Decreased Immunosuppression Associated with Antitumor Activity of 5-Deoxy-5-fluorouridine Compared to 5-Fluorouracil and 5-Fluorouridine

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ABSTRACT

5-Fluorouracil (5-FUra), 5-deoxy-5-fluorouridine (5’dFUrd), and 5-fluorouridine were compared for their relative antitumor activity, their capacity to inhibit leukocyte exudation and macrophage (M0) killing of tumor cells in vivo and in vitro, and their ability to induce leukopenia and monocytopenia. 5’dFUrd was less toxic than 5-FUra and exhibited anti-Ehrlich ascites activity over a wider range of drug doses. Inflammatory exudates induced by thioglycollate or pyran were inhibited up to 91% by 5-FUra injection but were inhibited not more than 62% by 5’dFUrd. Pyran-induced M0 inhibition of Ehrlich ascites proliferation in vivo was diminished up to 5-fold by 5-FUra but was never diminished more than 2-fold by 5’dFUrd, while neither agent suppressed in vitro M0 cytotoxicity of in vivo pyran-activated M0. At high doses, 5-FUra reduced white blood cell counts 73%, in contrast to the 8% reduction caused by 5’dFUrd, while at their optimal anti-Ehrlich ascites doses, 5-FUra and 5’dFUrd both lowered white blood cell counts by only 20%. However, 5-FUra caused a severe monocytopenia not seen in animals given injections of comparable doses of 5’dFUrd. Therefore, 5-FUra appeared to inhibit the inflammatory response and antitumor activity by inhibiting the influx of immature M0 into the peritoneal cavity, not by inhibiting the function of mature effector cells.

INTRODUCTION

It is possible that the ultimate success of cancer chemotherapy may be dependent upon the preservation of the patient’s immune system (6). In general, antitumor drugs are quite effective in reducing tumor burden. However, to reach a state of long-term remission, an intact immune system may be essential in eradicating residual drug-resistant tumor cells (14). A limited number of chemotherapeutic agents like cyclophosphamide (5), Adriamycin (19), and methotrexate (22) has been reported to actually enhance immune activity. Most anticancer drug regimens, however, do not enhance immune activity but suppress it to some degree (13). There is, however, a growing awareness that chemotherapeutic regimens must be effective enough to reduce tumor bulk but not so toxic that the hosts' immune response is eliminated. This approach has been maintained with varying degrees of success in studies involving melphalan (24), 6-mercaptopurine (25), and cyclophosphamide (20). In some cases, aggressive chemotherapy has been shown to have a positive therapeutic effect. This has, in part, been attributed to inhibition of humoral blocking reactions (12) or sensitive tumor-induced T-suppressor cells.

5-FUra, one of the principal drugs examined in this paper, is a toxic anticancer drug (27). It is rare that 5-FUra-induced immunosuppression has been minimized and antitumor activity maintained (26). The therapeutic effectiveness of 5-FUra, however, has been limited not only by its high level of toxicity (2) but also because of its immunosuppressive activity (7, 21). Unlike cyclophosphamide, which may act in apparent synergy with such immunoadjuvants as Corynebacterium parvum (23) or Bacillus Calmette-Guérin (10), 5-FUra-induced immunosuppression does not appear to be reversible by C. parvum treatment (10). Theoretically, the ideal fluoropyrimidine should possess the antitumor capabilities of 5-FUra without its inherent immunosuppressive qualities.

In this paper, "inflammation" is not measured in classic biochemical terms (i.e., prostaglandin release) but is defined as the influx of leukocytes into the peritoneal cavity following i.p. thioglycollate or pyran injection. Also, use of the term "immunocompetence" does not reflect a specific T- or B-cell response but is defined as pyran-mediated antitumor activity presumably involving the macrophage arm of host defense. With those definitions in mind, we have demonstrated that 5’dFUrd, an agent reported to have less toxicity and greater antitumor activity than other fluoropyrimidine drugs (2), is also far less immunosuppressive than 5-FUra or 5-Furid. This decreased immunosuppression may be in part accounted for its high level of antitumor activity and low level of toxicity.

