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Phase I trial of sulofenur (LY186641) given orally on a daily × 21 schedule

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Sulofenur (LY186641), a diarylsulfonylurea, was evaluated clinically utilizing either a daily × 21 schedule or a daily × 5 (with 2 days off) for 3 weeks schedule. Eighteen patients with refractory solid tumors received 47 evaluable courses of sulofenur given p.o. daily × 21 every 28 days at five dose levels while 14 received 29 courses of sulofenur given daily × 5 for 3 weeks every 28 days at three dose levels. Toxicities included anemia, methemoglobinemia and hemolysis. One patient experienced a fatal subendocardial infarction on the daily × 21 schedule. One partial response was observed in a patient with a sertoli cell tumor on the daily × 5 for 3 weeks schedule. Daily × 5 for 3 weeks is the schedule recommended for phase II trials.

Key words: Sulofenur, phase I, sulfonylurea, methemoglobinemia, hemolysis, pharmacology.

Introduction

Sulofenur [LY186641, *N*-(5-indanylsulfonyl)-*N'*-4-(chlorophenyl)-urea] is a member of a novel class of orally administered antitumor compounds, the diarylsulfonylureas, demonstrating favorable preclinical antitumor activity with no evidence of hypoglycemic activity. Antitumor activity has been seen against several murine tumors (e.g., colon-26, M-5 ovarian, C3H mammary), human xenografts (e.g., LX-1 lung, VCR 5 colon, GC3 colon) and in a human tumor cloning system at drug concentrations of 300 μg/ml (e.g., lung, breast, colon, kidney, ovary).[1] Complete regressions in advanced rhabdomyosarcoma xenografts have been described in each of six human cell lines studied.[2] Efforts to determine the mechanism of action of sulofenur and related sulfonylureas have failed to show any evidence for cell cycle specificity or for inhibition of protein, RNA or DNA synthesis.[3] Mitochondria have been suggested as a site of sequestration and potential site of action for sulofenur.[4] Structure–activity studies of a series of diarylsulfonylurea compounds have suggested that the lipophilicity of substituents for the aryl domain correlates with antitumor activity of a given compound.[3]

Early phase I trials (p.o. weekly and p.o. daily × 7 schedules) showed dose-limiting toxicities of methemoglobinemia and hemolysis.[5,6] In an effort to maximize the concentration × time product of sulofenur and to try to minimize the toxicities, we have explored a daily × 21 schedule. The objectives of this study included: determination of the maximally tolerated dose (MTD) of sulofenur when given orally on a daily × 21 schedule repeated every 28 days; determination of the qualitative and quantitative toxicities of sulofenur on this schedule; determination of the pharmacokinetics of sulofenur on this schedule; and the search for preliminary evidence for antitumor activity of sulofenur. Once these objectives were met, an alternate schedule was explored in an effort to ameliorate the observed toxicities. Sulofenur was then administered orally daily × 5 for 3 weeks, with the cycle repeated every 28 days.

This study was supported partially by a grant from Eli Lilly and Company, the clinical services of Audie Murphy VA Hospital, and a National Institutes of Health Training Grant (grant RR01346). This study is dedicated to the loving memory of Gerald B Grindey, PhD, who contributed so much to the care of patients with cancer. May he rest in peace.

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Materials and methods

Eligibility

Only patients with histologically diagnosed malignancies refractory to conventional therapy, or for whom no effective therapy was known, were selected as candidates for entry into this study. Prior to study entry, a complete history was taken and a physical examination was performed. Height, weight, performance status and tumor measurements were recorded. Initial laboratory data obtained included a complete blood count (CBC), prothrombin time, partial thromboplastin time, serum electrolytes and chemistries, and urinalysis. A chest radiograph and electrocardiogram were also performed. Eligibility criteria included: (i) a histologically confirmed diagnosis of advanced non-hematologic malignancy; (ii) age of 18 years or greater; (iii) an Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1 or 2 (ambulatory and able to perform self-care with patient ambulatory for >50% of waking hours); (iv) recovery from major surgery or toxicity from prior chemotherapy or radiotherapy for a minimum of 2 weeks; (v) adequate bone marrow function (white blood cell count $\geq 3000/mm^3$, granulocyte count $\geq 1500/mm^3$, hemoglobin ≥ 10 g% or hematocrit >30%, platelet count >100 000/mm^3), renal function (serum creatinine < 2.0 mg/dl, normal urinalysis), metabolic function (serum electrolytes within 10% of normal) and hepatic function (serum total bilirubin ≤ 2.0 mg/dl, SGOT < 2.0 × upper limits of normal unless liver is involved with malignancy); (vi) no history of hemolysis (serum haptoglobin greater than lower limits of normal, negative direct and indirect Coomb's tests); (vii) no red blood cell transfusions within the previous 21 days; (viii) serum methemoglobin $\leq 1.5\%$; (ix) a negative G6PD screen; (x) no history of cardiac disease within the previous year; and (xi) no history of diabetes mellitus requiring medications. Informed consent was obtained from all patients according to federal, state and institutional guidelines.

