5-Ethynyluracil (776C85): Modulation of 5-Fluourouracil Efficacy and Therapeutic Index in Rats Bearing Advanced Colorectal Carcinoma

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ABSTRACT

5-Ethynyluracil (EU; 776C85) is a potent inactivator of dihydropyrimidine dehydrogenase, the enzyme that rapidly degrades 5-fluourouracil (FUra). We have investigated the antitumor activity and toxicity of FUra alone and in combination with EU in rats bearing advanced colon carcinoma. Two schedules were studied: (a) FUra daily for 4 days i.v. push (daily × 4); and (b) FUra administered i.v. push weekly for 3 weeks (weekly × 3). EU was administered at 1 mg/kg 1 h before FUra and for two additional days post-FUra therapy. The maximum tolerated doses of FUra alone were 35 and 100 mg/kg/day and for FUra plus EU were 10 and 15 mg/kg/day for the daily × 4 and weekly × 3 schedules, respectively. The dose-limiting toxicities were diarrhea and stomatitis both for FUra alone and for FUra in combination with EU. Although EU was not toxic and not active as an antitumor agent, it markedly improved the efficacy and therapeutic index of FUra. The antitumor activity of FUra was schedule dependent, yielding 13% complete and sustained tumor regression on the weekly schedule and no complete and sustained tumor regression on the daily schedule. The combination of FUra and EU produced 100% complete and sustained tumor regression on both schedules. The therapeutic index was 8 ± 1 for FUra alone and 6 for FUra with EU. EU was considerably more effective than either leucovorin or N-(phosphonacetyl)-L-aspartate as a modulator of FUra. Leucovorin or N-(phosphonacetyl)-L-aspartate induced minimum improvements on the daily schedule and only increased the therapeutic index to 1.5 on the weekly schedule. Because a 4-day continuous infusion of FUra alone at the maximum tolerated dose did not improve FUra therapy, we conclude that the improvements by EU involve additional modulations that complement the enhanced exposure of FUra.

INTRODUCTION

FUra has been used for more than 30 years for the treatment of various types of cancers (1–4). It remains the standard chemotherapy for gastrointestinal cancer, in particular colorectal cancer, although the response rate is only 15–20% (5–7). Investigators have attempted to biochemically modulate the metabolism of FUra to improve its antitumor activity. Early studies showed that thymidine, uridine, allopurinol, hydroxurea, and dipyridamole provided limited benefit (8–12). More recently, PALA and leucovorin have been shown to more successfully modulate FUra (13–17). PALA improves FUra therapy by increasing the level of FUra incorporated into cellular DNA (18, 19), whereas LV provides a source of reduced folate to stabilize the ternary complex with fluorodeoxyuridine monophosphate and thymidylate synthase, yielding greater inhibition (20, 21).

FUra is metabolized predominately by the catabolic pathway, which degrades over 90% of the injected dose (22). DHPDase (uracil reductase; EC 1.3.1.2), the first enzyme of this pathway, catalyzes the rapid reduction of FUra. DHPDase is found in a variety of human tissues including kidney, liver, lung, and intestinal mucosa (23, 24). Because the highly variable catalytic activity of DHPDase correlates with the rate of FUra clearance (25), inhibitors of DHPDase can have a profound effect on the pharmacokinetics of FUra.

EU, an irreversible inhibitor of DHPDase and (E)-5-(2-bromovinyl)-2'-deoxyuridine, a produg of BUra, increases the therapeutic activity of FUra against s.c. implants of MOPC-315 and adenocarcinoma 755 tumor cells in mice (26, 27). EU is a mechanism-based, irreversible inactivator of DHPDase with considerably greater potency than BUra (28, 29). An oral dose of 1.8 µg/kg in rats inactivates 50% of liver DHPDase (29). EU at 1 mg/kg provides complete, protracted inactivation of DHPDase and thereby preserves systemically administered FUra for at least 6 h (30). EU also increases the therapeutic index of FUra 2- to 4-fold in two tumor-bearing mouse models (30).

In the present study, we evaluated EU as a modulator of FUra in rats bearing advanced colorectal carcinoma. The results were even more striking with these larger animals (30). EU greatly improved the efficacy and the therapeutic index of FUra and was considerably better than either LV or PALA as a modulator. These findings were presented in part at a recent American Association for Cancer Research meeting (31).