MATERIALS AND METHODS

Animals. Male CF-1 mice or female C57BL/6J mice were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.), or The Jackson Laboratory (Bar Harbor, Maine).

Culture Medium and Target Cell Types. Culture medium used in all experiments was Eagle’s minimal essential medium containing 100 units of penicillin per ml, 100 μg of streptomycin per ml, and 20% fetal bovine serum. The Lewis lung carcinoma cell line was prepared as a target for in vitro cytotoxicity experiments by treatment with 0.25% trypsin con...
taining 0.02% EDTA for 10 min at 37°. After trypsinization, the cells were washed in Eagle’s minimal essential medium and resuspended at 5 × 10^6 cells/ml. The Ehrlich ascites tumor cell line used in in vivo experiments was serially passed by weekly inoculation of 1 × 10^6 cells into the peritoneal cavity of C57BL/6J or C3H/HeN mice.

Drugs. 5′dFUrd and 5-FUrd were supplied by Dr. W. E. Scott, Hoffman-LaRoche, Inc. (Nutley, N. J.). 5-FUra was purchased from Sigma Chemical Co. (St. Louis, Mo.). The purity of 5′dFUrd and 5-FUrd was established using high-pressure liquid chromatography. All drugs were dissolved in 0.15 m NaCl and used within 4 days. For short-term storage, drugs were kept in the dark at 4°. The drugs were used initially in a survival study over a range of antitumor doses. The optimal antitumor dose was fixed at the dose giving the highest percentage of tumor-free survivors and the lowest weight loss. Once the optimal antitumor dose of each drug was established, it was doubled to obtain the designated “high” dose and halved to obtain the “suboptimal” dose. “Low” dose was defined as one-tenth of the highest dose of drug. In Tables 2 to 5, the difference between the “high” and “optimal” 5-FUra doses was halved, making the high dose 37.5 mg/kg instead of 50 mg/kg. It was necessary to decrease the high dose of 5-FUra, because few animals survived the 5-FUra treatment at 50 mg/kg.

Survival Study for Fluoropyrimidine-treated Tumor Bearers. Male CF-1 mice were weighed and then given i.p. inoculations of 3.8 × 10^6 EATU cells on Day 0 (Table 1). An appropriate dose of fluoropyrimidine was injected i.p. on Days 1 to 7, and animals were weighed again on Day 8. All survivors were killed on Day 45 and examined for gross evidence of tumor. A swollen abdomen and an opaque peritoneal exudate were the clearest evidence of tumor growth. Morphological examination of the PEC was used to identify EATU cells.

PEC. PEC were removed from mice by lavage with 7 to 8 ml of 0.15 m NaCl or HBSS. To obtain an “activated” tumoricidal inflammatory exudate (Table 2), mice were given i.p. injections of 30 mg pyran per kg (NSC 46105; Ben Venue Laboratories, Inc., Bedford, Ohio) on Day 0. Pyran was dissolved in 0.15 m NaCl and used immediately. On Day 7, animals were killed, and the PEC were counted. If used in an in vitro cytotoxicity assay, cells were diluted and dispensed appropriately. To test fluoropyrimidine suppression of the inflammatory response, pyran-treated mice were given i.p. injections of 5-FUra, 5′dFUrd, or 5-FUrd on Days 0 to 3. To measure in vivo suppression of pyran-induced tumoricidal activity (Table 3), pyran was injected i.p. on Day 0, fluoropyrimidine drug was injected on Days 0 to 3, and EATU cells (1 × 10^6) were injected on Day 4. Animals were killed on Day 9.

In Vitro Determination of Antitumor Activity. To assay inhibition of AM0 tumoricidal activity in vitro (Table 4), inflammatory PEC from animals treated with pyran and fluoropyrimidine were obtained by peritoneal lavage with 8 ml of HBSS or 0.15 m NaCl. Cells were washed, resuspended in culture medium, and counted in a hemacytometer, and the mean was derived from the counts of 5 individual animals ± S.E. PEC (0.1 ml) were distributed into microtiter wells (Flow Laboratories, Hambden, Conn.) at a concentration of 1 × 10^4 cells/ml, 5 × 10^4 cells/ml, and 1 × 10^5 cells/ml. After a 60-min incubation at 37°, PEC in microtiter plates were washed vigorously with HBSS to remove nonadherent cells. These adherent PEC were then used in a 48-hr in vitro cytotoxicity test against [H]thymidine (6.7 Ci/mmol; New England Nuclear, Boston, Mass.)-labeled Lewis lung targets.

