Attenuation of the Antitumor Activity of 5-Fluorouracil by (R)-5-Fluoro-5,6-dihydrouracil

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Abstract

5-Ethynyluracil (5-EU; 776C85) is a potent mechanism-based inactivator of dihydropyrimidine dehydrogenase that improves the antitumor activity of 5-fluorouracil (5-FU) to a greater extent than can be accounted for by the improved 5-FU pharmacokinetics that result from preventing the catabolism of 5-FU. We therefore tested the effects of (R)-5-fluoro-5,6-dihydrouracil (5-FUH2), the 5-FU catabolite extensively formed in the absence of 5-EU, on the antitumor activity and toxicity of 5-FU in 5-EU-treated rats bearing large s.c. tumors. Rats were dosed once weekly for 3 weeks with the following regimens: 100 mg/kg 5-FU (maximum tolerated dose), 10 mg/kg 5-FU 1 h after 1 mg/kg 5-EU, or 10 mg/kg 5-FU plus 90 mg/kg 5-FUH2 1 h after 1 mg/kg 5-EU. The latter regimen was designed to approximate the exposure produced from 5-FU in the absence of 5-EU, where >80% of the dose is catabolized. 5-FU produced complete and sustained tumor regressions in 94% of the animals pretreated with 5-EU. In contrast, 5-FU in combination with 5-FUH2 produced complete regression in only 38% of the 5-EU-treated rats, which was similar to the antitumor activity of 5-FU in the absence of 5-EU. All treatments resulted in 7–11% transient weight loss. 5-FU produced no other notable toxicity in 5-EU-treated rats. However, 5-FUH2 added to this regimen caused transient diarrhea and stomatitis in 13% of the animals, which was similar to the toxicity produced by 5-FU in the absence of 5-EU. Thus, 5-FUH2 or other downstream catabolites of 5-FU, impaired the antitumor activity and slightly increased the toxicity of 5-FU. Accordingly, 5-EU appeared to improve the efficacy of 5-FU by preventing the formation of 5-FU catabolites.

Introduction

5-EU2 (776C85) is a potent mechanism-based inactivator of DPD (EC 1.3.1.2, uracil reductase) in vitro (1) and in vivo (2). Low doses of 5-EU inactivate DPD in all mammals studied and, thereby, preserve the physiological substrates, uracil and thymine, which become highly elevated in plasma (3, 4). Furthermore, 5-FU, a widely used cancer agent that is an excellent substrate for DPD (5–7), is not catabolized. 5-FU is rapidly converted to 5-FUH2 and eventually to FBAL in animals treated with 5-EU (8, 9). In the absence of 5-EU, >80% of the dose is catabolized. 5-FUH2 is rapidly converted to 5-FUH2 and eventually to FBAL (10–12) according to the catabolic pathway diagrammed in Fig. 1.

5-EU significantly improves 5-FU therapy in mice and rats bearing s.c. tumors (8, 13). As expected, 5-EU eliminates the problems associated with DPD. It increases the plasma half-life and the total exposure to 5-FU, induces a linear relationship between the dose of 5-FU and the corresponding 5-FU plasma concentration × time curve, and increases the oral bioavailability of 5-FU up to 100% (8). However, for unknown reasons, 5-EU also increases the therapeutic index of 5-FU by 2–4-fold in mice (8) and by 6-fold in rats (13) and improves the absolute efficacy of 5-FU in rats (13). In rats bearing advanced colon carcinoma, the combination of 5-FU with 5-EU showed considerable more efficacious than 5-FU alone, even when 5-FU is dosed by continuous infusion (13).

Thus, the improvements in 5-FU therapy conferred by 5-EU involve more than improving the pharmacokinetics of 5-FU. We therefore speculated that the biochemical consequences resulting from inactivating DPD, which include elevated plasma concentrations of uracil and thymine and the absence of 5-FU catabolites, could affect the efficacy and/or toxicity of 5-FU (3, 4, 8, 13). Because 5-FUH2 is the first catabolite of 5-FU, we tested the effects of 5-FUH2 on the antitumor activity and toxicity of 5-FU in 5-EU-treated rats with large tumors. Thus, the initial catabolite and, presumably, the other downstream catabolites were added to a regimen that normally prevents their formation. The results are reported herein.