Dosage and formulation

Sulofenur was provided for this study by Eli Lilly and Company (Indianapolis, IN) in the form of opaque white capsules in 5, 25, 100 and 250 mg strengths. Drug was administered with 6–8 oz of water. Patients refrained from oral intake for 1 h pre-dosing and 15 min post-dosing. In those patients providing blood samples for pharmacokinetic analysis, oral intake was prohibited from 4 h before to 2 h after oral drug administration. However, patients were allowed to drink water *ad libitum* except for the period from 1 h before to 30 min after drug administration.

Study design

The initial objective of this study was to investigate a daily × 21 schedule for sulofenur. The starting dose of 180 mg/m^2 represented two-thirds of the lowest toxic daily dose in monkeys when administering sulofenur on a daily × 90 schedule. Drug was given by mouth, once daily for 21 days, every month. Once an MTD was established with the daily × 21 schedule, a variation of this schedule was explored (daily × 5 for 3 weeks, repeated every 28 days). Drug was continued in individual patients if there was no evidence for disease progression, and if toxicity was acceptable. An attempt was made to treat all patients for at least a 2 month period. Dose escalation or reduction for individual patients was allowed.

Patients were seen weekly while on study, with a complete history taken and physical examination performed at each visit as well as the recording of weight and performance status. The routine laboratory studies noted above were also obtained at each visit. Tumor measurements were performed every 4 weeks with Southwest Oncology Group (SWOG) criteria used for response evaluation.[7] Toxicity was graded according to SWOG criteria. Dose-limiting toxicity was generally defined as toxicity of grade 3 or higher by SWOG criteria. The MTD was defined as the dose level where approximately 75% of patients achieved a reversible grade 3 toxicity.

Pharmacokinetics

Sample collection. Blood samples were collected for pharmacokinetic analyses from at least two patients at each dose level. Nine milliliters of heparinized whole blood were obtained on days 1 and 21 before drug administration, and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24 and 28 h after drug administration. On weeks 1, 2 and 4 blood levels were also obtained for trough levels of sulofenur and its metabolites. The blood samples were centrifuged at 2500 r.p.m. for 10 min. Following this, plasma was removed, and the samples were flash frozen and stored at −20°C

until analysis. Blood samples for the daily × 5 for 3 weeks schedule were collected pre-dose on days 1, 5, 8, 12, 15 and 19, and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h following drug administration on day 19.

Chromatographic procedure. A gradient high-performance liquid chromatographic (HPLC) methodology was developed for the analysis of sulofenur and its hydroxy and keto derivatives. A structurally related analog, LY186642, was added (50 µl, 1 mg/ml) to each 0.2 ml plasma sample or standard as an internal standard (IS). The samples were then acidified with 50 µl of 2N HCl and immediately applied to a pre-conditioned C-2-extraction column (1 ml, 100 mg size Bond-Eluts®; Varian, Harbor City, CA). The columns were preconditioned with 3 ml of HPLC grade methanol followed by 3 ml of distilled, deionized water (DDH_2O). Immediately following the addition of the sample mixture, the column was washed with 2 ml of DDH_2O. The absorbed sulofenur and IS were eluted with 1 ml of 0.01N methanolic solution into tubes containing 100 µl of 0.15N sodium hydroxide. The tubes were vortexed to assure adequate mixing and neutralization of the acid. The extraction efficiencies from plasma for sulofenur, the hydroxy and keto derivatives and IS averaged 86, 91, 85 and 78%, respectively.

