Synergistic Antitumor Effect of Fluoropyrimidines and Polyinosinic-Polycytidylic Acid against L1210 Leukemia

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INTRODUCTION

5-Fluorouracil (FUra) has been widely used clinically in the chemotherapeutic treatment of gastrointestinal cancer (1). Although it is not highly efficacious as a single agent, its use in combination with other drugs or metabolites appears to yield an enhanced therapeutic effect (2-11). Recently, a report by Stolfi et al. (12) indicated that the toxicity to mice resulting from treatment with FUra could be alleviated significantly by coadministration of poly(I):poly(C). (2',5')oligo(A) synthetase activity, an indication of interferon activity, was markedly depressed in the spleen and bone marrow following treatment with FUrd; however, poly(I):poly(C) administered together with FUrd returned (2',5')oligo(A) synthetase activity to normal levels. These data indicate that poly(I):poly(C) ameliorates the host toxicity of fluoropyrimidines, possibly via an interferon-mediated effect, and thereby results in enhanced therapeutic efficacy of the antime-tabolite as an antitumor agent.

RESULTS

Antitumor Activity. Groups of mice bearing s.c. implanted L1210 leukemia cells were treated i.v. on days 1, 5, and 9 after tumor inoculation with varying doses of FUra (Chart 1) or FUrd (Chart 2) either alone or in combination with poly(I):poly(C), 5 mg/kg. In these experiments, the maximum ILS of mice treated with FUra or FUrd was 54 and 76%, respectively. Coadministration of poly(I):poly(C) with FUra or FUrd increased the ILS to 95 and 115%, respectively, and these effects were statistically significantly greater (P < 0.01) than optimal dosages of either fluoropyrimidine alone. The enhancement in the antitumor activity of FUra or FUrd produced by poly(I):poly(C) was particularly noticeable at high doses of fluoropyrimidine where the maximum tolerated dose was increased from 50 to 100 mg/kg for FUra

MATERIALS AND METHODS

Materials. [α-32P]ATP (760 Ci/mmol) was purchased from New England Nuclear, Boston, MA. Poly(I):poly(C) and poly(I):poly(C)-agarose were obtained from P-L Biochemicals, Milwaukee, WI. FUra and FUrd were provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, National Cancer Institute. Mouse interferon-α (2.6 × 10^6 units/mg) and mouse interferon-β (2.5 × 10^6 units/mg) were purchased from Lee Biomolecular, San Diego, CA, and recombinant mouse interferon-γ (2 × 10^6 units/mg) was obtained from Genetech, San Francisco, CA.

Mice. Groups of 5 or 6 mice (CDF1) weighing 20–23 g were given implants either s.c. or i.p. with 10^6 L1210 leukemia cells per animal.

Treatment. Twenty-four h after s.c. tumor implantation, poly(I):poly(C), FUra, FUrd, or the combination of poly(I):poly(C) and FUra or FUrd were injected i.v. Under conditions in which the tumor was implanted i.p., drugs were administered i.p. The average life span was determined as the average number of days that the mice in each group survived after tumor implantation. The percentage of ILS represents the percentage increase in life span of the treated group relative to the control (saline-injected) group.

FUrd Incorporation into RNA. FUrd (50 mg/kg) or FUrd and poly(I):poly(C) (5 mg/kg) was injected i.v. 24 h after s.c. tumor implantation. On the fifth day, mice were given injections of [3H]FUrd (2.5 mgCi/50 mg/kg) or labeled FUrd plus poly(I):poly(C). Twenty-four h after the last injection, mice were killed, and the spleen, bone marrow, and small intestine were removed and homogenized in 2 ml of RNA extraction buffer (0.02 M sodium acetate; 0.14 M NaCl; polyvinyl sulfate, 10 μg/ml; 0.2% sodium dodecyl sulfate) in a Polytron homogenizer (12). Tissue extracts were mixed with 2 ml of water-saturated phenol containing 0.1% 8-hydroxyquinoline; the aqueous phase was separated by centrifugation at 10,000 × g for 10 min at 4°, and RNA was precipitated at -20° with 3 volumes of 2% (w/v) potassium acetate in 95% ethanol.

