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Phase I clinical trial of 5-fluoro-pyrimidinone (5FP), an oral prodrug of 5-fluorouracil (5FU)

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Summary

Purpose: 5-Fluoro-Pyrimidinone (5FP) is an oral pro-drug of 5-Fluorouracil (5FU), and is converted to 5FU by hepatic aldehyde oxidase. Preclinically, 5FP demonstrated anti-tumor activity against colon 38 and P 388 leukemia models in mice. Using an accelerated titration trial design with one patient cohorts and initial 100% escalations, a Phase I trial was conducted to determine the maximum tolerated dose (MTD) of 5FP and describe its toxicity and pharmacokinetic profile.

Patients and methods: 5FP was administered orally once daily for 5 days every 4 weeks. The initial dose level was 23 mg/m^2/d. Using single patient cohorts, escalation proceeded according to accelerated titration 4B design, initially by 100% and subsequently 30–35% escalations (exact escalation determined by pill size) until dose limiting toxicity was observed. A total of 19 patients were enrolled with a median age of 56 years and median performance status of 1. Most patients were heavily pre-treated with chemotherapy, radiation therapy, or both, and patient population included a wide variety of tumor types.

Results: Dose escalation proceeded rapidly to 1715 mg/m^2/d with the only toxicities observed being nausea and vomiting. The large number of pills necessary at that point required a formulation change, which resulted in appreciable hematologic toxicity. This led to rapid de-escalation of dose in subsequent patients, with the MTD finally being determined to be 625 mg/m^2/d. The DLTs observed were grade 4 neutropenia for greater than 5 days and grade 3 anemia. Other toxicities included nausea, vomiting, fatigue, constipation and mucositis. Pharmacology studies confirmed that 5FP was converted to 5FU in humans at all dose levels. However, the extent of conversion decreased over the five daily treatments, but returned for the subsequent cycle. The hematologic toxicity was not related to 5FU exposure per course.

Conclusion: 5FP is a tolerable oral outpatient therapy. Accelerated titration was an efficient way of conducting this phase I trial. The recommended phase 2 dose is 625 mg/m^2/d orally for 5 days every 28 days.

Introduction

5FU is a widely used anti-metabolite having moderate activity in the treatment of a variety of malignancies of epithelial origin. Since its initial synthesis by Heidelberger over 30 years ago, much work has been done to elucidate its cellular pharmacology and mechanism of action [1]. There is evidence for the entry of fluorouracil into the cells via a carrier-mediated process [2]. Intracellularly, 5FU is converted to FUMP and FUTP, and subsequently FdUMP and FdUTP. The cytotoxicity of fluorouracil is reported to be related to several mechanisms, including: inhibition of thymidylate synthetase by FdUTP [6–8]; incorporation of FUTP into cellular RNA [3–5]; and incorporation of FdUTP into

cellular DNA [6,10]. The relative contribution of each of these mechanisms to cellular toxicity is not clear.

5FP differs from 5FU chemically only in that it lacks a keto-group at position 4 of the ring structure (Figure 1). The keto-group is introduced when absorbed 5FP is converted to 5FU by hepatic aldelyde oxidase [15,16].

Guo and colleagues described the pharmacology of 5FP in BDF1 mice [16]. The oral bioavailability of 5FP in BDF1 mice varied between 78 and 100%, depending on dosage and dosing regimen, and the $t_{1/2}$ of 5FP was slower than that of corresponding doses of 5FU. Clearance rate of 5FP was three-fold greater than that of 5FU. There was rapid conversion of 5FP to 5FU in vivo and steady state plasma levels of the resulting 5FU were sustained for at least 4 hours [16]. These data suggested that orally administered 5FP may be able to mimic administration of 5FU by continuous infusion. Guo et al. also reported that oral 5FP was active in the colon 38 and P388 leukemia models in mice indicating that conversion to and delivery of 5FU occurs in a biologically effective manner. They also suggested that twice the milligram dose of 5FP must be administered to achieve the same effects of 5FU. Toxicology studies identified that the profiles of 5FU from 5FP were the same as 5FU alone when administered in a dose ratio of 2:1 that achieved equitoxicity.