The chromatographic procedure consisted of injecting (ISS-100 autoinjector; Perkin-Elmer, Norwalk, CT) 10–200 µl of the extract onto an ambient C18 column (25 cm × 4.6 mm i.d., Supelco, Bellefonte, PA; LC-18-DB, 5 µm) preceded by a Waters guard column packed with pellicular C18 packing (CO-Pell ODS; Whatman, Hillsboro, OR). A pre-column (25 cm × 4.6 mm i.d.) packed with silica (BioSil® A, 100-200 mesh; Bio-Rad, Melville, NY) positioned between the pump (Perkin-Elmer Series 4, Norwalk, CT) and autoinjector was also utilized. The mobile phase consisted of 0.025 M sodium phosphate buffer (pH 7) and acetonitrile (CH_3CN). Elution began with a mixture of 75% buffer, 25% CH_3CN and increased to 35% CH_3CN by 10 min according to the gradient curve 4 on the Perkin-Elmer gradient elution program. From 10 to 20 min, the CH_3CN was increased from 35 to 50% following curve 0.3. The initial conditions were then re-established and allowed to equilibrate (5 min) prior to the next injection. The flow rate was kept constant at 1.5 ml/min. UV absorbance (Kratos, Model 773, Kratos, Ramsey, NJ) was monitored at 254 nm. Chromatograms and peak areas were stored and analyzed on a Hewlett Packard 1000-based computer system. Sulofenur eluted at 17.2 min, the IS at 18.1 min, and the hydroxy and keto metabolites at 5.9 and 9.2 min, respectively. The assay was linear over a range of 1–600 µg/ml with respective relative standard deviations of 11.3–5.8%.

Pharmacokinetic analysis. Elimination rate constants (k_e) were estimated by linear regression analysis of data points on the terminal log-linear portion of the plasma concentration (C_p) versus time curve. Terminal half-lives were calculated by $0.693/k_e$. The area under the plasma concentration versus time curve was calculated by the trapezoidal method up to the last data point and extrapolated to infinity. Clearance was calculated by assuming complete absorption (F) and calculated from the equation, Cl/F = dose/$AUC_{0-\infty}$. The apparent volume of distribution (V_d area) was estimated by the equation clearance/k_e.

Results

Nineteen patients were entered on the daily × 21 schedule. Patient characteristics are shown in Table 1. Patients with colon and lung cancers accounted for over 75% of patients treated.

Fourteen patients were entered on the daily × 5 for 3 weeks schedule. Patient characteristics are shown in Table 1. Patients with lung, colon and renal cancers accounted for over 70% of patients treated.

Table 1. Patient characteristics

	Schedules	
	daily × 21	daily × 5 for 3 weeks
Number of patients	19	14
Male : female	16 : 3	11 : 3
Age (median)	59	60
Performance status (median)	1	1
Tumor type		
colon	5	3
lung	10	4
renal	2	3
unknown primary	2	0
prostate	0	1
breast	0	1
testicular	0	1
sarcoma	0	1
Prior therapy		
chemotherapy	15	11
radiotherapy	9	6
both	8	6
neither	3	3

Table 2. Dose escalation (daily × 21)

Dose (mg/m^2)	No. of patients (evaluable)	No. of courses (evaluable)
280	3 (3)	8 (8)
300	3 (3)	8 (8)
450	3 (3)	12 (12)
630	7 (6)[a]	16 (15)
810	3 (3)	4 (4)

[a]Drug discontinued on day 10 due to disease-related hypercalcemia.

Toxicities

Daily × 21 schedule. Eighteen patients received 47 evaluable courses of therapy on the daily × 21 schedule as shown in Table 2. Drug discontinuation was necessary in one patient on day 10 due to disease-related hypercalcemia. The MTD on this schedule was 810 mg/m^2, with a recommended phase II dose of 630 mg/m^2.

