Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 11064–11068, December 1993
Medical Sciences

5-Ethynyluracil (776C85): A potent modulator of the pharmacokinetics and antitumor efficacy of 5-fluorouracil

(biochemical modulation/therapeutic index/potentiation)

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Communicated by George H. Hitchings, July 29, 1993

ABSTRACT **5-Ethynyluracil (5-EU, 776C85) is a mechanism-based irreversible inhibitor of dihydropyrimidine dehydrogenase (EC 1.3.1.2), the rate-determining enzyme in 5-fluorouracil (5-FU) catabolism. In the present study, 5-EU was found to be a potent modulator of 5-FU catabolism in mice and rats. Liver extracts prepared up to 6 hr after a 5-EU dose (2 mg/kg) were >96% inhibited in their ability to catalyze 5-FU degradation. 5-EU treatment increased the elimination $t_{1/2}$ and the area under the plasma concentration–time curve of 5-FU. 5-FU oral bioavailability was ≈100% in rats pretreated with 5-EU. Consequently, 5-EU induced a linear relationship between the area under the plasma concentration–time curve and the oral dose of 5-FU. As expected from the preservation of plasma 5-FU, 5-EU potentiated the antitumor activity and the toxicity of 5-FU in two mouse tumor models (Colon 38 and MOPC-315). However, 5-EU potentiated the antitumor activity to a greater degree and thereby increased the therapeutic index of 5-FU 2- to 4-fold.**

5-Fluorouracil (5-FU) is an effective antineoplastic agent used in the treatment of various solid tumors, including gastrointestinal, breast, and ovarian carcinomas (1). The drug is usually administered by bolus i.v. injection or by continuous i.v. infusion; p.o. dosing is avoided because of significant patient-to-patient variations in oral bioavailability (2). Drug catabolism is an important factor in chemotherapy with 5-FU. Between 60% and 90% of administered 5-FU is catabolized (3). Dihydropyrimidine dehydrogenase (DPD; EC 1.3.1.2) catalyzes the reduction of 5-FU in various human tissues (4). Its catalytic activity correlates with the rate of 5-FU clearance (5). Humans with normal levels of DPD eliminate 5-FU with a $t_{1/2}$ of 13 min and excrete only 10% of the dose as unchanged drug in the urine. However, people genetically deficient in DPD eliminate 5-FU with a $t_{1/2}$ of 159 min and excrete 90% of the dose unchanged in the urine (6). Genetic DPD deficiency also results in elevated levels of endogenous uracil and thymine in urine and plasma (7). In addition to variations of DPD activity between patients, a circadian pattern in DPD levels within individual patients causes cyclical variations in plasma 5-FU level during continuous drug infusions (8). Thus, inhibitors of DPD can have significant effects on the pharmacokinetics of 5-FU (9–12).

5-Ethynyluracil (5-EU, 776C85) is a mechanism-based irreversible inhibitor of DPD (13). It binds to the enzyme with a K_m of 1.6 μM and inactivates it by covalent modification with a first-order rate constant of 20 min^{-1} (enzyme $t_{1/2}$ = 2 sec). Although other inhibitors and inactivators of DPD have been identified (14–18), 5-EU is the most potent inactivator of this enzyme discovered to date (13). Very low doses of 5-EU rapidly inactivate (ED_{50} = 1.8 μg/kg) liver DPD in rats and markedly elevate (ED_{50} = 10 μg/kg) endogenous plasma uracil and thymine levels (ref. 33; D. J. Nelson and L. Frick, personal communication). In the present study, we show that 5-EU increased the $t_{1/2}$ and the area under the plasma concentration–time curve (AUC) of 5-FU. 5-EU-treated rats exhibited significantly less variation in the oral bioavailability of 5-FU and had a linear relationship between AUC and dose of 5-FU. 5-EU also increased the therapeutic index of 5-FU against s.c. implants of Colon tumor 38 and MOPC-315 tumors in mice.

MATERIALS AND METHODS

Animals and Drugs. CD-1 and BALB/c female mice (18–20 g) and CD male rats (150–175 g) were obtained from Charles River Breeding Laboratories. C57BL/6 × $C3HF_1$ ($B6C3F_1$) mice were obtained under contract from various commercial suppliers. 5-EU was synthesized at the Wellcome Research Laboratories. [6-^{14}C]5-FU (56 mCi/mmol; 1 Ci = 37 GBq) was purchased from Moravek Biochemicals (Brea, CA). 5-FU and other reagents were purchased from Sigma.

Cell Culture. MOPC-315 mouse myeloma tumor cells (American Type Culture Collection) were grown in RPMI 1640 medium (GIBCO/BRL) containing 2 mM L-glutamine and supplemented with 10% fetal bovine serum (HyClone) and 1 mM sodium pyruvate. Colon 38 carcinoma was obtained from the Development Therapeutics Program Tumor Repository (Frederick, MD).

