A Phase I and Pharmacokinetic Study of Squalamine, an Aminosterol Angiogenesis Inhibitor[1]

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ABSTRACT

Purpose: **The purpose of this study was to assess the feasibility and characterize the pharmacokinetics of squalamine administered as a continuous i.v. infusion daily for 5 days every 3 weeks.**

Experimental Design: **Patients with advanced solid malignancies were treated with escalating doses of squalamine as a 5-day continuous i.v. infusion every 3 weeks. Doses were initially escalated in 100% increments from a starting dose of 6 mg/m^2/day, with a single patient treated at each dose level until moderate toxicity was observed, at which time additional patients were treated.**

Results: **Thirty-three patients were treated with 73 courses of squalamine at 13 dose levels ranging from 6 to 700 mg/m^2/day. Hepatotoxicity, characterized by brief, asymptomatic elevations in transaminases and hyperbilirubinemia, was the principal dose-limiting toxicity of squalamine. At 700 mg/m^2/day, two of three patients developed grade 4 hyperbilirubinemia, which precluded further dose escalation. At 500 mg/m^2/day, one of seven patients experienced dose-limiting grade 4 hyperbilirubinemia and grade 3 neurosensory changes, which resolved soon after treatment. Squalamine pharmacokinetics were dose-proportional. At 500 mg/m^2/day, the mean (percentage coefficient of variation) clearance, half-life, and volume of distribution of squalamine were 2.67 liters/h/m^2 (85%), 9.46 h (81%), and 36.84 liters/m^2 (124%), respectively, and steady-state concentrations [20.08 μg/ml (13%)] were well above those that inhibit angiogenesis in preclinical models.**

Conclusions: **At the recommended Phase II dose of 500 mg/m^2/day, squalamine is well tolerated and results in plasma concentrations at least an order of magnitude higher than those required for prominent antiangiogenic effects in preclinical studies.**

INTRODUCTION

Squalamine (Fig. 1), an aminosterol originally purified from the tissue of the dogfish shark, is a potent inhibitor of growth factor-mediated endothelial cell proliferation and migration (1, 2). Its antiangiogenic activity does not appear to be mediated by altering production of VEGF[3] or by blocking the VEGF receptor (3). Instead, experimental evidence indicates that squalamine inhibits NHE-3, the endosomal isoform of the sodium-proton exchange pump, thereby affecting endothelial cell volume, pH, growth, and motility (4). Recent work demonstrates that squalamine may alter cellular responses to growth stimuli that increase intracellular calcium by binding to NHE-3 and redistributing calmodulin, a calcium transducer within endothelial cells (5).

Squalamine has demonstrated prominent antiangiogenic activity in several preclinical models including the chick chorioallantoic membrane model, in which treatment with squalamine concentrations as low as 0.1 μg/ml for 1 h significantly decreased blood vessel caliber (3). Similarly, squalamine strongly inhibited growth factor-mediated proliferation and migration of stimulated rat brain endothelial cells (3).

Squalamine's inhibitory actions on angiogenesis are thought to underlie its antitumor activity because squalamine causes prominent tumor growth inhibition *in vivo*, despite a lack of intrinsic cytotoxic activity against tumor cells *in vitro*. For instance, squalamine reduced the formation of new blood vessels and significantly inhibited growth of syngeneic rabbit VX2 carcinoma implants in the rabbit cornea, even though the agent did not inhibit growth of rabbit VX2 carcinoma cells *in vitro* (3). Likewise, systemic administration of squalamine to rats bearing

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[3] The abbreviations used are: VEGF, vascular endothelial growth factor; CIV, continuous i.v. infusion; MTD, maximum tolerated dose; AST, aspartate amino transaminase; ALT, alanine amino transaminase; mCRM, modified Continual Reassessment Method; DLT, dose-limiting toxicity; C_{max}, maximum plasma concentration; C_{ss}, steady-state plasma concentration; CL, clearance; $t_{1/2}$, apparent half-life of elimination; CV, coefficient of variation; PK, pharmacokinetics; AUC, area under the time-concentration curve; CI, confidence interval.

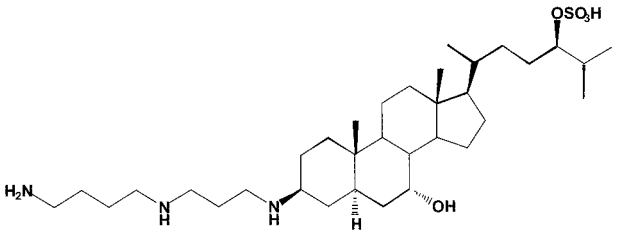

Fig. 1 Structure of squalamine.