Materials and Methods

Materials. 5-EU was synthesized at the Wellcome Research Laboratories. 5-FU was purchased from Hoffman LaRoche Inc. (Nutley, NJ). The biological formed (R)-enantiomer of 5-FUH2 was synthesized and purified as described (7). Six- to 7-week-old female Fisher 344/HSD rats were obtained from Harlan Sprague-Dawley Inc. (Indianapolis, IN).

Antitumor Therapy. Ward colorectal carcinoma tumor fragments were transplanted s.c. into 150–200-g rats and treatment was initiated 14–16 days later when tumor weights were approximately 2.5–3.0 g as described previously (13). Each group had four rats per experiment and each experiment was repeated four to nine times. Tumor regression was expressed as partial regression when tumor weight was temporarily reduced by at least 50% and as CR when tumors were completely resolved and were not detectable for at least 90 days.

Drug Dosing. 5-FU was administered by i.v. push once weekly for 3 weeks. 5-FU was dissolved in sterile saline adjusted to pH 10 with NaOH and was dosed i.p. at 1 mg/kg 1 h prior to 5-FU and for 2 additional days post 5-FU therapy. 5-FUH2 was dissolved in sterile saline and the pH was adjusted to 7 with NaOH. 5-FUH2 and 5-FU were dosed together.

Results

In our previous study (13), rats with Ward tumor fragments were treated after the tumor mass reached 3 g. The MTD of 5-FU produced no complete and sustained tumor regressions (CR) on a once-a-day for 4-day (daily) schedule, CR in only 13% of the rats on a once-a-week for 3-week (weekly) schedule, and CR in 14% of the rats on a 4-day continuous infusion schedule. In contrast, doses of 5-FU at, and considerably below, its MTD produced CR in 100% of the rats treated with 5-FU on both weekly and daily schedules. To test the effect of 5-FU catabolites on the antitumor efficacy and toxicity of 5-FU, we administered 5-FUH2 along with 5-FU in 5-EU-treated rats on the weekly schedule. Rats were dosed with 5-EU and then with 10 mg/kg 5-FU or with 10 mg/kg 5-FU plus 90 mg/kg 5-FUH2. The doses in the latter regimen were chosen to approximate the systemic exposure that occurs in rats after dosing with 100 mg/kg/day (the MTD) 5-FU in the
5-FUH2 impairs 5-FU therapy

Fig. 1. The catabolic pathway of 5-FU and its blockade by 5-EU. The reactions are catalyzed by: 1, DPD (uracil reductase, EC 1.3.1.2); 2, dihydropyrimidinase (EC 3.5.2.2); and 3, β-ureidopropionase (β-alanine synthase, EC 3.5.1.6).

Discussion

In the present study, we added 5-FUH2 to the 5-FU treatment regimen in 5-EU-dosed rats to test the effects of the first 5-FU catabolite on the antitumor activity and toxicity of 5-FU. This study demonstrated that 5-FUH2 impaired the antitumor activity of 5-FU without greatly affecting toxicity in this tumor model system. Thus, 5-EU appears to improve the antitumor efficacy of 5-FU by blocking the formation of 5-FUH2, or possibly the downstream catabolites of 5-FU. DPD initiates the catabolic pathway by catalyzing the reduction of 5-FU to 5-FUH2. Dihydropyrimidinase then catalyzes ring opening to form FUPA, and β-alanine synthase catalyzes the elimination of CO2 and NH3 to form FBAL (Fig. 1). Studies with purified enzymes show that ring opening of 5-FUH2 is kinetically favored over oxidative conversion to 5-FU (7). Thus, 5-FU is rapidly converted to FBAL via 5-FUH2 and FUPA. Because FBAL has a prolonged half-life of 33 h, it accumulates in humans (11) and rats (14). We do not know which catabolite(s) is responsible, either directly or indirectly, for interfering with 5-FU antitumor activity. We are attempting to synthesize the biologically active stereoisomers of FUPA and FBAL to address this question. In any case, pretreatment with 5-EU permanently eliminates the first enzyme of the catabolic pathway and thereby prevents the formation of all 5-FU catabolites. 5-EU, a highly specific and efficient irreversible inactivator of DPD, has advantages over competitive inhibitors that are rendered less effective by metabolism and elimination or by competing substrates.