Animal Dosing and Sample Collection. Drugs were dissolved in alkaline saline (pH 8.5–10) and administered to mice in a volume of 10 ml/kg. 5-EU was administered s.c. or p.o. 30 min or 90 min before 5-FU and is equally effective by either route (D. J. Nelson and L. Frick, personal communication). Whole blood (≈0.4 ml per mouse, three mice per time point) was obtained by cardiac puncture of CO_2-anesthetized mice with a syringe containing 5% EDTA. For rat studies, a catheter tube was inserted into the right jugular vein (19). The animals were then placed in individual metabolic cages and fed chow and water overnight. Animals were fasted 24 hr prior to p.o. drug administration. 5-EU was dosed p.o. 1 hr before 5-FU. Both drugs were administered (at 5 ml/kg) at approximately the same time of day in each study to control for potential circadian variations in DPD activity.

Uracil and 5-FU Analysis. Uracil and 5-FU were quantitated following resolution by reverse-phase HPLC. Plasma or urine samples were thawed and diluted 1:1 with water. Protein was removed by ultrafiltration using the Centrifree micropartition system (Amicon). HPLC was performed on a reverse-phase Microsorb C_{18} column (250 mm × 4.6 mm i.d.; Rainin, Woburn, MA) with a Dynamax axial compression guard column. A Waters model 712 WISP automated sample

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Abbreviations: 5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; 5-EU, 5-ethynyluracil; AUC, area under the plasma concentration–time curve; MED, minimum effective dose; BVUra, (*E*)-5-(2-bromovinyl)uracil; BVdUrd, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine.

injector was used for sample injection, and microcomputer-controlled LKB Bromma 2150 HPLC pumps delivered the mobile phases: 50 mM ammonium acetate buffer, pH 4.8/0.5% acetonitrile (buffer A), and 50 mM ammonium acetate buffer, pH 4.8/60% acetonitrile (buffer B). A 25-min isocratic elution in buffer A was followed by a 2.5-min linear gradient to 60% buffer B and then by a 5-min isocratic elution in 60% buffer B. Flow rates were 0.5 ml/min for the first 25 min and 1 ml/min for the remainder of the gradient. The effluent was monitored by UV absorption at 265 nm using a Waters model 991 photodiode array detector.

DPD Assays. Mouse and rat livers were homogenized (1:3, wt/vol) in ice-cold 20 mM potassium phosphate buffer (pH 8.0)/1 mM EDTA/1 mM 2-mercaptoethanol using a Brinkman model PT3000 Polytron (three cycles at 30,000 rpm for 20 sec). The homogenates were centrifuged to remove cell debris and then clarified by ultracentrifugation (100,000 × *g* for 1 hr). Liver extracts from 5-EU-treated animals were fractionated on a fast desalting gel filtration column (Pharmacia) to remove unbound 5-EU.

DPD activity was determined using minor modifications of the method described by Naguib *et al.* (4). Reaction mixtures contained 20 mM potassium phosphate buffer (pH 8.0), 1 mM EDTA, 20 μM [6-^{14}C]5-FU (56 μCi/μmol), 1 mM dithiothreitol, and enzyme in a final volume of 50 μl. 5-FU and its catabolites were separated on silica gel TLC sheets with fluorescent indicator (Macherey & Nagel, Polygram Sil G/UV$_{254}$) as described (20). One unit is defined as the amount of enzyme that catalyzes the disappearance of 1 nmol of 5-FU per min. Protein concentration was determined by the method of Lowry *et al.* (21).

Pharmacokinetic Analysis. The elimination portion of semilogarithmic plasma concentration vs. time plots was analyzed by linear regression. AUC values were determined by the linear trapezoidal method and extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal elimination rate constant.

Antitumor Studies. *In vivo* antitumor testing with murine Colon 38 was conducted under contract at Southern Research Institute (SRI), Birmingham, AL. B6C3F$_1$ mice were implanted s.c. with 70-mg tumor fragments on day 0 and were treated on days 1 through 9. 5-FU was administered at approximately the same time each day. 5-EU (2 mg/kg, i.p.) was dosed 30 min prior to 5-FU. Typical 5-FU doses are indicated in Fig. 3. Tumor weights were calculated three times per week from measurements of tumor length and width. Antitumor activity was expressed as the days delay in tumor growth (T − C). To calculate T − C, the difference in the median time (days) for the tumors of treated (T) and control (C) groups to grow to 500 mg was averaged with the difference in the median time for the tumors of T and C groups to grow to 1000 mg. Tumor-free survivors were excluded from T − C calculations. Plots of T − C vs. 5-FU dose and mortality vs. 5-FU dose were analyzed by iteratively fitting the data to a logistic plot (SigmaPlot, Jandel, San Rafael CA) to calculate the dose that produces 10-days delay (minimum effective dose, MED) and LD$_{50}$ values, respectively.