The dose-limiting toxicities observed were reversible methemoglobinemia and hemolytic anemia. Hematologic toxicities are summarized in Table 3. Anemias were grade 1 or 2 except for one patient at a dose level of 630 mg/m^2 with grade 3 anemia (Hgb < 7.0 g%). Red blood cell transfusions were given to three of six patients at 630 mg/m^2 and three of three patients at 810 mg/m^2. Four patients required drug discontinuation due to anemia and methemoglobinemia (two patients at 630 mg/m^2 on days 8 and 19, and two patients at 810 mg/m^2 on days 7 and 19). A majority of patients at the 630 and 810 mg/m^2 dose levels experienced hemolysis as documented by peripheral smear examination and changes in serum haptoglobin and plasma free hemoglobin. Most patients demonstrated peak blood methemoglobin levels > 10% at the 630 and 810 mg/m^2 dose levels. At the 630 mg/m^2 dose level, peak blood methemoglobin levels ranged from 5.6 to 20.1%, with a mean value of 12.6% for the six patients treated. At the 810 mg/m^2 dose level, peak blood methemoglobin levels ranged from 15.1 to 25.2%, with a mean value of 20.3% for the three patients treated. Peak methemoglobin levels for patients receiving sulofenur at a dose level of 630 mg/m^2 occurred on day 22 (four patients) and on day 15 (one patient), with one patient discontinuing treatment on day 8. Blood methemoglobin levels normalized within 1 to 2 weeks following drug discontinuation. Figure 1 shows plots of hemoglobin, reticulocyte count and blood methemoglobin levels for the six patients receiving 630 mg/m^2 during their first course of treatment.

Two patients received methylene blue intravenously to treat methemoglobinemia with some improvement. Methylene blue was administered in the usual fashion, as a 1% solution, at a dose of 1 mg/kg intravenously over 5 min.[8] One patient treated at the 810 mg/m^2 sulofenur dose level was admitted on day 8 of course 1 with moderately severe dizziness, lethargy, a blood hemoglobin of 8.1 g/dl and a blood methemoglobin level of 20.7%. Sulofenur was discontinued on day 8. The patient was given methylene blue 40 mg (1 mg/kg) intravenously on day 8 and also received 2 units of packed red blood cells by transfusion. By day 9, the blood methemoglobin level had fallen to 13.6% and by day 15 the level had dropped to 9.2%, with all symptoms resolving by day 10. A second patient treated with sulofenur 630 mg/m^2 was admitted on day 8 of course 1 with a blood methemoglobin level of 20.1% and associated mild dyspnea. Sulofenur was discontinued on day 8 and methylene blue was administered intravenously at a dose of 70 mg on days 9 and 10. The blood methemoglobin level fell to 1.9% and symptoms completely resolved by day 12.

Non-hematologic toxicities are listed in Table 4. The most common non-hematologic toxicities included cyanosis, lethargy/confusion, nausea and vomiting, and dyspnea. A direct correlation seemed evident between these toxicities and methemoglobinemia. All non-hematologic toxicities were grade

Table 3. Hematologic toxicities (daily × 21)

Dose (mg/m^2)	No. of patients	Anemia		Hemolysis	No. of patients with methemoglobinemia (peak blood metHgb)
		grade 1–2	grade 3		
180	3	3	0	0	2 (2.6%, 4.0%)
300	3	1	0	0	3 (1.7%, 2.7%, 7.5%)
450	3	1	0	1	3 (2.1%, 3.1%, 6.5%)
630	6	5	1	4	6 (5.6%, 9.9%, 10.5%, 12.6%, 17.0%, 20.1%)
810	3	3	0	3	3 (15.1%, 20.7%, 25.2%)

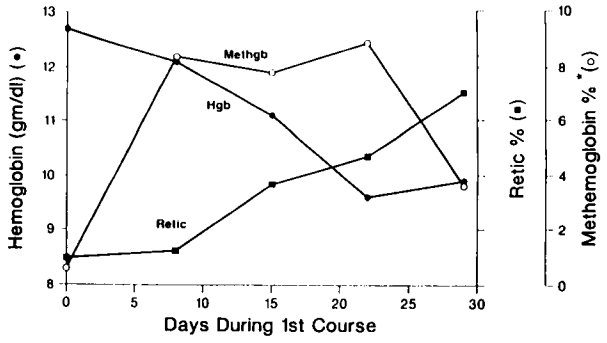

Figure 1. Hematologic parameters for six patients receiving sulofenur 630 mg/m^2 daily × 21 days during the first treatment course. All values are means.

2 or less with the exception of one patient with grade 3 fatigue (at the 810 mg/m^2 dose level) and one patient treated with 300 mg/m^2 who suffered a fatal subendocardial infarction. This patient developed grade 1 anemia and mild reversible methemoglobinemia (peak levels $\leq$ 7.5%) during three courses of treatment. On day 6 of course 4, the patient was admitted with hypercalcemia (corrected serum calcium of 13.5 mg/dl) and treated with aggressive hydration. Sulofenur was discontinued on day 7, at which time the blood hemoglobin was 8.3 g/dl and the blood methemoglobin level was 6.7%. On day 8 the patient suffered a subendocardial infarction complicated by atrial fibrillation and pulmonary edema. Despite aggressive supportive care measures, the patient expired on day 13.