Lewis lung tumor targets (10^3 cells) were labeled overnight with 200 μCi of [H]thymidine. Targets were removed from flasks with 0.25% trypsin in phosphate-buffered saline. Cells were washed, counted, and dispensed in 0.2-ml aliquots at a concentration of 5 × 10^6 cells/ml into microtiter wells containing washed adherent PEC so that the final adherent PEC:target ratios were 10:1, 5:1, and 1:1. After a 48-hr incubation, 0.1 ml of supernatant was removed from each well, added to 10 ml of Biocount scintillation fluid (New England Nuclear), and counted in a liquid scintillation counter. To obtain “spontaneous release,” 0.1 ml of supernatant was removed from wells containing labeled targets in 0.2 ml of medium. To obtain maximum release, 0.1 ml of supernatant was removed from labeled targets in 0.2 ml of a 2% sodium dodecyl sulfate solution. In a morphological assay of tumoricidal activity, 5 × 10^4 Lewis lung tumor targets were plated over 1 × 10^6 PEC. After 72 hr, the slides were fixed, stained, and assessed morphologically for the suppression of tumor cell growth as described previously (16).

Staining Procedures. To determine the percentage of EATU cells in the peritoneal exudate of drug-treated, tumor-bearing controls (Table 3), PEC were stained for esterase activity with naphthyl butyrate and counter-stained with hematoxylin (Sigma). Three fields were read with 100 cells/field. EATU cells could be distinguished from host cells based on their multiple nucleoli, large nucleus, regular almost spherical shape,
and small size. In contrast, M02 possessed no nucleoli, a smaller bean-shaped nucleus, more cytoplasm, and an irregular shape. To determine the percentage of M02 in the peritoneal exudate of animals treated with pyran and fluoropyrimidine (Table 4), a cytocentrifuged cell preparation was fixed with methanol and stained with “Camco Quik Stain” (Scientific Products, Malaw Park, Ill.).

To assess leukopenia and monocytopenia in animals treated with pyran and fluoropyrimidine (Table 5), 20 μl of blood were removed by retroorbital bleeding with a capillary pipet. The blood was diluted with 10 ml of “Isoton” (Coulter Diagnostics, Hialeah, Fla.). Two drops of “Zap fluid” was sometimes tinged with red from bleeding. and lacked a cloudy peritoneal exudate although the peritoneal fluid was sometimes tinged with red from bleeding.

To compare the relative immunosuppressive properties of 5-FUra, 5′dFUrd, and 5-FUrd, it was first necessary to establish the optimal antitumor dose for each drug. Table 1 represents the results from a survival study using tumor-bearing mice given injections of varying concentrations of fluoropyrimidines. The optimal antitumor dose was defined as that drug dose which resulted in the greatest number of tumor-free survivors and the lowest level of host toxicity as measured by weight loss. For 5-FUra, the optimal antitumor dose was 25 mg/kg, although only 3 of 8 (37%) animals survived under this drug regimen. In contrast, the optimal 5′dFUrd dose of 200 mg/kg led to a survival rate of 88% (8 of 9 tumor-free survivors). 5-FUrd had the lowest level of host toxicity as measured by weight loss. For 5-FUrd, the optimal antitumor dose was 25 mg/kg, although only 3 of 8 (37%) animals survived under this drug regimen. In contrast, the optimal 5′dFUrd dose of 200 mg/kg led to a survival rate of 88% (8 of 9 tumor-free survivors). 5-FUrd had the lowest level of host toxicity as measured by weight loss. For 5-FUrd, the optimal antitumor dose was 25 mg/kg, although only 3 of 8 (37%) animals survived under this drug regimen. In contrast, the optimal 5′dFUrd dose of 200 mg/kg led to a survival rate of 88% (8 of 9 tumor-free survivors). 5-FUrd had the lowest level of host toxicity as measured by weight loss. For 5-FUrd, the optimal antitumor dose was 25 mg/kg, although only 3 of 8 (37%) animals survived under this drug regimen. In contrast, the optimal 5′dFUrd dose of 200 mg/kg led to a survival rate of 88% (8 of 9 tumor-free survivors). 5-FUrd had the lowest level of host toxicity as measured by weight loss.