MOPC 315 is a murine myeloma that grows preferentially in BALB/c mice (22). Cells grown *in vitro* were harvested in midlogarithmic phase, washed with sterile Dulbecco's phosphate-buffered saline, and implanted s.c. (1 × 10^6 cells per mouse, 3.3 × 10^6 cells per ml) in the right axillary region. Mice, housed in microisolator cages (eight per cage), were maintained on a 6:00 a.m. to 6:00 p.m. light cycle. When solid tumors reached a measurable size of 150–250 mg (between 10 and 20 days after implantation), single-dose 5-FU therapy was administered i.p. without or with 5-EU pretreatment (2 mg/kg, i.p., 30 min before 5-FU). Typical 5-FU doses are indicated in Fig. 4. 5-FU was administered at approximately the same time of day in each experiment. Plots of percent inhibition of tumor growth vs. 5-FU dose and mortality vs. 5-FU dose were analyzed by iteratively fitting the data to a logistic plot to calculate ED$_{50}$ values and LD$_{40}$ values, respectively.

RESULTS

***In Vivo* Inhibition of DPD by 5-EU.** Liver extracts from mice and rats were tested for their ability to catalyze 5-FU degradation in the *in vitro* enzyme assay. The specific activities of mouse and rat liver DPD were 0.7 ± 0.1 unit/mg and 0.9 ± 0.1 unit/mg, respectively. After a single 5-EU dose (2 mg/kg, p.o.), liver DPD activity was inhibited >96% at the time points tested (1 and 4 hr postdose in mice; 1 and 6 hr postdose in rats). These times were the approximate beginning and end points of subsequent 5-FU pharmacokinetic experiments.

Effect of 5-EU on 5-FU Clearance in Mice and Rats. 5-FU was rapidly cleared ($t_{1/2}$ = 4.5 min) from the plasma of mice dosed with 50 mg/kg, i.p., and plasma drug levels were <10 μM within 30 min (AUC = 70 μM·hr). In contrast, pretreatment with 5-EU (2 mg/kg, p.o.) significantly preserved plasma 5-FU. The $t_{1/2}$ and AUC of 5-FU increased to 34 min and 400 μM·hr, respectively. 5-EU also increased the elimination $t_{1/2}$ of p.o. 5-FU from 5 min (5-FU alone) to 38 min and increased the AUC of 5-FU from 20 μM·hr (5-FU alone) to 340 μM·hr.

More detailed pharmacokinetic studies were performed in rats because these animals can be cannulated to permit sequential blood sampling. Fig. 1*A* shows the plasma 5-FU concentration vs. time profiles for 6 rats dosed p.o. with 5-FU (50 mg/kg) alone. The individual plasma concentration profiles were highly variable. Peak plasma concentrations ranged from 0 to 300 μM at 10–20 min postdose, and plasma 5-FU levels were at the limit of detection after 1–1.5 hr. In contrast, 5-FU plasma profiles were considerably less variable after pretreatment with 5-EU (Fig. 1*B*). Peak plasma 5-FU concentrations ranged from 160 to 280 μM, and 5-FU was detectable in plasma for at least 5 hr. The mean AUC values (± standard error) of 5-FU in rats dosed without and with 5-EU were 90 ± 50 and 440 ± 60 μM·hr, respectively; and the elimination $t_{1/2}$ values of 5-FU were 9 ± 4 min and 100 ± 40 min, respectively. In crossover studies not shown, rats

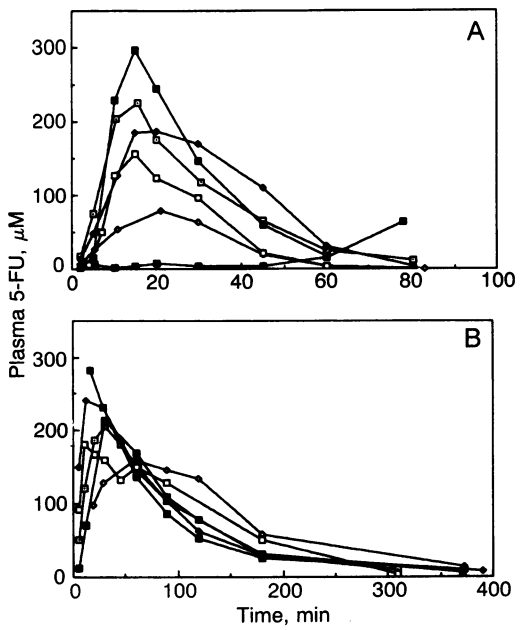

FIG. 1. Effect of 5-EU on plasma levels of 5-FU in rats. Individual plasma concentration vs. time profiles for six rats dosed with 5-FU (50 mg/kg, p.o.) (*A*) or for six rats pretreated with 5-EU (2 mg/kg, p.o.) 1 hr before 5-FU (50 mg/kg, p.o.) (*B*).