Daily × 5 for 3 weeks schedule. Fourteen patients were treated on the daily × 5 for 3 weeks schedule and received 29 courses of therapy at three dose levels (see Table 5). On this schedule the MTD was 1080 mg/m^2 with a recommended phase II dose of 810 mg/m^2.

Hematologic toxicities observed with the daily × 5 for 3 weeks schedule are detailed in Table 6. Anemias were classified as grade 1 or 2 in all but four patients: two patients at the 950 mg/m^2 dose level and two patients at the 1080 mg/m^2 dose level experienced grade 3 anemias (Hgb × 7.0 g/dl). One of three patients treated at the 950 mg/m^2 dose level and three of six patients treated at the 1080 mg/m^2 dose level required red blood cell transfusions. Overt hemolysis, documented by peripheral smear, reduced serum haptoglobin and/or elevated plasma free hemoglobin, was observed in three patients. A reticulocytosis was noted in all 15 episodes of anemia. Methemoglobinemia was seen in all patients treated on the daily × 5 for 3 weeks schedule. At the 810 mg/m^2 dose level, peak met-hemoglobin levels ranged from 4.7 to 31.3%, with a mean value of 10.9%. At the 950 mg/m^2 dose level, peak serum methemoglobin levels ranged from 12.3 to 18.4%, with a mean value of 13.2%. At the 1080 mg/m^2 dose level, peak serum methemoglobin levels ranged from 6.5% to 31.9%, with a mean value of 17.1%. Sulofenur treatment was discontinued after 2 weeks of therapy in two patients, both at the 1080 mg/m^2 dose level, due to anemia and methemoglobinemia.

Non-hematologic toxicities are listed in Table 7. The majority of these toxicities were classified as grade 2 or less with the most frequently observed toxicities including fatigue/malaise, dyspnea, nausea and vomiting, cyanosis, anorexia, hepatotoxicity, rigors, and chest pain. One patient developed

Table 5. Dose escalation (daily × 5 for 3 weeks)

Dose (mg/m^2)	No. of patients (evaluable)	No. of courses (evaluable)
810	7 (7)	38 (38)
950	3 (2)[a]	3 (2)
1080	6 (6)	8 (7)[b]

[a]Drug discontinued on day 11 due to disease-related small bowel obstruction.
[b]One course discontinued after day 5 due to disease-related small bowel obstruction.

Table 4. Non-hematologic toxicities[a] (daily × 21)

Dose (mg/m^2)	Evaluable patients	Cyanosis	Nausea/ vomiting	Dizziness	Lethargy/ confusion	Dyspnea	Fatigue[b]
180	3	0	0	0	0	0	0
300	3	0	1	0	0	0	0
450	3	0	0	0	0	0	0
630	6	1	1	2	2	1	0
810	3	3	2	1	1	2	1

[a]All toxicities were grade 2 or less except for fatal subendocardial infarction and grade 3 fatigue.
[b]Other toxicities (one patient each): subendocardial infarction at 300 mg/m^2, muscle cramps at 450 mg/m^2 and dermatitis at 630 mg/m^2.

Table 6. Hematologic toxicities (daily × 5 for 3 weeks)

Dose (mg/m^2)	No. of patients	Anemia		Hemolysis	No. of patients with methemoglobinemia (peak blood metHgb)
		grade 1–2	grade 3		
810	7	6	0	2	7 (4.7%, 6.4%, 6.8%, 7.0%, 8.6%, 11.6% 31.3%)
950	3	1	2	1	3 (8.8%, 12.3%, 18.4%)
1080	6	4	2	1	6 (6.5%, 9.7%, 15.4%, 17.3%, 21.6%, 31.9%)

reversible cholestatic jaundice consistent with a sulfonylurea hepatitis. This patient completed the first course of sulofenur at a dose level of 1080 mg/m^2, with a peak blood methemoglobin level of 31.9% on day 12. The blood hemoglobin level fell to 6.0 g/dl on day 25, and the patient also manifested a depressed serum haptoglobin and a reticulocytosis, necessitating transfusion with 3 units of packed red blood cells. On day 25, the patient presented with jaundice and elevated liver function studies showing a total bilirubin of 23.5 mg/dl, alkaline phosphatase of 745 IU/l and SGOT of 353 IU/l. Ultrasonography did not show evidence for biliary duct dilation and a nuclear medicine scan was consistent with hepatocellular dysfunction. On day 58 the hematologic indices for the patient had normalized and liver function studies revealed a total bilirubin of 1.8 mg/dl, alkaline phosphatase of 211 IU/l and SGOT of 51 IU/l.