Catabolites of 5-FU are generally considered to be toxic. For example, the neurotoxicity of 5-FU to dogs and cats (15–18) has been attributed to FBAL (15, 19, 20) and is prevented by pretreatment with 5-EU (21). However, this unusual dose-limiting neurotoxicity differs from the typical limiting bone marrow suppression and gastrointestinal disorders that occur in humans (22) and in tumor-bearing rodents treated with 5-FU (13). Thus, the toxicity of 5-FU catabolites in these species is not well understood. In the present study, 5-FUH2 was not

Table 1 Antitumor efficacy of the 5-FU-therapeutic regimens*

<table>
<thead>
<tr>
<th>Drug (mg/kg/dose)</th>
<th>CR (%)</th>
<th>PR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU (100)</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td>5-EU (1) + 5-FU (10)</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>5-EU (1) + 5-FU (10) + 5-FUH2 (90)</td>
<td>38</td>
<td>44</td>
</tr>
</tbody>
</table>

* Rats were dosed with the indicated regimens once-a-week for 3 weeks as described in "Materials and Methods" and in the legend of Fig. 2. Tumor volume was assessed.

Table 2 Toxicity of the 5-FU-therapeutic regimens*

<table>
<thead>
<tr>
<th>Drug (mg/kg/dose)</th>
<th>MWT &lt; SD (%)</th>
<th>Diarrhea (%)</th>
<th>Stomatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU (100 MTD)</td>
<td>9 ± 2</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>5-EU (1) + 5-FU (10)</td>
<td>7 ± 3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-EU (1) + 5-FU (10) + 5-FUH2 (90)</td>
<td>11 ± 3</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

* Rats were dosed with the indicated regimens once a week for 3 weeks as described in "Materials and Methods" and in the legend of Fig. 2. Toxicity and weight change were assessed.

a Transient maximal weight loss.
unduly toxic, inducing diarrhea and stomatitis in only 13% of the rats. Therefore, the toxicity of 5-FU catabolites is probably less important than their detrimental effect on antitumor efficacy of 5-FU in rats.

By preventing the catabolism of 5-FU, 5-EU lowers the efficacious and the toxic doses of 5-FU (8, 13). Fortunately, it lowers the efficacious dose more than the toxic dose, and thereby increases the therapeutic index of 5-FU in mouse (8) and rat (13) tumor models. Our present findings could also account for the mechanism of the increased therapeutic index. By blocking the formation of 5-FU catabolites, 5-EU unfetters the antitumor activity of 5-FU and makes it efficacious in the nontoxic dose range. However, other biochemical modulations that are secondary to inactivating DPD, such as elevated plasma uracil and thymine, could also contribute to the beneficial modulations that are secondary to inactivating DPD, such as elevated plasma uracil and thymine, could also contribute to the beneficial effects of 5-EU. The combination of 5-EU and 5-FU is currently being studied in Phase I clinical trials. We hope that eliminating 5-FU effects of 5-EU unfetters the antitumor activity of 5-FU and makes it efficacious dose more than the toxic dose, and thereby increases the therapeutic index of 5-FU in mouse (8) and rat (13) tumor models. Our present findings could also account for the mechanism of the increased therapeutic index. By blocking the formation of 5-FU catabolites, 5-EU unfetters the antitumor activity of 5-FU and makes it efficacious in the nontoxic dose range. However, other biochemical modulations that are secondary to inactivating DPD, such as elevated plasma uracil and thymine, could also contribute to the beneficial effects of 5-EU. The combination of 5-EU and 5-FU is currently being studied in Phase I clinical trials. We hope that eliminating 5-FU catabolites will also improve the therapeutic efficacy of 5-FU in humans.

References


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