received 5-FU p.o. 1 day and i.v. the next. The AUC values indicated that the oral absorption of 5-FU was 100% in 5-EU-treated animals vs. 65% for 5-FU administered alone.

Inhibition of 5-FU catabolism by 5-EU in rats was also evident from analyses of 24-hr urine samples. Over 80% of i.v. dosed 5-FU was recovered as intact 5-FU in the urine of 5-EU-treated rats, whereas only 13% was recovered from rats dosed with 5-FU alone. 5-EU treatment also led to a 20- to 25-fold increase in the amount of uracil excreted into urine.

We also investigated the linearity of oral 5-FU disposition kinetics in 5-EU-treated rats. AUC increased linearly over a dose range up to 30 mg/kg (Fig. 2*A*). The proportionality was decreased slightly at the 50 mg/kg dose. A similar relationship was observed between C_{max} and dose (Fig. 2*B*).

Effect of 5-EU on the Antitumor Efficacy of 5-FU. The effect of 5-EU on the antitumor efficacy of 5-FU was studied in s.c. tumor models of Colon 38 and MOPC-315 myeloma. In the Colon 38 model, mice were dosed with 5-FU either p.o. or i.p. Fig. 3 shows the combined data for three separate experiments with the p.o. route. Antitumor activity and toxicity were monitored as tumor delay (T − C) and percent drug-related deaths, respectively. A comparison of the abscissa scales shows that 5-EU shifted the efficacy and toxicity dose–response curves of 5-FU to greatly reduced doses. However, the efficacy was shifted more than the toxicity. 5-EU administered alone did not inhibit tumor growth. The results of the individual experiments with 5-FU administered p.o. and i.p. are quantitatively analyzed in Table 1. The therapeutic index of 5-FU, calculated as the ratio of LD_{50} to MED, was increased 2- to 4-fold by 5-EU. 5-EU also improved the therapeutic index of 5-FU when calculations were based on LD_{10} and LD_{20} values (data not shown).

Mice implanted with MOPC-315 cells were treated with a single i.p. dose of 5-FU. Antitumor activity and toxicity were monitored as percent inhibition of tumor growth and percent drug-related deaths, respectively. Effective, but nonlethal, doses of 5-FU generally inhibited tumor progression for about 8 days, and then growth resumed. 5-EU administered alone did not inhibit tumor growth. Fig. 4 shows that 5-EU shifted the efficacy and toxicity dose–response curves of single-dose 5-FU to reduced doses and that efficacy was shifted more than toxicity. The results of this and two additional experiments are quantitatively analyzed in Table 2. 5-EU increased the therapeutic index 2- to 4-fold. 5-EU also improved the therapeutic index of 5-FU when calculations were based on LD_{10} and LD_{20} values (data not shown).

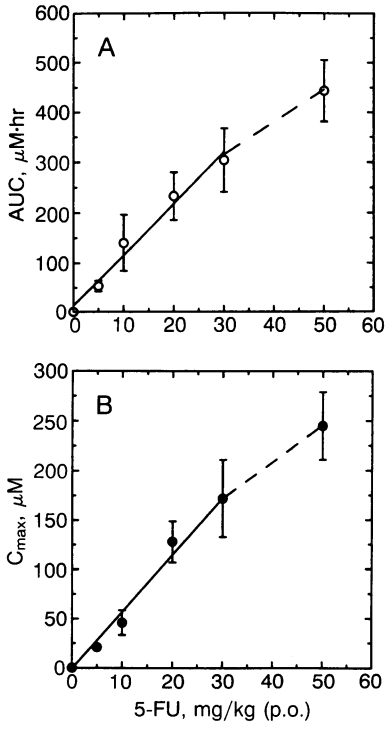

FIG. 2. Oral absorption of 5-FU in 5-EU-treated rats. Rats (two to six per group) were dosed p.o. with 5-FU 1 hr after dosing with 5-EU (2 mg/kg, p.o.). Plasma concentrations were determined at various times. The data are plotted as AUC vs. 5-FU dose (*A*) and C_{max} vs. 5-FU dose (*B*). Range or standard error of the mean is shown by bars.