Since 5FP was shown to be orally bioavailable in animal studies, it potentially represents a convenient method of delivery of systemic 5FU, especially for prolonged administration schedules. In addition, since the active drug, 5FU, is formed in the liver, the administration of 5FP may lead to higher hepatic 5FU levels and a greater anti-tumor effect against liver metastases or primary liver cancer. 5FP was advanced to Phase I clinical evaluation with the following objectives: To demonstrate 5FP is a prodrug in humans, being converted to 5FU; To determine the MTD of oral 5FP on a daily $\times$ 5, q 4 weekly schedule; To describe the toxicity associated with 5FP administration; To characterize the 5FU exposure resulting from the first and fifth days of 5FP treatment.

Materials and methods

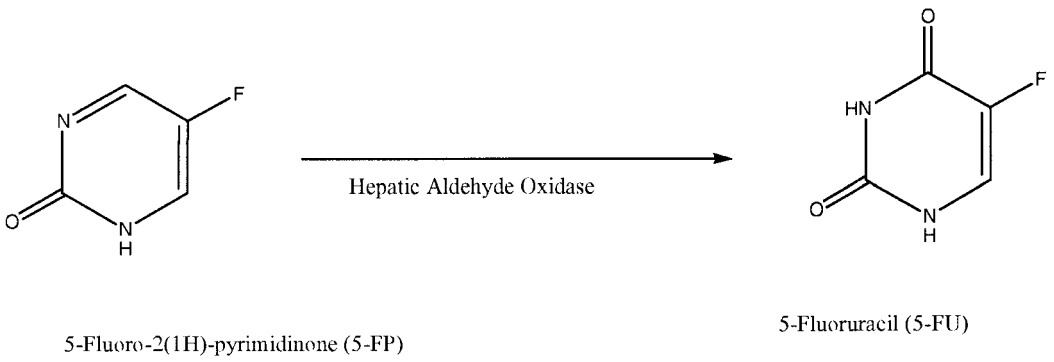
Patient eligibility criteria

Patients with histologically confirmed solid tumors refractory to conventional therapy or for whom no effective therapy was known were eligible for this study. Eligibility criteria included: anticipated survival time of at least 12 weeks; Zubrod Performance Status $\leq$2; no chemotherapy or radiotherapy within 28 days of study entry (42 days for mitomycin C or nitrosourea); age $\geq$18 years; no evidence of brain metastasis on computed tomographic scan; adequate bone marrow reserve (WBC count $\geq$3,000/μL, platelet count $\geq$100,000/μL); adequate liver (serum bilirubin $\leq$1.5 mg/dL) and renal function (serum creatinine $\leq$1.5 mg/dL); negative pregnancy test in women of childbearing potential; no history of malabsorption or gastric resection; no additional coexistent medical problems of sufficient magnitude to jeopardize compliance with the study. The eligibility criteria included no limit on the extent of prior treatment with chemotherapy or radiotherapy including prior 5FU. The study was approved by the Wayne State University Human Investigations Committee and was conducted in accordance with the Declaration of Helsinki. In compliance with institutional as well as Food and Drug Administration guidelines, patients were informed of potential toxicities and all provided written consent to participate in this Phase I study.

Toxicity and response evaluation

Pre-treatment tests and measurements included a complete history and physical examination. Laboratory studies included a complete blood count (CBC) with differential, prothrombin time, partial thromboplastin time, electrolytes, multiphasic chemistry profile (total protein, albumin, blood urea nitrogen, creatinine, lactic dehydrogenase, alkaline phosphatase, calcium, uric acid, total and direct bilirubin, serum alanine aminotransferase), and urinalysis. Baseline chest X-ray, electrocardiogram, and pertinent radiographic studies for assessment of evaluable/measurable disease were also obtained. Interim history and physical examinations were performed weekly to evaluate toxic effects. CBC was monitored weekly initially, then twice weekly when myelosuppression was noted. Multiphasic chemistry profile and serum electrolytes were assessed weekly.

Tumor assessment was performed at least every 2 months, more frequently if disease progression was suspected. Clinical responses were determined according to the following criteria. A complete remission (CR) required total disappearance of all measurable lesions with no new lesions and normalization of any elevated tumor markers for at least 4 weeks. A par-



5-Fluoro-2(1H)-pyrimidinone (5-FP)

5-Fluoruracil (5-FU)

Figure 1. The structure of 5FP and its enzymatic conversion to 5FU by hepatic aldehyde oxidase.

tial remission (PR) required a decrease by at least 50% in the sum of the products of the perpendicular diameters of all measured lesions without appearance of new lesions for at least 4 weeks. Stable disease (SD) was defined as a decrease in lesion size from 0% to <50% lasting at least 4 weeks or a steady state not qualifying for increasing disease of at least 8 weeks duration, again without the appearance of new lesions. Progressive disease (PD) was defined as an increase of at least 25% in the sum of the products of the perpendicular diameters of all measured lesions or appearance of new lesions.