MATERIALS AND METHODS

Rats. Six- to 7-week-old female Fischer 344/HSD rats (body weight, 150–200 g) were obtained from Harlan (Indianapolis, IN) and kept four per cage with water and food ad libitum according to an institutionally approved animal protocol.

Tumor. The chemically induced Ward colorectal carcinoma was used in this study (32). Nonnecrotic tumor pieces (0.1 g) were transplanted s.c. via trocar under slight ether anesthesia. Treatment was initiated 14 to 16 days later when tumor sizes were approximately 2.5–3.0 g. Four rats were used per experiment with 10–12 points per treatment group. The data points represent the average of all experiments.

Chemotherapeutic Agents. FUra was purchased from Hoffmann-La Roche Inc. (Nutley, NJ). The stock solution of 50 mg/ml was diluted in sterile 0.9% NaCl. Rats were dosed by i.v. push (or by continuous infusion where specifically indicated). EU was synthesized at Burroughs Wellcome Co. (Research Triangle Park, NC). Stock solutions of 0.25 mg/kg of EU were prepared by adding sterile saline to dry EU, adjusting the pH to 10 with NaOH, and sterile filtering. EU was stable for one month at 4°C. In an experiment involving other modulators, PALA (100 mg/kg) was administered i.v. push 24 h prior to FUra on the daily × 4 schedule and weekly × 3 schedule; LV (200 mg/kg) was administered by 2-h infusion daily × 4 or weekly × 3 with FUra administered i.v. push at 1 h of the LV infusion.

Treatment Schedules. Two schedules were used. FUra was administered i.v. push daily (or continuous infusion (33) for 4 days (daily × 4) and by i.v push weekly for 3 weeks (weekly × 3). EU (1 mg/kg) was administered i.p. 1 h before FUra and for an additional 2 days after FUra therapy for both schedules.

MTD and MED. The MTD was defined as the maximum dose that did not cause drug-related lethality in tumor-bearing rats. The MED was defined as the dose where 10 to 25% of rats bearing colon carcinoma have sustained CR on day 90 posttreatment.
Tumor Measurements and Body Weights. Two axes (mm) of tumor (L, longest axis; W, shortest axis) were measured with the aid of a Vernier caliper. Tumor weight (mg) was estimated as

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\text{Tumor weight} = \frac{1}{2}(L \times W^2)
\]

Tumor measurements were taken at least three times a week during the first 3 weeks and once a week thereafter. For each experiment, the same observer made all measurements to minimize variations in caliper measurements. As a general policy, rats were sacrificed when the tumor size exceeded 10 g. As an indication of drug toxicity, body weights of the animals were also recorded at the time of tumor volume measurement.

Tumor Regression. CR was defined as the inability to detect tumor by palpation at the initial site of tumor appearance for more than 90 days posttherapy. Rats with CR were sacrificed 3 months after achieving CR. Tumor regrowth after CR occurred in less than 5% of rats. The regression rate was expressed as the percentage of animals in the group. All studies were performed in accordance with IACUC guidelines and under the approved Roswell Park Cancer Institute protocol 208R.

RESULTS

Toxicity of FUra ± EU. To identify the MTD of FUra with or without EU, rats were treated with different FUra doses on the daily × 4 and weekly × 3 schedules. FUra produced steep dose-response lethality curves with both schedules but was toxic at lower doses on the daily schedule (Fig. 1). EU shifted the dose-response curve to lower doses on both schedules. However, EU did not alter the pattern of reversible toxicity. The dose-limiting toxicities for some animals at the MTD were reversible stomatitis on the daily schedule and reversible diarrhea on the weekly schedule for FUra with or without EU. Furthermore, reversible weight loss occurred at the MTD and lower doses of FUra with and without EU (Figs. 2 and 3).

Effect of EU on the Antitumor Efficacy and Therapeutic Index of FUra. To identify the MED of FUra and the efficacy at the MTD, antitumor activity was evaluated at various doses of FUra with or without EU. The data in Figs. 2 and 3 indicate that EU, which exhibited neither toxicity nor intrinsic antitumor activity, greatly improved FUra therapy.