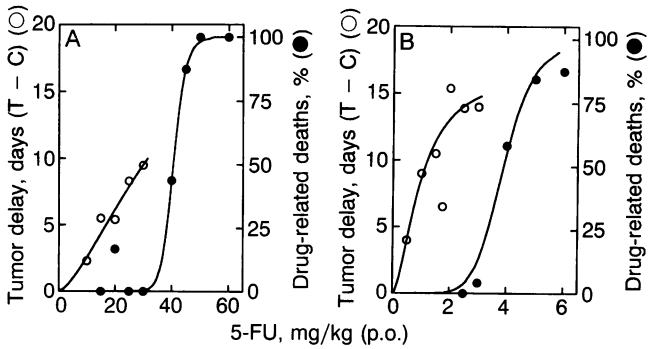

FIG. 3. Effect of 5-EU on the efficacy and toxicity of 5-FU against s.c. implants of Colon 38 in mice. Mice with s.c. implants of Colon 38 were treated daily with p.o. 5-FU (±2 mg of 5-EU per kg, i.p.) for 9 days and monitored for antitumor efficacy (○) and toxicity (●) as described in *Materials and Methods*. (*A*) 5-FU alone. (*B*) 5-FU plus 5-EU. Each point is the average of the individual doses used in experiments 1–3 (Table 1) weighted by the number of times that dose was studied.

DISCUSSION

5-EU is an exceedingly potent mechanism-based inactivator of DPD (13). Very low doses of 5-EU inactivate DPD *in vivo* (33) and cause plasma uracil and thymine levels to rise

Table 1. Effect of 5-EU on the therapeutic index of 5-FU in mice implanted s.c. with Colon 38

Treatment	5-FU MED,* mg/kg	5-FU LD_{50}, mg/kg	Therapeutic index (LD_{50}/MED)	Increase in therapeutic index,† fold
Experiment 1‡				
5-FU alone	28	40	1.4	
5-FU + 5-EU	0.66	3.7	5.6	4.0
Experiment 2‡				
5-FU alone	25	42	1.7	
5-FU + 5-EU	0.89	3.9	4.4	2.6
Experiment 3‡				
5-FU alone	>38	38	<1	
5-FU + 5-EU	1.5	4.7	3.1	>3.1
Experiment 4§				
5-FU alone	18	34	1.9	
5-FU + 5-EU	0.58	4.3	7.4	3.9
Experiment 5§				
5-FU alone	18	33	1.8	
5-FU + 5-EU	1.6	4.9	3.1	1.7
Experiment 6§				
5-FU alone	30	39	1.3	
5-FU + 5-EU	1.3	5.8	4.5	3.5

*Dose that produced a median 10-day delay of tumor growth compared to the growth of tumors in untreated mice.
†Ratio of the therapeutic index for 5-EU plus 5-FU to the therapeutic index of 5-FU alone.
‡p.o. dosing.
§i.p. dosing.

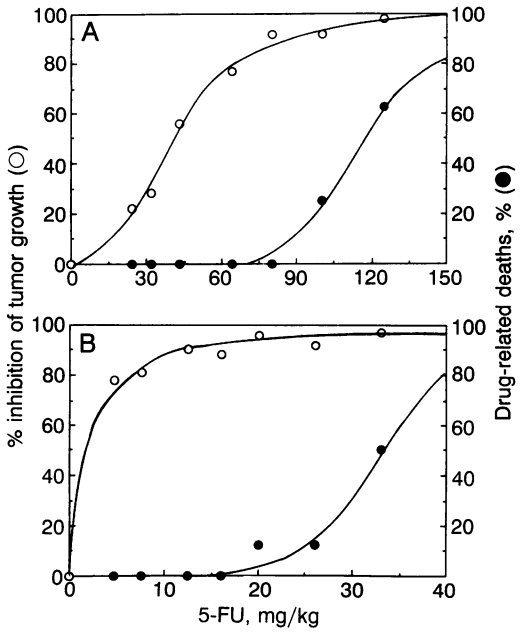

FIG. 4. Effect of 5-EU on the efficacy and toxicity of 5-FU against s.c. implants of MOPC-315 in mice. Mice with s.c. implants of MOPC-315 were treated once with i.p. 5-FU (±2 mg of 5-EU per kg, p.o.) and monitored for antitumor efficacy (○) and toxicity (●) as described in Table 2 and in *Materials and Methods*. (*A*) 5-FU alone. (*B*) 5-FU plus 5-EU.

substantially. For example, 5-EU treatment of rats increases plasma uracil from 4 μM to 70 μM and plasma thymine from <1 μM to 15 μM (D. J. Nelson and L. Frick, personal communication). We confirmed that extracts of livers from animals treated with 2 mg of 5-EU per kg did not catalyze the conversion of 5-FU to 5,6-dihydro-5-fluorouracil. Thus, 5-EU permitted us to study the pharmacokinetics and the antitumor effects of 5-FU under conditions where DPD did not catalyze the destruction of 5-FU.

