A Possible Cause and Remedy for the Clinical Failure of 5-Fluorouracil Plus Eniluracil

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Abstract

Background: Eniluracil is a potent inactivator of dihydropyrimidine dehydrogenase (uracil reductase), the enzyme that rapidly catabolizes 5-fluorouracil (5-FU). Although eniluracil in combination with 5-FU was promising in phase I and II studies, in 2 multicenter phase III colorectal cancer studies, eniluracil dosed in a 10-to-1 ratio to 5-FU produced less antitumor benefit than the standard regimen of 5-FU/leucovorin without eniluracil. The current study tested whether the high eniluracil doses caused the clinical failure. The effect of excess eniluracil versus adequate eniluracil on 5-FU antitumor activity was studied in rats bearing large transplanted colon tumors. **Materials and Methods:** The rats were divided into 3 groups: group A received no treatment, group B was treated with eniluracil 1 mg/kg (adequate) 1 hour before 5 mg/kg 5-FU, and group C was treated identically to group B but also received eniluracil 25 mg/kg (excess) 5 minutes before 5-FU administration. **Results:** The rate of complete tumor regression (cure) was 0% in group A (no treatment), 88% in group B (adequate eniluracil), and 25% in group C (excess eniluracil). Toxicity was minimal. Only slight weight loss occurred in all groups. **Conclusion:** When the eniluracil dose is in 5-fold excess to 5-FU (as in the phase III clinical trials), the antitumor activity of 5-FU was significantly diminished. Moreover, to maximize the antitumor activity of 5-FU, eniluracil must not be present in excess over 5-FU in rats bearing tumors. Simple strategies to achieve optimal antitumor efficacy are presented.

Clinical Colorectal Cancer, Vol. 9, No. 1, 52-54, 2010; DOI: 10.3816/CCC.2010.n.007
Keywords: 5-FU, Antitumor efficacy, Dihydropyrimidine dehydrogenase, DPD

Introduction

Eniluracil (5-ethynyluracil, 776C85) is a potent inactivator of dihydropyrimidine dehydrogenase (DPD; uracil reductase), the enzyme that rapidly catabolizes 5-fluorouracil (5-FU).[1,2] Low doses of eniluracil enable 5-FU to be administered orally and convert the route of 5-FU elimination from metabolism to renal excretion. Accordingly, the 5-FU half-life is increased from 10-20 minutes to 4.5-6.5 hours.[3-8] Moreover, eniluracil prevents the formation of 5-FU catabolites, such as α-fluoro-β-alanine, that might cause neurotoxicity and hand-foot syndrome and also attenuate the antitumor activity of 5-FU.[1,2] Furthermore, by eliminating DPD, an enzyme present in greatly variable levels, the highly variable nonlinear pharmacokinetics of 5-FU become highly predictable and the relationship between the 5-FU dose and the 5-FU plasma levels is perfectly linear.[5] Eniluracil significantly potentiated the antitumor efficacy of 5-FU and increased the chemotherapeutic index in laboratory animals bearing tumors.[1,9] Eniluracil produced promising results and was well tolerated in phase I and II studies.[3,5-8,10-12]

Subsequently, 2 multicenter phase III studies were conducted in patients with colorectal cancer (CRC) using a combination pill containing eniluracil in 10-fold excess to 5-FU. Patients received eniluracil 10 mg/m^2 and 5-FU 1 mg/m^2 every 12 hours for 28 days. The cycle was repeated after 1 week off drug. Unfortunately, this regimen tended to produce less antitumor benefit than the standard regimen of 5-FU/leucovorin (LV) without eniluracil.[13] This outcome was unexpected because preclinical[1,2] and early clinical data[11,12] were very encouraging. Eniluracil and 5-FU even produced tumor responses in patients with advanced CRC that were refractory to 5-FU treatment.[7]

Eniluracil and 5-FU are structurally related compounds, differing only by the 5-substituent. We therefore considered the possibility that excess eniluracil might be interfering with the antitumor activity of 5-FU by inhibiting its anabolic activation. The following study was performed in rats bearing advanced Ward colorectal tumors to test this hypothesis.

