inhibition of colony-forming cell proliferation (10, 23) and since the coadministration of poly I-poly C and FUra does not lead to increased toxicity, but rather to a saving of bone marrow function in comparison with treatment with FUra alone, we conclude that the poly I-poly C is causing an inhibition of DNA synthesis without cytotoxicity. Further, the data indicate that DNA synthesis inhibition is transitory and that cells reenter the synthetic cycle at some time between 42 and 66 hr after administration of poly I-poly C. Although the accumulation of marrow cells in G1 and the reduction in the percentage of cells in S phase during the first 42 hr after poly I-poly C administration was statistically significant, the absolute magnitude of the changes observed was small (3 to 5%). However, if one considers that on the average only 13.5% of normal bone marrow cells were in the S phase of the cycle (Table 3), then the specific reductions of S-phase cells in marrow from poly I-poly C-treated mice were 21 and 32% at 18 and 42 hr, respectively (Table 3). Further, it is possible that inhibition of DNA synthesis in a particular subpopulation of marrow cells, such as granulocyte precursors, may be even more profound than indicated by cytofluorometric measurements because of the heterogeneity of the marrow cell population.

This data would tend to support the hypothesis that, if DNA synthesis was delayed in cells exposed to FUra for a sufficient time to allow for repair (presumably the generation of new, undamaged, molecules of RNA, and/or the synthesis of free thymidylate synthetase) before a critical phase in the proliferation cycle of the cell, then the lethal event would be circumvented. The temporal relationship between the administration of poly I-poly C and FUra was found to be a critical determinant of the intensity of toxic manifestations. In relation to FUra alone, poly I-poly C could enhance (when administered 48 hr before FUra), diminish (when administered together with FUra), or not affect (when administered 48 hr after FUra) the degree of resultant toxicity. These results may be understood in light of the kinetic effect of poly I-poly C on the phase cycling of bone marrow cells in these mice discussed above.

When poly I-poly C was given 48 hr before FUra, the period of DNA synthesis inhibition was ending when the FUra was administered, and in fact, greater numbers of cells were entering S phase. Cells entering S phase would be expected to have greater need for the synthesis of critical enzymes that may be blocked by the presence of FUra in RNA and greater need for thymidylate synthetase activity. Under such circumstances, FUra-associated toxicity would be expected to be increased, and results indicate that this indeed does occur. In contrast, when poly I-poly C was administered together with FUra, the approximate 48-hr period of DNA synthesis inhibition would be expected to permit substantial replacement of FUra-containing RNA as well as the synthesis of new molecules of thymidylate synthetase, resulting in a decrease in the toxic manifestations of FUra, and this result has been demonstrated consistently. When poly I-poly C was administered 48 hr after FUra, the FUra-associated damage already would have occurred, and therefore no effect would be expected, and none was observed.

To our knowledge, this is the first demonstration of the influence of interferon on the cytotoxic activity of FUra. As discussed above, the influence varied depending upon the temporal relationship between the administration of poly I-poly C and FUra; therefore, it appears that interferon can function as a positive or negative modulator of FUra activity. Because of the clinical importance of FUra in cancer therapy, this observation may have significant practical application; however, the utility of this approach will depend upon the existence of a differential in the antiproliferative susceptibility to interferon between tumor cells and normal host target cells. For example, if the tumor cells are not susceptible or are less susceptible to the antiproliferative action of interferon compared to normal host tissues, then the coadministration of poly I-poly C (or purified interferon) with FUra will permit the use of higher doses of FUra than normally tolerable, with a resultant increase in the therapeutic efficacy of FUra. If, in contrast, the tumor cells are more susceptible than normal host cells to the antiproliferative action of interferon, then, under circumstances where interferon is administered appropriately before FUra (e.g., 48 hr before FUra in this murine system), larger numbers of tumor cells would be reentering cycle at the time of administration of FUra (as compared to host cells); therefore, susceptibility to FUra would be specifically enhanced in tumor as compared to normal host tissues. However, if interferon does not exert differential antiproliferative activity in normal versus malignant cells, there is the danger that concomitant administration of interferon with FUra or with FUra-containing chemotherapeutic combinations (and possibly with other chemotherapeutic agents) may diminish the anticancer activity otherwise.
obtainable with those agents. These theoretical considerations currently being applied in CDF, mice bearing spontaneous breast tumors.

REFERENCES


Modulation of 5-Fluorouracil-induced Toxicity in Mice with Interferon or with the Interferon Inducer, Polyinosinic-Polycytidylic Acid