Treatment

5FP was supplied for oral administration by Sparta Pharmaceuticals in hard gelatin capsules containing initially 20 mg, and 50 mg, and subsequently 100 mg (new formulation prompted by large number of pills) of the drug. All doses were rounded to the nearest 20 mg, and only whole capsules were administered. The drug was administered with water (8 ounces) after an overnight fast.

This was an open-label Phase I dose-escalation and pharmacokinetic study of 5FP. The schedule of administration was once daily × 5 days with a 23-day recovery period prior to the next cycle.

The accelerated titration 4B design [18] was used. The starting dose was 23 mg/m^2/dx5. Using the 4B accelerated titration design, each initial cohort consisted of 1 patient, and dose was escalated between patients by 100% with each new course until the first instance of DLT (≥ grade 3 non-hematologic toxicity, excluding nausea, vomiting, alopecia or fatigue, or grade 4 myelotoxicity) was encountered at any course or the second instance of any course grade 2 toxicity of any type was encountered in any course (except nausea, vomiting or alopecia). Thereafter, 25%–35% increments were used until the MTD was reached. New cohort patients were not treated until the previous patient completed the 4-week cycle. Cohort size was expanded to 3 patients per dose level when the second instance of any grade 2 toxicity of any type (other than nausea, vomiting or alopecia) was observed. Patients who experienced toxicity (≥ grade 2) continued treatment at the current dose level. Patients were de-escalated to the lower dose level if they experienced DLT with the current course. Cohort size was expanded to 6 patients when DLT was encountered. With 5FP, the DLT consisted of myelotoxicity, specifically > 500 neutrophils nadiring for ≥ 5 days. At the dose level of 1715 mg/m^2, the large number of pills necessitated a formulation change. The MTD was defined as the dose level below that which produced drug-attributable, dose-limiting toxicity in at least 2 of a minimum of 6 patients receiving either their first or second course of 5FP. Dose limiting toxicity was defined as any of the following: ≥ Grade 2 neurologic, renal, or cardiac toxicity, ≥ Grade 3 other non-hematologic toxicity (excluding alopecia, nausea and vomiting), or Grade 4 hematologic toxicity. Toxicities were graded according to NCI Common Toxicity Grading Criteria [19].

Pharmacokinetics

Sample collection

Six to eight mL of blood was collected at pre-dose, 5 min, 10 min, 15 min, 20 min, 30 min, 45 min, 60 min, 75 min, 90 min, 120 min, 150 min, 180 min, and at 360 min after administration on days 1 and 5 of the treatment in heparin-sodium vacutubes. Plasma was isolated by centrifugation of the tubes for 10 min at

2500 rpm, spiked with chlorouracil internal standard, and stored at –20 °C for HPLC analysis.

Separation 5 FP and 5-FU from plasma

Both 5FP and 5FU were separated from plasma by solid phase extraction. Briefly, a 1 mL Supelclean LC_SCX column (Suplenco, USA) was primed with 1 mL of methanol, followed by 1 mL 0.1M of Copper (II) solution, and finally 2 mL of 50 mM potassium monophosphate buffer (pH 7). Three hundred μL of plasma was loaded onto the column and washed sequentially with 2 mL of 50 mM potassium monophosphate buffer (pH 7), 2 mL of methanol, and 1 mL ether. The prodrug (5FP), its active metabolite (5FU) and internal standard were eluted with 700 μL of 1.8M aqueous ammonia solution. The elute was evaporated to dryness under a stream of nitrogen in a water bath at 60 °C and reconstituted in 150 μL of distilled water by vortexing.