RESULTS

Antitumor Dose Response for 5-FUra, 5′dFUrd, and 5-FUrd.

To measure cytotoxicity (Table 4)

\[
\text{% of cytotoxicity} = \left( \frac{\text{test cpm} - \text{spontaneous release}}{\text{maximum cpm} - \text{spontaneous release}} \right) \times 100
\]
Table 4

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug dose (mg/kg)</th>
<th>Total PEC counts/animal (&lt;10^6)</th>
<th>% of antitumor activity in vitro ([1]H]thymidine release for each effector:target ratio</th>
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</thead>
<tbody>
<tr>
<td>Resident</td>
<td>1.3 ± 0.1b</td>
<td>NDc</td>
<td>ND, ND, ND</td>
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<td>0.9% NaCl solution</td>
<td>6.8 ± 1.0</td>
<td>22.3 ± 1.1d</td>
<td>22.4 ± 4e, 5.7 ± 3.9</td>
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<td>Pyran</td>
<td>13.6 ± 1.8</td>
<td>19.2 ± 1.2f</td>
<td>ND</td>
</tr>
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<td>5'-FUra + pyran</td>
<td>High (37.5)</td>
<td>8.7 ± 0.7f</td>
<td>18.5 ± 1.2f, 11.8 ± 3.3e, 2.8 ± 1.0</td>
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<tr>
<td>5'-dFUrd + pyran</td>
<td>High (400)</td>
<td>2.0 ± 0.5g</td>
<td>22.0 ± 1.2f, 21.5 ± 3.1e, 0</td>
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<tr>
<td>5'-FUrd + pyran</td>
<td>High (5.0)</td>
<td>7.9 ± 0.4f</td>
<td>36.2 ± 2.9f, 26.1 ± 5.1f, 5.7 ± 1.2</td>
</tr>
<tr>
<td>5'-FUrd + pyran</td>
<td>Optimal (25)</td>
<td>12.6 ± 1.1f</td>
<td>19.8 ± 1.7f, 14.6 ± 2.8f, 13.9 ± 3.1</td>
</tr>
<tr>
<td>5'-FUrd + pyran</td>
<td>Optimal (200)</td>
<td>4.1 ± 1.6f</td>
<td>26.1 ± 2.3f, 16.0 ± 3.5, 5.3 ± 3.3</td>
</tr>
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</table>

a Lewis lung tumor targets (10^6 cells) were labeled overnight with 200 μCi of [1]H]thymidine (6.7 Ci/mmol). Targets were removed from flask with 0.25% trypsin in phosphate-buffered saline. Lewis lung tumor targets (1 x 10^6) were washed and dispersed in microculture wells with various ratios of adherent PEC cells. Each well contained a volume of 0.2 ml Eagle's minimal essential medium: 20% fetal bovine serum. After a 48-hr incubation, 0.1 ml was removed from each well and counted in a scintillation counter. Each number represents the mean of 6 wells.