(2',5')oligo(A) Synthetase. Tissues were homogenized using a Polytron homogenizer in a buffer containing: 20 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (pH 7.4), 5 mM MgCl2, 120 mM KCl, 1 mM dithiothreitol, 10% glycerol, and 0.5% Nonidet P-40. Extracts were centrifuged at 15,000 × g for 15 min at 4°, and the supernatant fluid was used to assay for (2',5')oligo(A) synthetase activity (13).
ANTITUMOR EFFECT OF FUra AND poly(l)-poly(C)

Chart 1. Effect of poly(l)-poly(C) on the antitumor activity of FUra against s.c. L1210 leukemia. Tumor-bearing mice were given injections i.v. on days 1, 5, and 9 with varying doses of FUra (□) or FUra + poly(l)-poly(C), 5 mg/kg (●). Each value is the mean of 5 mice, where the standard error varied from 2-4% of the mean.

Chart 2. Effect of poly(l)-poly(C) on the antitumor activity of FUrd against s.c. L1210 leukemia. Tumor-bearing mice were given injections i.v. on days 1, 5, and 9 with varying doses of FUrd (□) or FUrd + poly(l)-poly(C), 5 mg/kg (●). Each value is the mean of 5 mice, where the standard error varied from 2-4% of the mean.

and from 20 to 50 mg/kg for FUrd. Experiments were also conducted using i.p. administration of FUrd to mice bearing i.p. implanted tumor (Chart 3). In this instance, the maximally effective dose was increased from 10 to 25 mg/kg by coadministration of poly(l)-poly(C), but the increase in ILS was less impressive, namely, an increase of 22% at the optimal dose of FUrd for each treatment regimen.

The toxicity of FUrd and poly(l)-poly(C) in non-tumor-bearing mice was also evaluated (Chart 4). In agreement with data of Stolfi et al. (12), the mortality of mice treated with a toxic dose (100 mg/kg) of FUra was significantly reduced by concurrent treatment with 5 mg/kg of poly(l)-poly(C).

In other experiments, interferons-α and -β or interferon-γ were administered concurrently with FUrd (Table 1). Under these protocols, neither interferon alone increased the life span of tumor-bearing mice. However, the combination of interferons with FUrd or poly(l)-poly(C) produced a significant, albeit marginal, increase in life span. Using this injection schedule, 10^6 units each of interferon-α + interferon-β did not further increase the effect on life span in combination with FUrd (results not shown).

Incorporation of FUrd into RNA. To determine whether or not the incorporation of FUrd into RNA was affected by poly(l)-poly(C), tumor-bearing animals were treated on days 1 and 5 with FUrd alone or with poly(l)-poly(C) and administered [3H]-FUrd in addition to unlabeled drug on day 5. The spleen, bone marrow, and small intestine were removed 1 day after the last injection, and [3H]-FUrd incorporated into RNA from these tissues was measured. Unfortunately, there was no palpable tumor at this time interval, and thus, the amount of [3H]-FUrd incorporated into tumor RNA could not be assessed. As depicted in Chart 5, coadministration of poly(l)-poly(C) with FUrd did not appreciably change the amount of drug incorporated into RNA of the bone marrow or small intestine, but it reduced by 23% the amount of drug incorporated into spleen RNA.

(2',5')Oligo(A) Synthetase Activity. A sensitive indicator of
Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (days)</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.3 ± 0.2</td>
<td>0</td>
</tr>
<tr>
<td>poly(l)-poly(C)</td>
<td>11.6 ± 0.2</td>
<td>15*</td>
</tr>
<tr>
<td>FUrd</td>
<td>11.2 ± 0.3</td>
<td>14*</td>
</tr>
<tr>
<td>IFN-α + IFN-β</td>
<td>10.3 ± 0.3</td>
<td>0</td>
</tr>
<tr>
<td>IFN-β</td>
<td>10.7 ± 0.6</td>
<td>4</td>
</tr>
<tr>
<td>IFN-α + IFN-β ± FUrd</td>
<td>12.8 ± 0.5</td>
<td>25*</td>
</tr>
<tr>
<td>IFN-α + IFN-β ± poly(l)-poly(C)</td>
<td>11.7 ± 0.3</td>
<td>14*</td>
</tr>
<tr>
<td>IFN-β + FUrd</td>
<td>11.7 ± 0.3</td>
<td>14*</td>
</tr>
<tr>
<td>IFN-α + poly(l)-poly(C)</td>
<td>11.5 ± 0.3</td>
<td>12*</td>
</tr>
</tbody>
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\*Statistically significant difference (P < 0.01) versus control.

Chart 6. Effect of FUrd and poly(l)-poly(C) on the activity of (2',5')-oligo(A) synthetase. Mice bearing s.c. L1210 leukemia were treated i.v. on days 1 and 5 with saline, poly(l)-poly(C), FUrd, or poly(l)-poly(C) + FUrd. On day 6, organs were removed and assayed for synthetase activity as described under "Materials and Methods." Each value is the mean of 4 mice. Bars, SE; numbers in columns, percent of control. *, P < 0.05.