Estimation of 5FP and 5FU in plasma

The concentration of 5FP and 5FU in plasma was estimated using a high performance liquid chromatography system consisting of 501 HPLC Pump (Waters Corporation), AS-100 autosampler systems (BioRad Laboratories), 490E multiwavelength detector (Waters Corporation) and Millennium^{32} version 3.05.01 software. Mobile phase consisted of 50 mM potassium monophosphate buffer (pH 7) and ran at the flow rate of 0.8 mL per minute with detection at 216 nm. A C-18 reverse phase column (Alltech Associates, USA) of 250 × 4.6 mm containing 5 μm size adsorbent as stationary phase was used. The concentrations in 50 μL of sample were determined by back extrapolation to standard curves relating increasing 5FU and 5FP to 5CU (I.S.) ratios and detector response. To minimize degradation, the autosampler was cooled to 4 °C and column was maintained at 15 °C.

Data analysis

Peak plasma concentration of 5FP and 5FU on days 1 and 5 were determined by direct measurement. Using WinNonlin^{TM}, version 1.5 (Scientific Consulting, Inc.), the area under the curve (AUC) was determined assuming a simple compartmental model.

Results

Patient characteristics are shown in Table 1. A total of 19 patients were enrolled on the trial. These patients had a variety of tumor types, and most of them were heavily pretreated. The number of patients on each dose level of 5FP are shown in Table 2. The majority of patients completed at least one cycle of drug. The total number of courses at each dose are also depicted in Table 2.

Table 1. Patient characteristics

Characteristic	Number
Total No.	19
Median age	56 (44–76)
Median P.S.	1 (0–1)
Male: Female	15:4
Prior treatment	
Chemotherapy	16
Radiation	12
Immunotherapy	7
Chemo+radiation	10
Chemo+radiation+immunotherapy	3
Tumor types	
Renal	5
Melanoma	4
Lung	3
Colorectal	2
Gastric	2
Thymus	2
Prostate	1

Table 2. Patients on each dose level of 5FP

Dose (mg/m^2/d)	New patients	Escalated	De-escalated	Courses
23	1	0		1
28.7	1	1		2
56	1	1		2
112	1	2		3
224	1	1		2
400	1	1		2
500	2	1		3
625	7(7 nf)*	2(1 of, 1 nf)	19	
875	3(3 nf)	2(1 of, 1 nf)	2(2 nf)	7
1225	1(1 nf)	1(1 of)		2
1715	0	2(1 of, 1 nf)		2

*nf: new dose formulation of the drug; of: old formulation (required large number of pills)

Hematologic toxicity

Myelosuppression (neutropenia) was the dose limiting toxicity in this study. The hematologic toxicity is displayed in Table 3. Patients at dose levels 23 mg/m^2 through 500 mg/m^2 tolerated the drug very well without appreciable toxicity. Patient # 6 was entered in the study at the dose of 400 mg/m^2. Since he tolerated it well, it was escalated to 500 mg/m^2, then to 625, 875, 1225, and 1715 mg/m^2. At this point he only experienced nausea and vomiting. The administration of a large number of pills at this dose forced a change in dosage of the capsules. The dose per capsule was changed from 50 mg to 100 mg hard gelatin capsules. He was begun on the new formulation at the 1715 mg/m^2 dose. The patient was hospitalized and experienced grade 4 neutropenia and leukopenia, grade 3 anemia, grade 1 thrombocytopenia and mucositis, grade 4 nausea and vomiting and grade 1 diarrhea at this dose. Although some may argue that this toxicity was cumulative, myelosupression was also observed when his dose was de-escalated, as well as in novel patients treated at both the 1225 and 875 mg/m^2 dose with the new formulation. There was no suggestion of such myelotoxicity in any patient treated with the old formulation. He subsequently underwent de-escalation to the dose of 875 mg/m^2 with the main toxicities at this dose being grade 4 neutropenia, grade 2 anemia, grade 2 nausea and vomiting, and grade 2 fever. When new capsules were administered, the number of administered capsules was reduced by at least half. When wet, the capsules tend to stick together due to the gelatin that forms the outer core of the capsule. This may result in slower drug release in the gastrointestinal tract. Reduction of the number of capsules (as a result of increased capsule size), will minimize this effect resulting in quicker drug release followed by faster absorption. In vitro dissolution studies usually do not predict these events as standard dissolution testing is performed using 10 capsules in 1000 mL of simulated gastric fluid. Other toxicities experienced with this new formulation (875 mg/m^2) included grade 4 neutropenia (2 pts.), grade 3 leukopenia (2 pts.), grade 1 thrombocytopenia (1 pt.), grade 2 constipation (1 pt.), grade 1 nausea (1 pt.) and grade 2 vomiting (2 pts.). At the 625 mg/m^2 dose, maximum toxicity was grade 2 (Table 3, 4). The MTD with this new formulation was determined to be 625 mg/m^2/d.