% of cytotoxicity = (test (cpm) - spontaneous release) / (maximum release (cpm) - spontaneous release) × 100

Results of 4 experiments with 3 to 6 replicates/group. Female C57BL/6J mice were given i.p. injections of pyran (30 mg/kg) on Day 0. Drugs were injected on Days 0 to 3. Animals were killed on Day 7. PEC were obtained by peritoneal lavage with 8 ml of HBSS. Cells were washed and resuspended in Eagle's minimal essential medium: penicillin: streptomycin: glutamine: 20% fetal bovine serum. A 0.1-ml volume of PEC was distributed into microtiter wells at a concentration of 1 x 10^6 cells/ml 5 x 10^6 cells/ml and 1 x 10^6 cells/ml. After a 60-min incubation at 37°, PEC in microtiter plates were washed vigorously with HBSS to remove nonadherent cells. These adherent PEC were then used in a 48-hr in vitro cytotoxicity test against [1]H]thymidine-labeled Lewis lung tumor targets. At least 3 fields were averaged with 100 cells/field in a Wright's stain differential to obtain percentage of macrophages. The percentage of macrophages in the whole PEC population ranged from 64% (for the resident population) to 95% (for the pyran-treated population).

5'-dFUrd. Note that 5'-dFUrd had considerable antitumor activity at all drug doses tested. Even at the low 40-mg/kg dose, 5'-dFUrd treatment led to a 25% survival rate. At the highest 400-mg/kg dose of 5'-dFUrd, only 2 of the 9 animals tested died from drug toxicity; the other 77% remained tumor free. On the other hand, because of its cytotoxicity, 5'-FUra treatment provided no survival优势 at all drug doses tested. Even at the low 40-mg/kg dose, 5'-dFUrd had considerably less toxicity than the high drug dose. At the 5-FUra suboptimal 25-mg/kg dose, 6 of the 8 (75%) mice died from tumor burden, while the comparable suboptimal 100-mg/kg dose of 5'-dFUrd produced 5 of 9 (55%) tumor-related deaths. Thus, 5'-dFUrd appeared to be less toxic at the high dose and also more tumoricidal at the lower doses.

Immunosuppression of the PEC Inflammatory Response by 5'-FUra, 5'-dFUrd, and 5'-FUrd. In our in vivo model of antitumor activity, the target was an EATU line. Since it is an ascites tumor, the PEC population of the host played a major role in controlling tumor growth after treatment with pyran (9). Therefore, we felt that a relevant parameter of drug-induced immunosuppression would be the measurement of the PEC inflammatory response in fluoropyrimidine-treated mice. Pyran was the inflammatory agent injected i.p. to induce the influx of tumoricidal "activated" M0. Inhibition of the inflammatory response was measured by comparing the control inflammatory response to the PEC count of animals treated with fluoropyrimidine drug and an inflammatory agent. In all cases, fluoropyrimidine inhibited the inflammatory response to some degree (Table 2). Similar results were found in drug studies using thioglycollate as the inflammatory agent (data not shown).

5-FUra inhibition at the optimal dose was more than 3 times that of 5'-dFUrd (77% versus 22%). At no dose of 5-FUra was the PEC count equal to that of the pyran control. In contrast, the PEC count was higher than that of the pyran control in 3 of the 4 doses of 5'-dFUrd tested.

The relatively low level of immunosuppression for some doses of 5-FUrd may arise from need to use the drug at very low doses. Because of its extreme toxicity, a nonlethal dose had not only little antitumor activity but also little immunosuppressive activity. in Vivo Immunosuppression of Pyran-mediated Antitumor
Activity by Fluoropyrimidines. Since 5-FUra strongly inhibited pyran-induced inflammatory activity in vivo (Table 2), we next asked whether it could also suppress pyran-induced antitumor activity in vivo. Mice were given i.p. injections of pyran on Day 0. Beginning on Day 0, animals received the fluoropyrimidine for 4 consecutive days. Twenty-four hr after the final drug injection, each group of 5 animals was given i.p. injections of EATU cells. Five days later, the animals were killed and lavaged, and the PEC from each individual animal were counted on a Coulter Counter.

Since preliminary studies at optimal and suboptimal doses of fluoropyrimidines showed 5-FUra to be more immunosuppressive than 5’dFUrd (data not shown), the comparison was expanded in several ways (Table 3). We compared 3 fluoropyrimidines (5-FUra, 5’dFUrd, and 5-FUrd) at 4 drug doses (high, optimal, suboptimal, and low). Slides of the PEC were stained for non-specific esterase activity to determine the percentage of EATU cells.