Antitumor activity

Antitumor activity was not observed on the daily × 21 schedule. On the daily × 5 for 3 weeks schedule, one partial response was noted in a patient with sertoli cell cancer metastatic to lung and skin, with a duration of 2 months. An evaluable but non-measurable response was noted in a patient with colon cancer metastatic to the liver, with a duration of 1 year. One minor response was noted in a patient with renal cell cancer.

Pharmacokinetics

On the daily × 21 schedule, samples were collected for pharmacokinetic studies from 10 patients at dose levels of 180, 300, 450, 630 and 810 mg/m^2. Pharmacokinetic parameters are summarized in Table 8. The harmonic mean half-life ($t_{1/2}$) was 30.1 h, with a mean clearance of (Cl/F) of 2.2 ml/min/m^2 and a mean volume of distribution (V_d area/F) of 5.8 l/m^2. Peak plasma concentrations of the parent drug ranged up to 258.9 μg/ml following day 1 drug administration and up to 342.4 μg/ml following day 21 drug administration. The mean plasma concentration versus time curves for sulofenur following day 1 and day 21 drug administration for patients treated at the 630 mg/m^2 dose level are illustrated in Figure 2. Parent drug elimination curves are similar for days 1 and 21. Figure 3 depicts a linear relationship between sulofenur dose and peak blood methemoglobin levels. A linear relationship was also observed between peak blood methemoglobin levels at each dose level. Figure 4 depicts the relationship between area under the concentration × time curves for sulofenur versus peak blood methemoglobin levels. Keto and hydroxy metabolites have been identified as the major metabolites. As shown in Figure 5, at the 630 mg/m^2 dose level the parent drug and metabolite trough levels were stable over the 21 day dosing interval.

On the daily × 5 for 3 weeks schedule, samples were collected for pharmacokinetic studies from six patients at the 810 mg/m^2 dose level. Pharmacokinetic parameters are summarized in Table 9. The $t_{1/2}$

Table 7. Non-hematologic toxicities[a] (daily × 5 for 3 weeks)

Dose (mg/m^2)	Evaluable patients	Fatigue/ malaise	Dyspnea	Nausea/ vomiting	Cyanosis	Anorexia	Hepatic	Rigors	Chest pain[b]
810	7	2	2	1	0	1	1	0	0
950	2	1	2	1	1	1	0	1	1
1080	6	4	2	2	2	1	2	1	1

[a]All toxicities were grade 2 or less except for grade 4 cholestatic hepatitis at 1080 mg/m^2.
[b]Other toxicities (one patient each): tachycardia at 950 mg/m^2, dizziness at 950 mg/m^2, headache at 1080 mg/m^2, impotence at 1080 mg/m^2 and constipation at 1080 mg/m^2.

Table 8. Pharmacokinetic parameters (daily × 21)

Patient	Dose	Day 1		Day 21		$t_{1/2}$ (h)	*Cl/F* (ml/min/m^2)	V_d area/*F* (lm/^{2})	Day 1	Day 21
		$C_{P_{Max}}$ (μg/ml)	T_{Max} (h)	$C_{P_{Max}}$ (μg/ml)	T_{Max} (h)				$AUC_{0\rightarrow\infty}$[a] (μg × h/ml)	$AUC_{0\rightarrow\infty}$[a] (μg × h/ml)
1	180	73.8	3	114.1	2	27.6	1.43	3.4	2124	2740
2	180	69.8	2	65.0	3	35.1	2.8	7.0	1327	1213
3	300	83.4	3	110.4	4	37.2	1.88	6.1	2652	1759
4	300	82.4	6	166.1	3	31.6	1.91	5.2	2618	3790
5	450	197.8	3	220.4	4	19.7	2.49	4.2	3023	3977
6	450	112.5	4	168.0	4	46.5	1.77	7.1	4240	4978
7	630	176.3	4	276.4	3	21.0	1.97	5.1	5309	7903
8	630	189.8	4	342.4	4	36.9	1.07	3.4	9817	10072
9	630	189.8	4	177.6	4	30.7	4.81	12.8	2175	3091
10	810	258.9	4	245.0	4	24.8	2.32	3.9	5810	10912
Mean (±SD)			3.7 (1.1)		3.5 (0.7)	30.1[b] (7.9)	2.2 (1.1)	5.8 (2.8)		

[a]AUC corrected = $AUC - \frac{C_p \text{ pre}}{K_{el}}$.