At this dose level, there were no admissions for neutropenic fever or sepsis, and none of the patients required platelet transfusions. One patient was admitted for nausea and vomiting secondary to small bowel obstruction (thought possibly due to capsule bezoar). There were no deaths from treatment in the study.

Other toxicities

Table 4 illustrates the non-hematologic toxicities observed with 5 FP. The main toxicities were gastrointestinal including nausea, vomiting, diarrhea, constipation, fatigue and mucositis. Most of these toxicities were mild except for nausea and vomiting which were grade 4 in 1 patient.

Pharmacokinetic results

Of the 19 patients investigated in the study, pharmacokinetic analysis was possible in 11 patients in the dose range of 500 mg/m^2 to 1225 mg/m^2. Treatment was discontinued in the remaining patients due to progression of the disease and not associated with drug administration. The data suggested that all the patients converted 5FP to 5FU on days 1 and 5 of therapy, and hence 5FP is a prodrug of 5FU (Table 5). Based on the sum of 5FP plus 5FU AUCs, increasing the 5FP dose increased the amount of absorbed drug (Figure 2, Table 5). However, the AUC of 5FU is highly variable and at times did not increase proportionally with an increase in dose (Figure 2c and 2d). Further, 5FU AUC was considerably lower and averaged 1500 μM-min on day 5 at all administered doses (Figure 2d, Table 6). Drug related hematotoxicity (neutropenia) observed out to 5 cycles did not correlate with C_{pmax} (Figure 3) or AUC of either 5-FP or 5-FU (data not shown).

Discussion

5FU remains one of the most commonly used agents for the treatment of a variety of neoplastic disorders and is used in a variety of schedules [11–14]. When given as a bolus dose, the doses range from 300 to 450 mg/m^2/d intravenously for five days every 28 days or 600 to 750 mg/m^2 intravenously weekly or every other week. Infusional 5FU has also been used extensively in schedules ranging from 1g/m^2/d for 4–5 days to prolonged infusions at the doses of 200–300 mg/m^2/d. Although higher response rates have been observed with continuous infusion 5FU, none of the different schedules have impacted survival [14].

This phase I study reports the first clinical experience with 5FP, which is a rationally designed oral fluoropyrimidine, converted to 5FU by hepatic aldehyde-oxidase. It offers an advantage over 5FU in

Table 3. Incidence of hematologic toxicity

Adverse events	Dose level (mg/m^2/d)[a]							
	625 N=9*		875 N=7*		1225 N=2*		1715 N=2*	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Anemia	1	0	1	1	1	0	0	1
Thrombocytopenia	0	0	1	0	0	0	1	0
Neutropenia	1	0	0	2	0	1	0	1

[a]patients at dose-levels 23 mg/m^2 through 500 mg/m^2 did not experience significant toxicity *N = number of patients per dose level

Table 4. Incidence of non-hematologic toxicity

Adverse events	Dose level (mg/m^2/d)[a]							
	625 N=9*		875 N=7*		1225 N=2*		1715 N=2*	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Nausea	6	1	4	0	1	0	2	0
Vomiting	3	1	3	0	0	0	2	0
Fatigue	4	0	4	0	0	0	1	0
Constipation	2	0	2	0	1	0	1	0
Diarrhea	4	0	0	0	0	0	0	0
Mucositis	3	0	0	0	1	0	1	0

[a]patients at dose-levels 23 mg/m^2 through 500 mg/m^2 did not experience significant toxicity; *N= total number of patients per dose level

Table 5. 5-FP to 5-FU conversion on day 1 of cycle 1

Administered dose (mg/m^2/d)	No. of patients	Total AUC* (mM-min)	± Stdev	5FU AUC (mM-min)	± Stdev	Ratio of 5-FU AUC: 5-FP AUC	% Prodrug converters
500	1	1565		987		1.7	100
625	6	6660	± 6586	2049	± 1852	0.44	100
875	3	21872	± 18110	3208	± 1111	0.17	100
1225	1	87498		651		0.0075	100