It was necessary to ascertain that each control group contained approximately the same percentage of tumor cells. In all cases, morphological determination indicated that drug-treated PEC control populations were composed of 85 to 95% EATU cells. This is in keeping with the immunofluorescence data (9) indicating that the PEC from mice killed 5 days after EATU cell (1 x 10⁶) injection contained between 90 and 95% EATU cells. A separate drug control was run at each dose of fluoropyrimidine tested, since the drug still possessed some antitumor activity on EATU cells injected 24 hr after the final drug injection. The average PEC count of the untreated tumor-bearing control was 11.7 ± 10², while the mean PEC cell count of the pyran-treated, tumor-bearing group was 1.1 ± 10². 5’dFUrd was less immunosuppressive than 5-FUra at all drug doses. Although the trend is evident at the higher drug doses, the contrast between the

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug dose (mg/kg)</th>
<th>% of suppression of the pyran inflammatory response</th>
<th>WBC counts/ml of blood (x 10⁹)</th>
<th>MD</th>
<th>Promonocytes</th>
<th>Lymphocytes</th>
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<td></td>
<td></td>
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<tr>
<td>Pyran</td>
<td></td>
<td></td>
<td>2.6 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>5-FUra</td>
<td>High (37.5)</td>
<td>0.7 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11 ± 2</td>
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<td>5’dFUrd</td>
<td>High (400)</td>
<td>2.4 ± 0.3</td>
<td>&lt;1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5-FUrd</td>
<td>High (5)</td>
<td>0.8 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>5-FUra</td>
<td>Optimal (25)</td>
<td>2.1 ± 0.5</td>
<td>6 ± 2</td>
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<td>5’dFUrd</td>
<td>Optimal (200)</td>
<td>2.1 ± 0.4</td>
<td>12 ± 2</td>
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<td>5-FUrd</td>
<td>Optimal (2.5)</td>
<td>2.2 ± 0.5</td>
<td>7 ± 2</td>
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<sup>a</sup> % of suppression = (1 - PEC counts of drug treated/PCE counts of untreated) x 100

<sup>b</sup> Twenty μl of blood were removed by orbital plexus bleeding using a calibrated micropipet. This was diluted in 10 ml of Isoton. After adding 2 drops of "Zap Isoton" to lyse erythrocytes, duplicate WBC counts were made on a Coulter Counter. All groups consisted of 5 animals, and the WBC of each individual counted arrived at the mean.

<sup>c</sup> In each group, 3 individual blood smears were made. Slides were stained with "Cameo Quik Stain," and the slides were assessed for percentage of lymphocytes, monocytes, and promonocytes. Percentage was based on 100 WBC/slide. Numbers represent the mean.

<sup>7</sup> When compared to pyran control or 5’dFUrd, p < 0.05 as shown by Student’s t test.
In Vivo-activated Macrophage Antitumor Activity following in Vivo Treatment with 5-FUra, 5’dFUrd, and 5-FUrd. In Table 3, we demonstrated that 5-FUra inhibited pyran-induced antitumor activity in vivo. Results from Table 4 showed that drug-mediated inhibition of the pyran-induced inflammatory response did not inhibit the ability of the remaining activated MØ to kill Lewis lung tumor cells in vitro. Lewis lung cells were used as targets to confirm the nonspecificity of PEC tumoricidal activity. These targets are also more amenable to in vitro AMØ-mediated lysis than is our EATU cell line. Although animals treated with pyran plus the high 5-FUra dose had 20 times fewer PEC than did the pyran control (5 X 10⁶ versus 1.3 X 10⁸), in vivo antitumor activity of the 5-FUra treated, pyran-activated MØ was actually superior to that of the pyran control. An in vitro morphological determination of antitumor activity using the 81-sq mm chambers of an 8-chambered slide (Lab-Tek Division, Naperville, Ill.) gave comparable results to the [³H]thymidine release study (data not shown). Since fluoropyrimidines did not directly affect the activity of mature activated MØ, and since 5-FUra reduced the number of PEC in the inflammatory exudate, it followed that 5-FUra may exert its immunosuppressive influence by inhibiting the influx of immature cells into the peritoneal cavity.