9L glioma xenografts inhibits tumor growth as effectively as the alkylating agent carmustine, despite the absence of growth-inhibitory effects against 9L glioma cells *in vitro* (3).

The rationale for the clinical development of squalamine included the agent's unique action as a selective inhibitor of angiogenesis, its impressive antitumor activity both as a single agent and in combination with cytotoxic agents in various human tumor xenografts, and its predictable and favorable toxicity profile in preclinical studies. A protracted administration schedule was selected for development based on squalamine's relatively rapid CL in some species and because more frequent dosing schedules of squalamine salts resulted in superior anticancer activity in preclinical studies (initial elimination half-lives in all species were <90 min[3]).[4] The principal objectives of this study were as follows: (*a*) to characterize the principal toxicities of squalamine administered as a CIV for 5 days repeated every 3 weeks; (*b*) to determine the MTD and recommend a dose for subsequent disease-directed trials; (*c*) to characterize the PK of squalamine; and (*d*) to seek preliminary evidence of antitumor activity.

PATIENTS AND METHODS

Patient Eligibility. Patients with histologically documented evaluable or measurable solid malignancies refractory to conventional chemotherapy or for whom no effective therapy existed were candidates for this study. Eligibility criteria also included: (*a*) age $\geq$ 18 years; (*b*) Eastern Cooperative Oncology Group performance status $\leq$ 2; (*c*) a life expectancy of $\geq$12 weeks; (*d*) no treatment with cytotoxic or investigational agents within 30 days of study entry (6 weeks if prior treatment included mitomycin C or nitrosoureas); and (*e*) adequate hematopoietic (WBC count $\geq$ 3000/μl, absolute neutrophil count $\geq$ 1500/μl, hemoglobin $\geq$ 9 g/dl, and platelet count $\geq$ 100,000/μl), hepatic [serum bilirubin $\leq$ 1.5 mg/dl; AST, ALT, and alkaline phosphatase levels $<$ 2 times institutional normal upper limit (transaminases $\leq$3 times and alkaline phosphatase $<$4 times the institutional upper normal limit for patients with liver metastases)], and renal function (serum creatinine $\leq$ 1.5 mg/dl or calculated creatinine CL $\geq$ 60 ml/min). Patients with coexisting medical problems that limited full compliance with the study were excluded. All patients gave written informed consent according to federal and institutional guidelines.

[4] Genaera Pharmaceuticals: MSI-1256F (squalamine lactate): Investigator's Brochure (3rd ed.). Plymouth Meeting, PA, October 20, 1999.

Table 1 Patient characteristics

Characteristic	No. of patients
No. of patients (evaluable)	33 (33)
Median no. of courses/patient (range)	2 (1–5)
Median age (range) (yrs)	59 (18–56)
Gender (male:female)	12:21
Median performance status (ECOG)	
0	9
1	18
2	6
No. of previous cytotoxic therapies	
0	3
1	2
2	3
$\geq$3	25
Primary tumor types	
Colorectal	8
Breast	4
Sarcoma	4
Lung (non-small cell)	3
Ovarian	3
Renal	3
Gastric	2
Cervix, endometrial, melanoma, pancreatic, testicular, lung (small cell)	1 each

Dosage and Drug Administration. Squalamine was administered as a 24-h CIV for 5 days (120 h) every 3 weeks. The starting dose was 6 mg/m^2/day, which represents one-tenth of the no-effect dose in rats (59 mg/m^2/day) on a 120-h CIV schedule.

The MTD was defined *a priori* as the highest dose at which a maximum of 20% of patients experienced DLT during the first treatment course. Dose escalation occurred in two stages: initially, single patients were accrued per dose level; and the dose was doubled for each new dose level until a grade 2 or greater toxicity was observed. Thereafter, the cohort size was increased to a minimum of three patients, and the dose levels were escalated in 40% increments. The second stage of dose escalation was guided by a mCRM, in which the increment between dose levels (40%), but not the number of dose levels, was fixed (6). Once DLT was observed for a particular patient during the first treatment course, the posterior distribution of the parameter determining the dose-toxicity curve was recalculated, and subsequent patients were treated at the dose closest to the current estimate of the MTD according to the mCRM. The investigators' judgment could take precedence over the mCRM at any time during the study. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (Version 1.0). DLT was defined as: (*a*) absolute neutrophil count $<$ 500/μl for $>$5 days and/or associated with fever ($\geq$38.5°C); (*b*) platelets $<$ 25,000/μl; (*c*) hemoglobin $<$ 6.5 mg/dl; and (*d*) grade 3 nonhematological toxicity excluding nausea and vomiting. After several patients experienced transient, isolated, asymptomatic elevations in hepatic transaminases at 255 and 357 mg/m^2/day, the criteria for DLT were amended so that only transaminase elevations of grade 4 severity or grade 3 transaminitis lasting $>$7 days was considered dose-limiting. Dose reduction by one level was permitted for individuals who experienced DLT. Intrapatient dose escalation was not permitted.