*Total AUC is the sum of 5 FP and 5 FU AUCs

Table 6. 5-FP to 5-FU conversion on day 5 of cycle 1

Administered dose ($mg/m^2/d$)	No. of patients	Total AUC* (mM-min)	± Stdev	5FU AUC (mM-min)	± Stdev	Ratio of 5-FU AUC: 5-FP AUC	% Prodrug converters
500	1	4565		1097		0.32	100
625	6	19153	± 16744	1420	± 928	0.08	100
875	3	52600	± 21487	1005	± 761	0.02	100
1225	1	78059		1302		0.017	100

*Total AUC is the sum of 5 FP and 5 FU AUCs

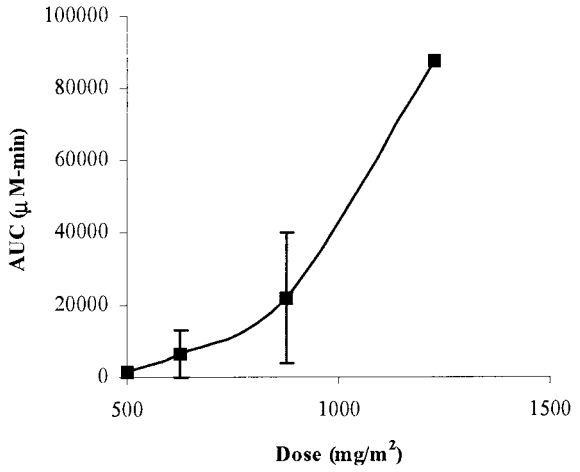


Figure 2a. Relationship between administered dose and total systemic exposure (5FP AUC plus 5FU AUC), indicating that increasing dose increased amount of absorbed drug. Measurement made in Cycle 1 on Day 1 (a) and day 5 (b) of treatment. Relationship between administered dose and systemic exposure to 5FU. Note that 5FU exposure was considerably less on day 5 (d) than day 1 (c).

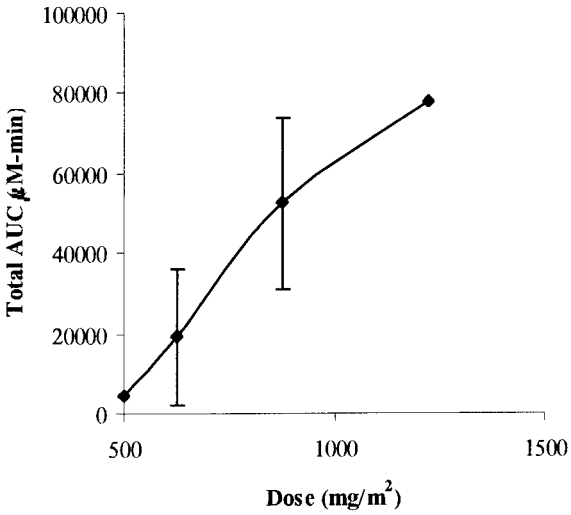


Figure 2b.

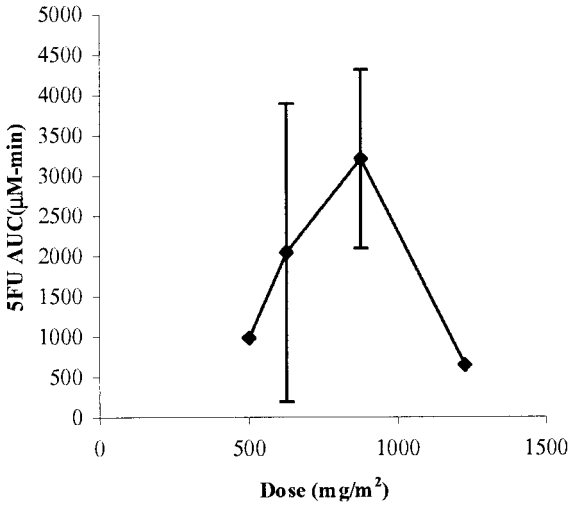


Figure 2c.

that it can be administered in a more convenient outpatient format compared to intravenous 5FU. More importantly, since the active drug, 5FU, is formed in the liver, the administration of 5FP may lead to higher hepatic 5FU levels and a greater anti-tumor effect against liver metastases or primary liver cancer.