5-EU pretreatment significantly increased the AUC and $t_{1/2}$ values of 5-FU in mice and rats. These effects of 5-EU are consistent with clinical observations that DPD influences the pharmacokinetic properties of 5-FU in patients. For example, Fleming *et al.* (5) observed a linear correlation between DPD activity in peripheral mononuclear cells and clearance of 5-FU in cancer patients, and Harris *et al.* (8) reported a

Table 2. Effect of 5-EU on the therapeutic index of 5-FU in mice implanted s.c. with MOPC-315 myeloma tumor cells

Treatment	5-FU ED_{50},* mg/kg	5-FU LD_{40}, mg/kg	Therapeutic index (LD_{40}/ED_{50})	Increase in therapeutic index,† fold
Experiment 1				
5-FU alone	57	75	1.3	
5-FU + 5-EU	4.0	17	4.2	3.2
Experiment 2				
5-FU alone	41	130	3.2	
5-FU + 5-EU	4.7	27	5.7	1.8
Experiment 3‡				
5-FU alone	54	110	2.0	
5-FU + 5-EU	4.0	32	8.0	4.0

*Dose that results in a 50% inhibition of tumor growth at 12–13 days post treatment.
†Ratio of the therapeutic index for 5-EU plus 5-FU to the therapeutic index of 5-FU alone.
‡5-EU was dosed at t = 0 hr and t = 8 hr on days 1–3. Therapeutic index was calculated from ED_{80} because the lowest 5-FU dose in the 5-EU-treated group resulted in 80% inhibition (see Fig. 4).

circadian rhythm of DPD activity that correlates inversely with circadian variations in plasma 5-FU levels during continuous infusions of 5-FU. However, the influence of DPD activity on the pharmacokinetics of 5-FU was most dramatically demonstrated by the inadvertent administration of 5-FU to a patient who was (at that time) not known to be genetically deficient in DPD (6). Subsequent pharmacokinetic studies showed that this person eliminated 5-FU with a $t_{1/2}$ of 159 min (12-fold greater than normal controls) and excreted 90% of the dose into urine as unchanged drug (compared to 10% for controls).

5-FU is not commonly administered via the oral route because of considerable patient-to-patient variations in oral bioavailability. Christophidis *et al.* (23) showed that oral administration of 5-FU (10–15 mg/kg) to 12 patients resulted in plasma drug levels that varied from 0 to 10.5 μg/ml and bioavailabilities that varied between 0 and 74%. Similar results were reported by others (24, 25). Our studies showed that the oral administration of 5-FU to rats also produced large interanimal variability in 5-FU plasma concentration vs. time profiles (Fig. 1*A*). The coefficient of variation of the mean AUC (90 ± 50 μM·hr) was 55%. Because the oral bioavailability of 5-FU was ≈100% in 5-EU-treated rats, the variability of the 5-FU AUC (440 ± 60 μM·hr; coefficient of variation = 14%) was considerably reduced (Fig. 1*B*).

Christophidis *et al.* (23) also showed that the bioavailability of p.o. and i.v. 5-FU is nonlinear vs. dose. The AUC of 5-FU markedly increases with increasing dose, suggesting that metabolism via DPD is a saturable process. Similarly, Abernethy *et al.* (26) found that a 2-fold increase in the p.o. dose of 5-FU (from 300 to 600 mg/m^2) resulted in a 6-fold increase in AUC. Disproportionate increases in AUC and half-life with increasing dose of i.v. 5-FU were observed in other clinical investigations (25, 27). Our studies with 5-EU-treated rats (Fig. 2) demonstrated linear disposition kinetics with p.o. 5-FU for doses up to 30 mg/kg. Since clinical doses of 5-FU are expected to be in this range, these observations suggest that pretreatment with 5-EU will lead to predictable 5-FU disposition pharmacokinetics in patients, and physicians will have more control maintaining plasma 5-FU between effective and toxic levels.