Table 2 Dose-escalation scheme

Squalamine dose (mg/m^2/day)	No. of patients			Total no. of courses	No. of patients with DLT	
	New	Reduced to this dose	Total		First course	All courses
6	1	0	1	2	0/1	0/1
12	1	0	1	2	0/1	0/1
24	3[a]	0	3	6	0/3	0/3
34	1	0	1	2	0/1	0/1
47.6	1	0	1	1	0/1	0/1
66.64	1	0	1	4	0/1	0/1
93	1	0	1	4	0/1	0/1
130	1	0	1	2	0/1	0/1
182	1	0	1	3	0/1	0/1
255	6	1	7	14[b]	1/6	3/7
357	6	1	7	14[c]	0/6	1/7
500	7	1	8	16[d]	1/7	3/8
700	3	0	3	3	2/3	2/3
Total	33		36	73		

[a] Before amending the criteria for DLT, one patient had a grade 3 elevation in hepatic transaminases that was considered dose-limiting, thus this dose level was expanded to three patients.
[b] The dose of squalamine was reduced in one patient from 357 to 255 mg/m^2/day.
[c] The dose of squalamine was reduced in one patient from 500 (1 course) to 357 mg/m^2/day (4 courses).
[d] The dose of squalamine was reduced in one patient from 700 to 500 mg/m^2/day.

Sualamine was supplied by Genaera Corp. (Plymouth Meeting, PA) in vials containing 50 mg of lyophilized drug. Individual doses of squalamine were prepared by adding 5 ml of sterile water to each 50-mg vial. The precise dose of concentrated squalamine was then reconstituted using a sufficient volume of 5% dextrose solution to produce a final squalamine concentration of 1–3 mg/ml. The solution was administered by CIV through a central venous catheter using an ambulatory infusion pump.

Pretreatment and Follow-Up Studies. A history, physical examination, and routine laboratory studies were performed pretreatment and on days 3, 5, 8, and 15 of each 3-week course. Concurrent medications were recorded throughout the study. Pretreatment studies included an assessment of relevant tumor markers, an electrocardiogram, and relevant radiological studies for evaluation of all measurable or evaluable disease. Radiographic imaging was repeated after every other course or as needed to confirm response. Patients were able to continue treatment in the absence of progressive disease. A complete response was defined as the disappearance of all active disease, whereas a partial response required at least a 50% reduction in the sum of the product of the bidimensional measurements of all documented lesions. Both complete and partial responses were confirmed on two measurements separated by a minimum period of 4 weeks. An increase in the size of any lesion by 25% or the appearance of a new lesion was considered disease progression.

Pharmacokinetic Sampling and Assay. During course 1, 3-ml whole blood samples were collected in heparinized tubes before the start of the squalamine infusion and at 0.5, 1, 2, 4, 8, 12, 16, 24, 48, 72, 96, and 120 h after the start of infusion. Samples were also collected at 7.5, 15, 30, and 45 min and at 1, 2, 4, 12, 24, and 48 h after the end of infusion. Samples were centrifuged at 3000 rpm for 15 min at 4°C immediately after collection, and then plasma was transferred to a sample tube and frozen at −20°C.

Quantitation of squalamine was achieved by high performance liquid chromatography using a Hewlett Packard Series II 1090L system (Hewlett-Packard Co., Rockville, MD) coupled with an electrospray mass spectrometer (Finnigan TSQ; Finnigan Corp., San Jose, CA) after solid-phase extraction. Chromatographic separations were performed with a YMC octadecanoyl sulfate (ODS-AQ) column (120 Å; 5 μm; 100 × 2 mm; YMC Inc., Wilmington, NC) fitted with a C18 guard column. Drugs were eluted in a gradient of mobile phase A (0.1% formic acid in 90:10 water:acetonitrile) in mobile phase B (0.1% formic acid in 90:10 acetonitrile:water). Squalamine and the internal standard eluted with a retention time of 2.40 and 2.50 min, respectively, with baseline resolution.