The dose limiting toxicity with 5FP was myelosuppression (Table 3). Other toxicities observed were gastrointestinal (nausea, vomiting, constipation, diarrhea, and mucositis – Table 4), and fatigue. There were no cases of hand-foot syndrome. This toxicity profile is similar to that seen with bolus administration of 5FU. The myelosuppression was readily reversible prior to the next cycle (no treatment delays) and patients were able to tolerate prolonged administration

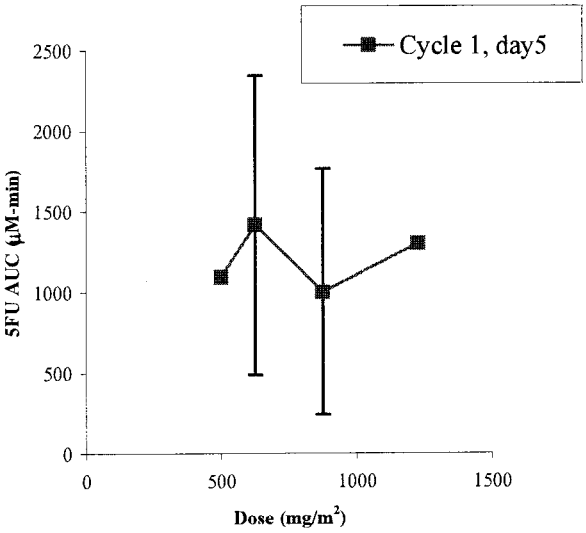


Figure 2d.

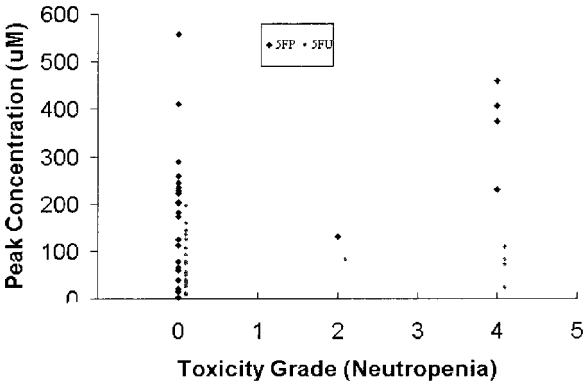


Figure 3. Lack of relationship between grade of neutropenia and peak plasma concentration of 5FP or 5FU (all courses).

of the drug. At the MTD (625 mg/m^2/d), a total of 19 courses of drug were administered among 9 patients. No responses were observed in this trial except for a minor response in a patient with thymoma. The maximum tolerated dose was identified as 625 mg/m^2/d orally for 5 days every 28 days. This dose is also the recommended phase 2 dose.

Pharmacokinetic studies confirmed that 5FP was absorbed and detectable levels were achieved in plasma. Also, 5FP conversion to 5FU was evident both on the first and fifth days of treatment at all dose levels (Tables 5 and 6). The extent of 5FP conversion to 5FU decreased over the five days as evident from the ratios of 5FP AUC to 5FU AUC (Table 5 and 6), but recovered by day one of the subsequent cycle, suggesting adverse drug effects on the activating enzyme that reversed within 23 days of therapy (before the subsequent cycle). This decrease suggests saturation of aldehyde oxidase as the 5FP dose is increased. At each dose level, lower relative conversion of 5FP to 5FU was observed after 5 days of treatment, suggesting further that the enzyme remains saturated with the higher 5FP AUCs achieved on day 5. Consequently, 5FU exposure achieved did not increased proportionally with increase in dose as shown in Figure 2. The hematologic toxicity did not seem to be related either to the 5FP or 5FU peak plasma concentration, since even some of the lowest exposures were associated with grade 4 toxicity and vice-versa (Figure 3). Accelerated titration 4B trial design (rapid intra-patient drug dose escalation) was followed in this trial. We believe that this was an efficient way of escalating the dose of 5FP in this trial. This design proved safe and reduced the number of patients who were under treated, sped the completion of the trial and provided a substantial increase in the amount of information obtained. Had a 3 patient cohort modified Fibonacci design been used, a minimum of 14 dose levels and 45 patients would have been needed to achieve the same endpoint. In this study, a total of 19 patients and 11 dose levels completed accrual. The initial overshoot of the doses beyond that which caused grade 3/4 toxicity was not due to the accelerated titration design, but rather due to the change in formulation.

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