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Submitted: May 11, 2009; Revised: Jul 17, 2009; Accepted: Sep 14, 2009

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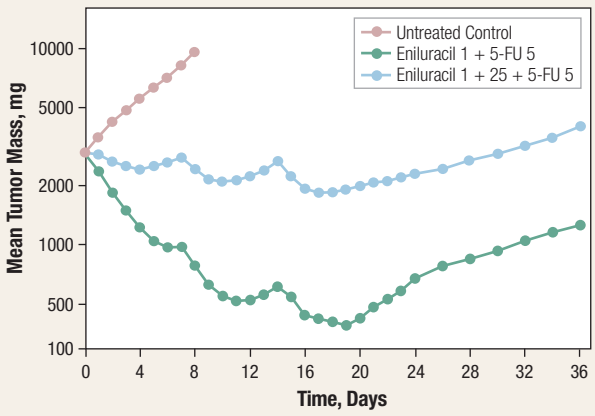
Figure 1 Effect of Eniluracil of the Antitumor Activity of 5-Fluorouracil (Average Response) in Rats Bearing Advanced Ward Colorectal Tumor



Rats bearing approximately 3000 mg of tumor mass were treated at days 0, 7, and 14 with the following treatments: group A, no treatment; group B, adequate eniluracil (t = 0) plus 5-FU (t = 60 minutes); group C, adequate eniluracil (t = 0) plus excess eniluracil (t = 55 minutes), plus 5-FU (t = 60 minutes). Doses (mg/kg) are indicated. Eight rats were used for each experimental group from 2 independent experiments. The mean tumor mass is indicated for each group.

Materials and Methods

Six-to-seven-week-old Fischer rats weighing 150-200 g were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). They were kept 4 to a cage with food and water ad libitum according to an institutionally approved protocol. The rats underwent transplantation with approximately 100-mg nonnecrotic Ward CRC tumor pieces and were treated once per week for 3 weeks after their tumors grew to 3000 mg in mass as previously described.[9] Each group was composed of 4 rats for each experiment. The study was independently repeated to produce data from a total of 8 rats per group. Eniluracil was dosed intraperitoneally, and 5-FU was dosed intravenously. Eniluracil was also dosed at 1 mg/kg to rats in groups B and C on days 2 and 3 of each weekly treatment. All materials and other methods are described elsewhere.[9]

Results

Rats bearing approximately 3000-mg tumor mass were treated as follows: group A, no treatment; group B, eniluracil 1 mg/kg (time [t] = 0) followed by 5-FU 5 mg/kg at t = 60 minutes; and group C, eniluracil 1 mg/kg at t = 0, followed by eniluracil 25 mg/kg at t = 55 minutes, and by 5-FU 5 mg/kg at t = 60 minutes. The eniluracil dose of 1 mg/kg was previously shown to completely inactivate DPD in rats.[14] The treatment for group C mimics the clinical trials in which high levels of eniluracil were present when 5-FU was dosed. However, the ratio of eniluracil to 5-FU was 5:1 in this study (vs. 10:1 in the clinical trial) because the dose of eniluracil used was the highest that could be physically administered. The 5-mg/kg 5-FU dose was used because it is the lowest dose that produced 100% complete response (CR) in eniluracil-treated rats bearing colorectal tumors.[9] Decreases in antitumor efficacy were most likely to be detected at this dose. Previously, 5-FU 2.5 mg/kg produced a 25% CR rate, and 5, 10, and 15 mg/kg produced a 100% CR rate. Toxicity was observed at 20 mg/kg. For comparison, 5-FU alone produced 13% CR at its maximum tolerated dose of 100 mg/kg with the same dosing schedule.

Table 1 Antitumor Efficacy and Toxicity of 5-Fluorouracil in Eniluracil-Treated Rats Bearing Advanced Ward Colorectal Tumors

Study Arm (Group)	CR,[a] %	MWL,[b] %
A (No Treatment)	0	0.9 ± 1.4
B (Adequate Eniluracil)	88	2.5 ± 1.2
C (Excess Eniluracil)	25	4.0 ± 1.3

[a]CR = complete tumor regression (cure).
[b]MWL = maximum weight loss during the study (mean ± SD). Each experimental group had 8 rats with 2 independent experiments.

In the current study, the tumors in group A rapidly grew to approximately 10,000 mg, and the rats were sacrificed. Tumors in group B were completely eliminated in 7 of the 8 rats. Moreover, 4 tumors were eliminated before the third dose of 5-FU. In contrast, tumors in group C were eliminated in only 2 out of 8 rats. The data in Figure 1 and Table 1 show that the excess eniluracil in group C significantly diminished the antitumor activity of 5-FU. Toxicity was minimal. Only slight weight loss occurred in all groups. No treatment-related deaths occurred.

Discussion

Likely Cause of Clinical Failure

The results in Figure 1 and Table 1 show that when eniluracil was present in excess amounts when 5-FU was dosed, the antitumor activity of 5-FU was attenuated. Group C was designed to mimic the clinical trials where high levels of eniluracil were present when 5-FU was dosed. In both the clinical trials and the current study, the groups receiving excess eniluracil experienced less antitumor activity than the respective comparative group. Although in the clinical trial, the ratio of eniluracil to 5-FU was 10:1 and was only 5:1 in the current study, the antitumor activity of 5-FU was still compromised.