Pharmacokinetic Analyses. Noncompartmental pharmacokinetic modeling and parameter estimation were performed using WinNonLin (Pharsight Corp., Mountain View, CA). Estimated pharmacokinetic parameters included: C_{max}; time to maximum plasma concentration (T_{max}); C_{ss}; terminal disposition volume (V_Z); CL; and $t_{1/2}$. The $AUC_{0-\infty}$ was calculated using the linear trapezoidal rule. The C_{ss} was calculated as the mean of the observed values at 72, 96, and 120 h for all patients who completed the entire 5-day infusion. The $t_{1/2}$ was calculated using log-linear regression of the terminal portion of the concentration-*versus*-time profile. CL was calculated by dividing the dose by the $AUC_{0-\infty}$.

Descriptive statistics were used to summarize all pharmacokinetic parameters for patients who completed the entire 5-day infusion. Dose proportionality was assessed by linear regression and by the power model (7). Linear regression was used to evaluate the relationship between CL and dose, as well as the relationship of CL and various clinical patient characteristics (age, weight, and body surface area). Student's *t* test was used to evaluate the relationship between CL and gender.

The relationships between indices of drug exposure ($AUC_{0-\infty}$ and C_{max} dose) and elevations in bilirubin, AST, and ALT during course 1 were explored. Elevations in bilirubin,

Table 3 Hepatic transaminase elevations and hyperbilirubinemia occurring on study ($n = 73$ courses)[a]

Squalamine dose level (mg/m^2/day)	AST			ALT			Bilirubin		
	Grade 1–2	Grade 3	Grade 3 > 5 days or grade 4	Grade 1–2	Grade 3	Grade 3 > 5 days or grade 4	Grade 1–2	Grade 3	Grade 4
6	1	0	0	0	0	0	0	0	0
12	1	0	0	0	0	0	0	0	0
24	0	2	0	2	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0
47.6	0	0	0	0	0	0	0	0	0
66.64	0	0	0	0	0	0	0	0	0
93	0	0	0	0	0	0	0	0	0
130	0	0	0	0	0	0	0	0	0
182	0	0	0	0	0	0	0	0	0
255	2	1	1	3	1	1	4	0	0
357	5	3	0	6	2	0	0	0	0
500	8	3	2	6	0	4	2	0	1
700	4	2	0	4	1	1	1	0	2

[a] Values represent the number of courses in which transaminase elevations or hyperbilirubinemia occurred. No distinction was made for causality.

AST, and ALT during the first course were evaluated by calculating the largest percentage increment in each parameter irrespective of causality, as follows: 100 × ([highest value of bilirubin/AST/ALT − pretreatment value of bilirubin/AST/ALT] ÷ pretreatment value of bilirubin/AST/ALT). The influence of the presence of liver metastases on elevations in each parameter in the first course was also assessed using Student's *t* test.

RESULTS

The relevant demographic characteristics of the 33 patients enrolled on this study are summarized in Table 1. In total, 73 courses of squalamine were administered at 13 dose levels ranging from 6–700 mg/m^2/day (Table 2). Three patients required dose reduction for DLT, which consisted of hyperbilirubinemia, elevated hepatic transaminases, and neurosensory symptoms. Seven patients received 4–5 courses (12–15 weeks) with stable disease as their best response. No major cytoreductive responses were observed.

Toxicity

Hepatic Toxicity. The principal dose-limiting effects of squalamine were hyperbilirubinemia and elevations in hepatic transaminases. Table 3 lists the numbers of courses associated with hepatic transaminase elevations and hyperbilirubinemia as a function of both grade and dose level. In general, the onset of hyperbilirubinemia was observed on day 1–3 of the squalamine infusion, peaked on day 3–5, and resolved by day 8. Fractionated bilirubin was measured in 5 of the 10 episodes of hyperbilirubinemia, of which direct bilirubin was elevated in three cases, indirect bilirubin was elevated in one case, and both were elevated in the remaining case. At the 500 mg/m^2/day dose level, one of seven patients developed grade 4 hyperbilirubinemia associated with both grade 3 elevations in hepatic transaminases and grade 3 neurosensory manifestations characterized by paresthesia and ataxia. All toxicities in the affected patient resolved after treatment was terminated on day 3, and subsequent treatment with 375 mg/m^2/day squalamine was well tolerated. At a squalamine dose of 700 mg/m^2/day, two of three patients experienced grade 4 hyperbilirubinemia on days 3–6. Concurrently, both patients had grade 3 or 4 elevations in hepatic transaminases; one patient also experienced anorexia and fatigue; whereas the other had perioral paresthesia and ataxia. Although the patient who experienced ataxia had ataxia before commencing squalamine, the symptoms worsened during treatment, and therefore squalamine could not be excluded as potentially contributory. In both patients, hepatic effects resolved completely by day 7–8.