On the daily × 4 schedule, FUra alone at 35 mg/kg/day, the MTD and the most effective dose, produced partial responses in 75% of the treated animals. However, the tumors regrew in all of these animals to the extent that animals had to be sacrificed. In contrast, in 100% of the animals pretreated with EU, FUra at 3.5 mg/kg produced complete tumor regression that was sustained for at least 90 days posttherapy (CR).

FUra was slightly more effective on the weekly × 3 schedule, producing 13% CR at the MTD (100 mg/kg/day). Again, EU increased the CR to 100% at 5 mg/kg/day of FUra. The dose-response of FUra in animals pretreated with EU is summarized in Table 1. Note that, although EU was dosed i.p. for these experiments, 100% CR was also produced for p.o. or i.v. administered EU in a study of 10 mg/kg/day FUra on the weekly schedule (data not shown).

We also dosed FUra by continuous infusion for 4 days to test whether the improvements by EU were simply the result of prolonged FUra exposure, as EU increases the plasma half-life of FUra by approximately 10-fold (30). Continuously infused FUra produced 14% CR at the MTD (35 mg/kg/day), which was similar to the CR rate on the weekly × 3 schedule but was far short of the results with EU modulation. The efficacy and therapeutic index, (MTD: MED ratio) for all regimens is summarized in Table 2. As a single agent, FUra had a therapeutic index of 1 or less. EU increased the index to 6 on both schedules.

Studies with LV and PALA. LV and PALA were also studied as modulators of FUra. Although LV added no benefit to the daily schedule, it did increase FUra CR rate to 63% on the weekly schedule. PALA increased the CR of FUra at the MTD to 13 and 75% on the daily and weekly schedules, respectively. PALA and LV increased the therapeutic index of FUra to 1.5 on the weekly schedule (Table 2).
treatment group had four rats and was the average of three to five experiments. Control
FUra ± EU. O, saline control; •, EU 1 mg/kg; A, FUra 10 mg/kg (MTD); V, FUra 1.25
mg/kg; •FUra 10 mg/kg + EU 1 mg/kg; A, FUra 15 mg/kg + EU 1 mg/kg (MTD). Each
represents 20 rats.

We also studied the effect of LV on FUra in rats pretreated with EU. FUra was dosed at 1.75 mg/kg/day on the daily X 4 schedule, the
weekly X 3 schedules, respectively. EU, which
increased the therapeutic index to 1.5.

In conclusion, these studies with a rat tumor model support the
earlier mouse tumor model (30) showing that EU is an effective
modulator of FUra therapy. It markedly increased the efficacy and the
therapeutic index of FUra and was considerably more effective than either LV or PALA as a
modulator of FUra therapy in this tumor model. Although MTD and
PALA were beneficial, especially when FUra was dosed on the weekly
X 3 schedule, they failed to increase the CR rate to 100% and only
increased the therapeutic index to 1.5.

As EU completely blocks the catabolism of FUra by inactivating
dHDHPHase, it preserves systemically administered FUra (30). Thus,
mice and rats pretreated with EU eliminate FUra approximately one-
tenth as fast as untreated animals. We therefore compared a 4-day
constant infusion of FUra at the MTD to modulation by EU to test
whether the effects of EU were simply caused by enhanced exposure
to FUra. The results indicated that constant infusion was not better
than i.v. push injection of FUra and that the effects of EU involved
more than enhanced exposure.

Although we do not know the mechanism by which EU improves
FUra therapy, we do know that EU produces additional biochemical
modulations. As a consequence of inactivating DHDHPHase, EU causes
plasma uracil and thymine levels to increase approximately 50-fold in
all laboratory animals studied. It would also prevent the formation of
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DISSUSSION

We used the Ward tumor model system in rats to study EU as a
modulator of the antitumor activity of FUra. To provide a stringent
test, therapy was not started until the tumor mass was 2.5-3.0 g. Although this tumor was sensitive to FUra therapy, few animals
survived long term. Thus, FUra at the MTDs produced significant
tumor regression but only resulted in a maximum of 0 or 13% CR with
the daily X 4 or the weekly X 3 schedules, respectively. EU, which
produced neither toxicity nor intrinsic antitumor activity, profoundly
improved FUra therapy. It enabled lower, nontoxic doses of FUra to
achieve 100% CR on both schedules and increased the therapeutic
index of FUras from \leq 1 to 6.

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