Elusive Mechanism

The most likely explanation is that excess eniluracil interfered with the metabolic activation of 5-FU. Uridine phosphorylase is a possible target that excess eniluracil might be inhibiting. It converts 5-FU to 5-fluorouridine and uracil to uridine. Eniluracil is a very weak inhibitor of human uridine phosphorylase with an IC_{50} of 375 μM.[14] However, because the maximum plasma concentration of eniluracil in the failed phase III clinical trial was only 5 μM,[3] eniluracil could not have significantly inhibited uridine phosphorylase. Moreover, eniluracil does not inhibit orotate phosphoribosyl transferase,[14] the other key 5-FU–activating enzyme. Nevertheless, eniluracil is an efficient substrate of human uridine phosphorylase and is converted to the corresponding nucleoside analogue (D. Baccanari, PhD, unpublished data, 1993). Perhaps an anabolite of eniluracil could be interfering with 5-FU activation.

In any case, the mechanism by which excess eniluracil diminishes 5-FU efficacy appears to be elusive. Its consequences could also be subtle. In both the phase III clinical trial[13] and our current study in rats, 5-FU, even in the presence of excess eniluracil, demonstrated very significant antitumor activity. For example, in the North Amer-

ica phase III clinical trial (where compliance was not a problem because dosing cards were used), eniluracil/5-FU was only slightly less effective that 5-FU/LV. Patients' median duration of survival was 13.3 months versus 14.5 months, and progression-free survival was 20 weeks versus 22.7 weeks. Thus, the mechanism(s) for these small but significant differences might prove difficult to determine.

The Remedy

The results in Figure 1 and Table 1 show that, to maximize the antitumor activity of 5-FU, eniluracil must not be present in excess amounts with 5-FU treatment. Because eniluracil is a very potent inactivator of DPD, it can be administered before 5-FU. Any eniluracil not covalently bonded to DPD should be given ample time to be partially—but importantly, not completely—cleared.

Because new DPD appears shortly after eniluracil has been eliminated,[15,16] it is important to administer an adequate dose of eniluracil that will also inactivate any DPD synthesized during the entire exposure to 5-FU. If variable amounts of DPD remain active, the regimen's highly predictable 5-FU pharmacokinetics and safety will be compromised. It is also important to ensure that the eniluracil dose is adequate in preventing the formation of neurotoxic 5-FU catabolites[17] in the central nervous system and other nervous tissues. Studies in rats have shown that the eniluracil dose required to inactivate DPD in the brain is 6-fold higher than the dose required to inactivate DPD in non-nervous tissues.[15] Thus, measurements of DPD in peripheral blood mononucleocytes could underestimate the degree of inhibition in nervous tissues. Total α-fluoro-β-alanine recovered in urine is a more accurate and meaningful determination of adequate DPD inhibition.

Mice and rats eliminate eniluracil considerably faster than humans eliminate it. The half-life of eniluracil is 10 minutes in mice and 34 minutes in rats, respectively.[1] Therefore, when the rats in group B were pretreated with eniluracil 1 hour before receiving 5-FU, only low levels of eniluracil were present by the time 5-FU was administered. The half-life of eniluracil is approximately 3.5 hours in human patients.[4,10] If excess eniluracil also decreases 5-FU antitumor activity in cancer patients, care must be taken to ensure that the levels of eniluracil are not in excess to 5-FU when 5-FU is administered. Previously, eniluracil 10-20 mg per day has been shown to adequately eliminate DPD in peripheral blood mononucleocytes and confer a 5-FU elimination half-life of 4.5-6.5 hours.[3-8] Patients treated in the phase III study received eniluracil 10 mg/m^2 simultaneously with 5-FU 1 mg/m^2 every 12 hour. Clearly, this high ratio of eniluracil to 5-FU can be easily avoided. If 30 mg to 40 mg eniluracil were dosed 12-16 hours before 5-FU, it would adequately inactivate DPD in both nervous and non-nervous tissues, and its concentration would be greatly decreased before the administration of 5-FU. This approach should be amenable for the 5-day or weekly eniluracil/5-FU dosing regimens that use 20-30 mg/m^2 5-FU.[7,11,12]

Conclusion

When eniluracil was present in excess to 5-FU, it diminished the antitumor activity in rats. Excess eniluracil might also be the reason that the phase III clinical trial did not achieve optimal efficacy. Because eniluracil inactivates DPD, the target enzyme, it can be predosed and partially cleared before 5-FU is administered. Thus, the antagonism of 5-FU by excess eniluracil would be avoided. We are hopeful that this strategy will significantly improve the efficacy of 5-FU in future clinical trials.

Disclosures

Thomas Spector has stock/equity ownership in Adherex Technologies Inc. Shousong Cao has no relevant relationships to disclose.

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