Since 1983, 10 phase II clinical trials have demonstrated that protracted (>30 days) constant i.v. infusion of 5-FU is more effective (30% vs. 7% average response) and less toxic than repeated i.v. bolus injections of 5-FU (28). Plasma drug levels in patients constantly infused with 5-FU (300 mg/m^2 per day) oscillate over a 24-hr period with peaks and troughs of 28 and 5 ng/ml, respectively, that inversely reflect the circadian periodicity of DPD (8). We simulated the pharmacokinetics of p.o. dosed 5-FU in humans predosed with 5-EU assuming that inactivation of DPD by 5-EU produced a pharmacokinetic profile of 5-FU equivalent to that in patients genetically lacking DPD ($t_{1/2}$ = 159 min, ref. 6) and that the oral bioavailability of 5-FU was 100%. The simulations show that in the patient described in ref. 6, 5-FU (1.25 mg, p.o.) dosed every 8 hr would produce plasma 5-FU levels that oscillate between 8 and 25 ng/ml. 5-FU (1 mg, p.o.) dosed every 6 hr would yield levels that ranged from 11 to 23 ng/ml (Soo Peang Khor, personal communication). Thus, patients predosed with 5-EU could take three or four pills of 5-FU per day and have smaller peak-to-trough oscillations in plasma 5-FU levels than would occur in patients on constant i.v. infusion of 5-FU alone.

5-EU potentiated the antitumor activity and the toxicity of 5-FU in two mouse tumor models. However, the antitumor activity was potentiated to a greater degree, such that the therapeutic index of 5-FU was increased up to 4-fold. In addition, Rustum *et al.* (29) showed that 5-EU increased the effectiveness and therapeutic index of 5-FU in rats with advanced s.c. colon carcinoma. All rats (100%) pretreated

with 5-EU and 5-FU on two dosing schedules had complete and sustained tumor regressions, whereas only 0–13% complete regressions occurred at the maximum tolerated dose of 5-FU alone.

5-FU has complex biochemical effects in normal and tumor cells. Its mechanism of action may involve tumor/host differences in the inhibition of thymidylate synthase by 5-fluorodeoxyuridine monophosphate, the incorporation of 5-FU into RNA, and/or its incorporation into DNA (1, 2, 30). Consequently, the mechanism by which 5-EU increased the therapeutic index of 5-FU may be difficult to evaluate. The dramatically increased plasma uracil and thymine concentrations generated by 5-EU treatment may play a role in selectively protecting the host against 5-FU toxicity or in increasing 5-FU efficacy. 5-EU would also prevent the formation of α-fluoro-β-alanine, a neurotoxic catabolite (31) that has a $t_{1/2}$ of about 33 hr in patients (3).

(*E*)-5-(2-bromovinyl)uracil (BVUra), another inactivator of DPD, increases the $t_{1/2}$ and AUC of 5-FU in rats (9). BVUra or (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVdUrd), a prodrug of BVUra, increases the antitumor activity of 5-FU against P388 leukemia and Lewis lung carcinoma (9, 12). Although the therapeutic index of 5-FU was not quantitated in these studies, BVdUrd was shown to improve the therapeutic index of 5-FU against Adenocarcinoma 755 and MOPC-315 (10, 32). However, BVUra is considerably less active than 5-EU as an inactivator of DPD (13). *In vivo*, BVUra and BVdUrd were administered at doses ranging from 42 to 100 mg/kg in the P388, Adenocarcinoma 755, and MOPC-315 studies (9, 10, 32), and three daily 10 mg/kg doses of BVdUrd were used against Lewis lung (12). In contrast, 5-EU was administered at 2 mg/kg in our studies.

Elevation of endogenous plasma uracil levels is another measure of DPD inhibition. Plasma uracil is elevated from a control level of 0.8 μM to a maximum of about 5 μM in mice dosed with 100 mg of BVdUrd per kg (10). In contrast, 5-EU doses of 0.5–1 mg/kg elevate mouse plasma uracil concentrations up to 60 μM and sustain uracil at this level for 4 hr (D. J. Nelson and L. Frick, personal communication), reflecting total inactivation of DPD (33).

Although 5-FU is the agent of choice for colorectal carcinoma, response rates for 5-FU alone in traditional bolus schedules are 15% or less (28). Therefore, new strategies to improve the safety and efficacy of 5-FU are important. The present report indicates that 5-EU may be useful for improving the response rate and the therapeutic index of 5-FU.

We thank Suzanne Joyner, Chris Richman Boytos, and Sharon Rudolph for their technical assistance; D. Nelson, L. Frick, and Soo Peang Khor for sharing some of their unpublished data; and B. Huber, J. Burchall, S. Jacobs, and T. Krenitsky for their support and interest.