[b]Harmonic mean.

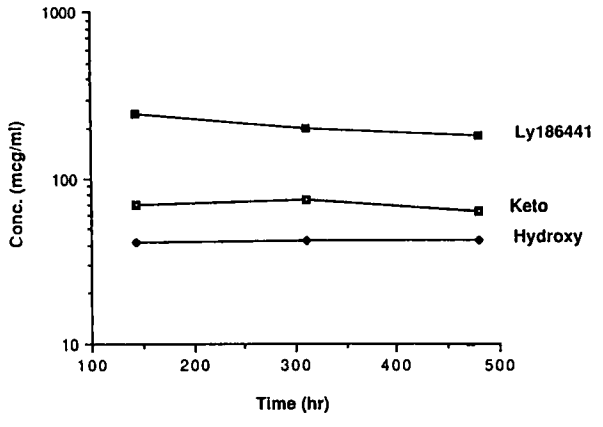

Figure 2. Sulofenur mean plasma concentration versus time curves following day 1 and day 21 drug administration for three patients treated with 630 mg/m^2 daily × 21 days.

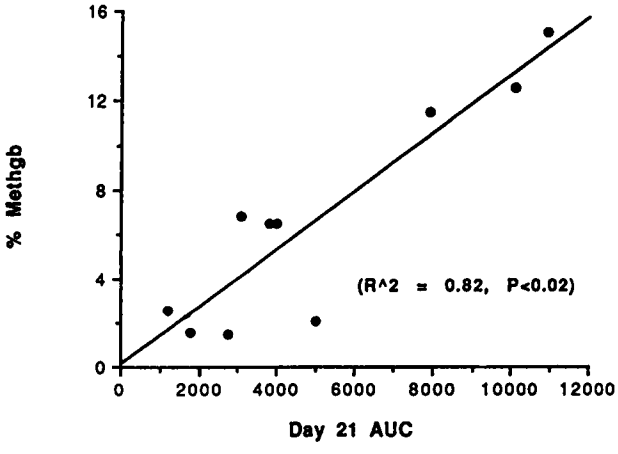

Figure 4. Sulofenur area under the concentration versus time curves versus peak blood methemoglobin levels.

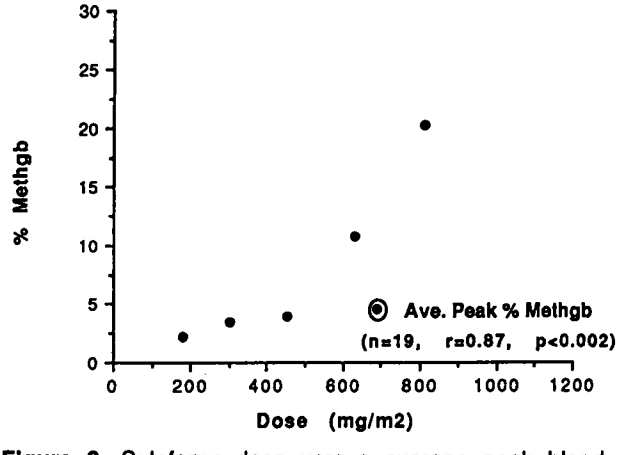

Figure 3. Sulofenur dose versus average peak blood methemoglobin levels.