Leukopenia and Monocytopenia in Pyran-treated Mice Given Injections of 5-FUra, 5’dFUrd, and 5-FUrd. If 5-FUra-induced immunosuppression operated either directly or indirectly at the stem cell level, then leukocyte counts in the peripheral blood would be expected to be reduced. Therefore, we gave mice injections of pyran and fluoropyrimidines and determined their PEC and WBC counts (Table 5). Like the 5-FUra-mediated suppression of the inflammatory response, 5-FUra treatment reduced the percentage of monocytes to <1 compared to the normal percentage of monocytes following 5’dFUrd treatment. At the high dose of 5-FUra, the 97% reduction in PEC counts was also paralleled by a reduction in the number of peripheral blood leukocytes. In contrast, the high dose of 5’dFUrd yielded only a 32% decline in PEC counts and an 8% reduction in the number of peripheral blood leukocytes. At the high dose of drug, there seemed to be a relationship between leukopenia and the reduction of PEC in the inflammatory exudate. This relationship did not hold true at the optimal drug dose where 5-FUra and 5’dFUrd inhibited the inflammatory response by 95 and 3%, respectively, while leukocyte counts were only reduced by ~20% for each drug. While the leukocyte counts showed that both drugs induced only a moderate reduction in WBC counts (2.1 X 10⁶ cells/ml), differential blood counts indicated that animals treated with an optimum 5-FUra dose continued to maintain a greatly reduced monocyte count compared to that of animals treated with 5’dFUrd. Therefore, inhibition of the pyran-induced inflammatory response appeared to be linked not so much to gross leukopenia but rather, more specifically, to monocytopenia.

DISCUSSION

This study was designed to compare the immunosuppressive activity of 5’dFUrd and 5-FUra at a range of antitumor doses. Previous papers from our laboratory (1, 2) and others (8, 17) have established the superior antitumor activity of 5’dFUrd over 5-FUra. Although studies have shown that 5-FUra is immunosuppressive at the T-cell (7), B-cell (7), and MØ level (11), few have compared the level of 5-FUra-mediated suppression to that induced by 5’dFUrd. Because of the data indicating that successful chemotherapy is often associated with an intact immune response (6), it is important to test possible chemotherapeutic agents for their immunosuppressive potential as well as for their antitumor activity. Ohta et al. (21) have examined 5-FUra-mediated immunosuppression of the T- and B-cell response and demonstrated that 5-FUra, when compared to 5’dFUrd, was a far stronger inhibitor of tumor allograft rejection, delayed-type hypersensitivity, and the antibody response to sheep erythrocytes. In this paper, we examined the MØ arm of host cell immunity and found, like Ohta et al. (21), that 5’dFUrd was less immunosuppressive than 5-FUra while possessing less toxicity and an antitumor activity over a wider range of drug dose.