DISCUSSION

The present investigation was initiated to assess whether the synthetic double-stranded RNA, poly(l)-poly(C), could enhance the antitumor activity of FUra or FUrd. It is apparent that poly(l)-poly(C) increased both the maximum tolerated dose of FUra or FUrd and the percentage of ILS in mice bearing L1210 leukemia s.c. following i.v. administration of the drugs. This model was felt to be more realistic in comparison to the standard protocol of i.p. injection of drug in mice implanted with the tumor i.p. Indeed, in the latter instance, poly(l)-poly(C) did not increase the life span of mice as significantly as it did in animals bearing the tumor s.c. and treated i.v. with the drugs, although the maximum tolerated dose was also enhanced. Poly(l)-poly(C) also protected non-tumor-bearing mice from the mortality produced by FUra, which is in confirmation of similar studies with FUra by Stolfi et al. (12).

The studies of Stolfi et al. (12) indicated that the protective effect of poly(l)-poly(C) might be mediated by a transient inhibition of the cycling of the stem cells in the bone marrow (12). Our study suggests that poly(l)-poly(C) may be protecting the bone marrow and spleen from the deleterious action of FUrd.

In contrast, FUrd treatment caused a pronounced decrease in (2',5')-oligo(A) synthetase activity in the spleen and particularly in the bone marrow, which suggests pronounced cytotoxicity to these tissues. Treatment of the tumor-bearing mice with both poly(l)-poly(C) and FUrd resulted in normal synthetase activity; i.e., poly(l)-poly(C) negated the inhibitory effect of FUrd. No statistically significant changes in synthetase activity occurred in the small intestine, although FUrd treatment alone did depress enzyme activity by 50%.

The interaction of interferon with a cell is the induction of the double-stranded RNA-dependent (2',5')-oligo(A) synthetase (15, 16). Thus, tissue extracts of the spleen, bone marrow, and small intestine were assessed for this enzyme activity following treatment on days 1 and 5 with poly(l)-poly(C), FUrd, or the combination of the two agents (Chart 6). Poly(l)-poly(C) produced a 2-fold increase in synthetase activity in the bone marrow and spleen but not in the small intestine. In contrast, FUrd treatment caused a pronounced decrease in (2',5')-oligo(A) synthetase activity in the spleen and particularly in the bone marrow, which suggests pronounced cytotoxicity to these tissues. Treatment of the tumor-bearing mice with both poly(l)-poly(C) and FUrd resulted in normal synthetase activity; i.e., poly(l)-poly(C) negated the inhibitory effect of FUrd. No statistically significant changes in synthetase activity occurred in the small intestine, although FUrd treatment alone did depress enzyme activity by 50%.

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effect does not appear to be mediated by interference with the metabolism of FUrd into RNA found previously under in vitro conditions (17), since this parameter was not affected in the bone marrow or small intestine and was reduced by only 23% in the spleen. On the other hand, poly(I)-poly(C) prevented the reduction of (2',5')oligo(A) synthetase activity of FUrd in the spleen and bone marrow, which indicated that it ameliorated the lymphocyte toxicity produced by the drug. Since high synthetase activity is associated with interferon production, particularly in lymphocytes (18), these data suggest that poly(I)-poly(C) induced interferon synthesis in the spleen and bone marrow and thus protected these peripheral tissues from the cytolytic effects of FUrd. The marginal effect produced by direct intravenous administration of interferons may be due to their rapid inactivation in the circulation and poor distribution to the spleen and bone marrow. The fact that interferons-α and -β protected mice from the toxicity of FUra using a prolonged schedule of administration (12) suggests that the same may be true for enhancement of the antitumor activity of FUra by interferons.

Although we were unable to directly measure (2',5')oligo(A) synthetase activity in the tumor due to its effective eradication from the implantation site, it was reported previously that poly(I)-poly(C) was an effective inducer of synthetase activity in both the spleen and tumor of mice bearing L1210 leukemia implanted i.p. (19). Therefore, it is possible that interferon induction in the tumor may have produced some direct protective effect. On the other hand, administration of interferon-α, -β, or -γ did not markedly affect the antitumor activity of FUrd. Moreover, we have not found any species of interferon to be synergistic with FUra or FUrd against human tumor cells in vitro (17). Thus, we feel the evidence points to a peripheral effect of poly(I)-poly(C) to reduce the host toxicity produced by FUra or FUrd, thereby allowing a greater maximum tolerated dose to be administered to achieve a prolongation in the life span of the animal.

REFERENCES


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