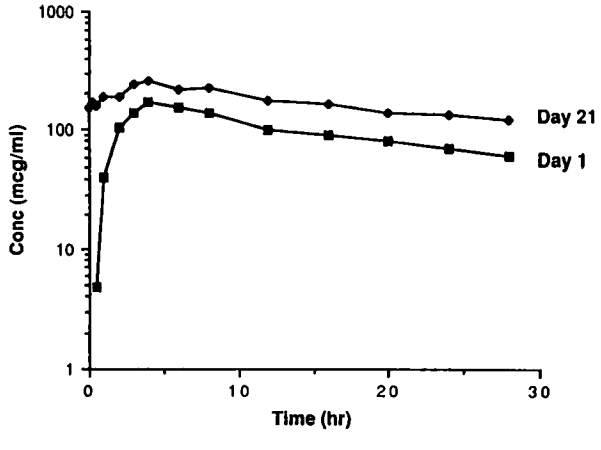

Figure 5. Sulofenur and metabolite trough levels for the 21 day dosing interval following a dose of 630 mg/m^2 (n = 2).

Table 9. Pharmacokinetic parameters (daily × 5 for 3 weeks): 810 mg/m^2

Patient	$C_{p_{Max}}$ (μg/ml)	T_{Max} (h)	$t_{1/2}$ (h)	AUC[a] (μg h/ml)
1	214.8	5	30.6	3766
2	304.3	4	36.5	3549
3	171.6	3	36.5	3842
4	221.5	2	43.3	3037
5	176.3	12	26.7	7599
6	344.7	4	33.0	9099
Mean (±SD)	238.9 (70.4)	5 (3.6)	33.7[b] (5.7)	5149 (2540)

[a]AUC corrected = AUC $- \frac{C_p \text{ pre}}{K_{el}}$.

[b]Harmonic mean.

was 33.7 h and peak plasma concentrations ranged up to 344.7 μg/ml.

Discussion

In this trial, sulofenur was administered on an oral daily × 21 schedule as well as on an oral daily × 5 for 3 weeks schedule. The dose-limiting toxicities identified with both schedules included reversible methemoglobinemia and hemolytic anemia. A direct correlation was observed between methemoglobinemia and the area under the concentration × time curve for sulofenur. The daily × 5 for 3 weeks schedule allowed for delivery of a higher dose level for sulofenur (810 mg/m^2) and the toxicity profile suggests that this regimen may be better tolerated. However, the dose intensity of this schedule and the areas under the concentration × time curves generated for sulofenur do not suggest an advantage for this alteration of the daily × 21 schedule. Additionally, the peak plasma concentrations achieved with this dosing regimen are generally less than the 300 μg/ml concentrations found to be active *in vitro*. However, some evidence of antitumor activity was observed on the daily × 5 for 3 weeks schedule.

Two additional sulofenur phase I trials have been reported in detail. Taylor and colleagues[6] evaluated an oral, daily × 7 schedule and identified dose-limiting toxicities of methemoglobinemia and hemolytic anemia and an MTD of 1200 mg/m^2/day for 7 days. One partial response was observed in a patient with ovarian cancer. An oral, weekly × 6 regimen reported by Hainsworth *et al.*[5] showed the same dose-limiting toxicities and identified an MTD of 2550 mg/m^2/week. No objective tumor responses were observed in that study. Pharmacokinetic parameters generated in those two trials (e.g., serum half-life, total body clearance) are consistent with the findings of the present study.

The results of phase II trials utilizing the daily × 5 for 3 weeks schedule of sulofenur have been reported in abstract form, using a sulofenur dose of 800 mg/m^2 in untreated patients with small cell lung and renal cell cancers.[9] This regimen was well tolerated. Six patients experienced grade 3 or greater anemia and required a total of 9 units of packed red blood cells. Unfortunately, no responses were observed in nine small cell lung cancer and 18 renal cell cancer patients.

Despite these early negative results, the novel structure of sulofenur, the apparently novel mechanism of action of the compound and the broad based pre-clinical antitumor activity seen with the drug make sulofenur an interesting agent for further development. Results suggest that, at tolerated doses and schedules, the drug has limited antitumor activity. Efforts are underway, however, to identify the mechanisms responsible for the dose-limiting hemolytic anemia and methemoglobinemia so that these toxicities might be circumvented or less toxic analogs might be developed. Of interest, sulofenur and related compounds have been identified as active antitumor agents via a murine xenograft screening system that emphasizes solid tumor lines, as opposed to the traditional emphasis on liquid tumor lines.[3]

Based on the results of this study, it is recommended that the daily × 5 (with 2 days off) for 3 weeks schedule be utilized for any screening phase II trials. This schedule offers an area under the concentration × time curve for sulofenur which is comparable to that achieved with alternate schedules and produces a favorable toxicity profile when administered at the recommended phase II dose of 810 mg/m^2.

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