1. Chabner, B. A. & Meyers, C. E. (1985) in *Cancer, Principles and Practice of Oncology*, eds. DeVita Jr., V. T., Hellman, S. & Rosenberg, S. A. (Lippincott, Philadelphia), pp. 287–328.
2. Meyers, C. E. (1981) *Pharmacol. Rev.* **33**, 1–15.
3. Heggie, G. D., Sommadossi, J.-P., Cross, D. S., Huster, W. J. & Diasio, R. B. (1987) *Cancer Res.* **47**, 2203–2206.
4. Naguib, F. N. M., el Kouni, M. H. & Cha, S. (1985) *Cancer Res.* **45**, 5405–5412.
5. Fleming, R. A., Milano, G., Thyss, A., Etienne, M.-C., Renée, N., Schneider, M. & Demard, F. (1992) *Cancer Res.* **52**, 2899–2902.
6. Diasio, R. B., Beavers, T. L. & Carpenter, J. T. (1988) *J. Clin. Invest.* **81**, 47–51.
7. Tuchman, M., Stoeckeler, J. S., Kiang, D. T., O'Dea, R. F., Ramnaraine, M. L. & Mirkin, B. L. (1985) *N. Engl. J. Med.* **313**, 245–249.
8. Harris, B. E., Song, R., Soong, S.-J. & Diasio, R. B. (1990) *Cancer Res.* **50**, 197–201.
9. Desgranges, C., Razaka, G., De Clercq, E., Herdewijn, P., Balzarini, J., Drouillet, F. & Bricaud, H. (1986) *Cancer Res.* **46**, 1094–1101.
10. Iigo, M., Araki, E., Nakajima, Y., Hoshi, A. & De Clercq, E. (1988) *Biochem. Pharmacol.* **37**, 1609–1613.
11. Fujii, S., Shimamoto, Y., Ohshimo, H., Imaoka, T., Motoyama, M., Fukushima, M. & Shirasaka, T. (1989) *Jpn. J. Cancer Res.* **80**, 167–172.
12. Iigo, M., Nishikata, K-I., Nakajima, Y., Hoshi, A. & De Clercq, E. (1990) *Eur. J. Cancer* **26**, 1089–1092.
13. Porter, D. J. T., Chestnut, W. G., Merrill, B. M. & Spector, T. (1992) *J. Biol. Chem.* **267**, 5236–5242.
14. Cooper, G. M. & Greer, S. (1970) *Cancer Res.* **30**, 2937–2941.
15. Gentry, G. A., Morse, P. A., Jr., & Dorsett, M. T. (1971) *Cancer Res.* **31**, 909–912.
16. Klecker, R. W., Jr., Jenkins, J. F., Kinsella, T. J., Fine, R. L., Strong, J. M. & Collins, J. M. (1985) *Clin. Pharmacol. Ther.* **38**, 45–51.
17. Naguib, F. M. N., el Kouni, M. H. & Cha, S. (1989) *Biochem. Pharmacol.* **38**, 1471–1480.
18. Porter, D. J. T., Chestnut, W. G., Taylor, L. C. E., Merrill, B. M. & Spector, T. (1991) *J. Biol. Chem.* **266**, 19988–19994.
19. Upton, R. A. (1975) *J. Pharm. Sci.* **64**, 112–114.
20. Iigo, M., Kuretani, K. & Hoshi, A. (1983) *Cancer Res.* **43**, 5687–5694.
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
22. Potter, M. (1972) *Physiol. Rev.* **52**, 631–719.
23. Christophidis, N., Vajda, F. J. E., Lucas, I., Drummer, O., Moon, W. J. & Louis, W. J. (1978) *Clin. Pharmacokinet.* **3**, 330–336.
24. Cohen, J. L., Irwin, L. E., Marshall, G. J., Darvey, H. & Bateman, J. R. (1974) *Cancer Chemother. Rep.* **58**, 723–731.
25. Finch, R. E., Bending, M. R. & Lant, A. F. (1979) *Br. J. Clin. Pharmacol.* **7**, 613–617.
26. Abernethy, D. R., Alper, J. C., Wiemann, M. C., McDonald, C. J. & Calabresi, P. (1989) *Pharmacology* **39**, 78–88.
27. Schaaf, L. J., Dobbs, B. R., Edwards, I. R. & Perrier, D. G. (1987) *Eur. J. Clin. Pharmacol.* **32**, 411–418.
28. Hansen, R. M. (1991) *Cancer Invest.* **9**, 637–642.
29. Rustum, Y. M., Cao, S. & Spector, T. (1993) *Proc. Am. Assoc. Cancer Res.* **34**, 283 (abstr.).
30. Parker, W. B. & Cheng, Y. C. (1990) *Pharmacol. Ther.* **48**, 381–395.
31. Okeda, R., Shibutani, M., Matsuo, T., Kuroiwa, T., Shimokawa, R. & Tajima, T. (1990) *Acta Neuropathol.* **81**, 66–73.
32. Ben-Efraim, S., Shoval, S. & de Clercq, E. (1986) *Br. J. Cancer* **54**, 847–851.
33. Spector, T., Harrington, J. A. & Porter, D. J. T. (1993) *Biochem. Pharmacol.*, in press.