MØ function was a very relevant measure of immunocompetence since, in a previous paper (9), we showed that AMØs were involved in in vivo antitumor activity against EATU cells. In this current paper, 5-FUra inhibited the PEC inflammatory response far more than did 5’dFUrd (Table 2). The 50 to 90% inhibition of pyran-induced inflammation (Table 2) is of importance, since pyran induces MØ-mediated in vivo tumoricidal activity (9). 5-FUra abrogation of the pyran-mediated inflammatory response was paralleled by in vivo suppression of pyran-mediated antitumor activity (Table 3). A survival study using drug plus pyran-treated tumor bearers (Table 3) also indicated that 5-FUra inhibited pyran-mediated antitumor activity to a greater degree than did 5’dFUrd. The differences are most pronounced at the lower drug doses, but it must be remembered that 5’dFUrd possessed antitumor activity even at the lowest drug dose tested. In a different model of immunosuppression, Ohta et al. (21) demonstrated that 5-FUra abrogated tumor cell allograft rejection. Although inoculation of tumor cells into fluoropyrimidine-treated controls was not begun until 24 hr after the last drug injection, these controls invariably showed some tumor inhibition when compared to tumor bearers receiving no treatment. This was surprising in light of the short in vivo half-life of fluoropyrimidines (18). Perhaps fluoropyrimidines trigger the release of some other toxic product which inhibits tumor proliferation temporarily or acts on a suppressor cell population, thereby allowing the immune response to react to the primary tumor graft. Residual metabolized fluoropyrimidine may also kill cells directly from the initial tumor cell inoculum. Peritoneal exudates from these fluoropyrimidine-treated, tumor-bearing controls were still predominantly EATU cells as determined morphologically by staining for esterase activity and counterstaining with hematoxalin (Table 3). Although the reason for the slightly reduced number of tumor cells in the fluoropyrimidine-treated controls is unknown, the crucial point is the contrast between the potent suppression of pyran-induced antitumor activity of 5-FUra and the lower level of immunosuppression of 5’dFUrd (Table 3). The survival study of tumor bearers treated with pyran and fluoropyrimidine also indicated that 5’FUrα inhibited the antitumor activity of pyran to a greater degree than did a comparable dose of 5’dFUrd (Table 3). Since 5’dFUrd did not abrogate antitumor activity in vivo (Table 3), it was expected that PEC from animals treated with pyran and 5’dFUrd would express antitumor activity in vitro (Table 4). What appeared unexpectedly was the in vitro antitumor activity of 5-FUra-treated inflammatory PEC. Apparently, 5-FUra blocks in vivo antitumor activity by decreasing the influx of immature cells into the peritoneal cavity rather than inhibiting the
antitumor activity of either the resident cells or the limited number of drug-treated, pyran-induced inflammatory cells. Thus, the animal succumbs to a tumor burden which overwhelms the few immunocompetent inflammatory PEC. To decrease the influx of cells into the inflammatory exudate, it was likely that 5-FUra suppressed a sensitive leukocyte precursor population, thus resulting in a decreased WBC count. This leukopenia occurred at the high 5-FUra dose (Table 5) in agreement with results from others (8, 21). In contrast, at the optimal antitumor dose of 5-FUra, the 95% inhibition of the inflammatory response correlated not with leukocyte reduction (~20%) but more closely with the decrease in monocytes (55%). Monocytopenia, as opposed to leukopenia, was not addressed by others in previous work (8, 21), because either no differentials were performed on peripheral blood smears, or the 5-FUra concentration was high enough to eliminate all leukocyte precursors. The data in this paper are consistent with the conclusion that 5-FUra acts at the stem cell level to eliminate the pool of immature, potentially inflammatory cells, thus abrogating antitumor AMØ activity in vivo. Alternatively, 5-FUra may not act at the stem cell level but may either inhibit release of promonocytes into the circulation or selectively act on monocytes in the peripheral blood. 5’dfUrd seems to be less toxic for MØ precursors and therefore has little or no effect upon the MØ inflammatory responses or its antitumor activity. This conclusion is appealing when the antitumor mechanisms of 5’dFUr and 5-FUra are examined.

5-FUra, when phosphorylated, can be converted into ribonucleotides which inhibit DNA synthesis and/or RNA function (27). However, 5’dFUr lacks the necessary hydroxyl group needed for phosphorylation. To manifest antitumor activity, it must be broken down into 5-FUra by a nucleotide phosphorylase (15). We have found 5’dFUr previously to be a “prodrug,” since we have been unable to detect active metabolites other than those derived from 5-FUra (2, 3). Therefore, the pharmacological effect of 5’dFUr is contingent upon the presence of host cell uridine phosphorylase which is present in high levels in EATU cells (2). In this regard, we have shown recently that bone marrow cells accumulate 5’dFUr but fail to metabolize it; presumably, they lack the necessary enzyme (4). Thus, the absence of MØ suppression following 5’dFUr injection may stem from the inability of MØ precursors to break 5’dFUr down into 5-FUra. The readiness with which EATU cells metabolize the drug makes 5’dFUr a desirable chemotherapeutic agent with high antitumor activity and low immunosuppressive activity.

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REFERENCES


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Decreased Immunosuppression Associated with Antitumor Activity of 5-Deoxy-5-fluorouridine Compared to 5-Fluorouracil and 5-Fluorouridine