Isolated elevations in hepatic transaminases were more common than hyperbilirubinemia (Table 3). The onset of transaminitis generally occurred during the 5-day infusion, peaked at a median of 6 days after treatment, and resolved, on average, by 5–6 days posttreatment. Most patients experienced grade 1–2 elevations in hepatic transaminases; however, one patient with liver metastasis who was treated with squalamine, 255 mg/m^2/day, experienced dose-limiting grade 4 transaminitis on day 3, necessitating immediate termination of treatment. The patient's AST and ALT decreased to grades 1 and 3, respectively, on day 8, with normalization documented on day 32. Before amending the DLT criteria, three additional patients [treated at the 255 mg/m^2/day (one patient) and 357 mg/m^2/day (two patients) dose levels] developed asymptomatic, grade 3 transaminase elevations that necessitated discontinuation of squalamine. The transaminases normalized thereafter.

Miscellaneous. Fatigue, experienced by 20 patients (61%), was typically reported as mild to moderate (grade 1–2) and occurred during and in the days immediately after squalamine treatment. However, one patient at each of the 255, 357, and 700 mg/m^2/day dose levels developed grade 3 fatigue commencing on days 2–3 of treatment. Fatigue in the patients treated with 357 and 700 mg/m^2/day squalamine resolved within 24–48 h after cessation of treatment. The patient treated at 255 mg/m^2/day experienced fatigue beginning on course 2, day 2 that promptly improved after a dose reduction to 182 mg/m^2/day for the remainder of the infusion. The same patient subsequently developed nonspecific grade 3 chest and back pain on course 2, day 6, for which squalamine could not be excluded as a potential cause. Other relatively common, albeit predominantly grade

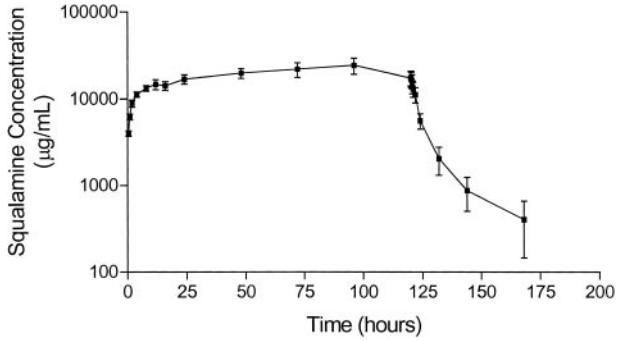

Fig. 2 A representative plasma concentration-time profile of squalamine using the mean plasma values (■) and CIs in patients treated at the 500 mg/m^2/day dose level.

1–2, nonhematological toxicities observed included anorexia (20 patients), nausea (13), vomiting (10), renal insufficiency (8), diarrhea (7), and chills (5). One patient treated at the 255 mg/m^2/day dose level developed grade 3 anorexia that responded to megestrol acetate. One patient at each of the 255 and 500 mg/m^2/day dose levels developed grade 3 nausea and vomiting in the absence of antiemetic premedication. Less common (≤10% of patients), potentially drug-related adverse effects, all of which were grade 1–2 in severity, included pain, fever, headache, injection site reactions, pallor, cyanosis, dehydration, tachycardia, mucositis, glossitis, weight loss, and constipation.

Hematological toxicity was generally uncommon. Nine patients experienced anemia (≤grade 3), three patients each developed grade 1–2 leukopenia or neutropenia, and one patient experienced grade 1 thrombocytopenia. Hematological toxicities did not appear to be related to either dose or cumulative treatment.

Pharmacokinetic Studies

A representative patient plasma concentration-time profile is shown in Fig. 2, and squalamine mean pharmacokinetic parameters are listed in Table 4. A large degree of interpatient variability was observed as indicated by the large CV of the total plasma CL (85%), terminal disposition volume of distribution (124%), and apparent half-life of elimination (81%). As shown in Fig. 2, plasma concentrations increased rapidly after the infusion was begun and decreased rapidly after cessation of treatment. Dose proportionality was assessed using two methods. By standard linear regression, squalamine generally exhibited dose-proportional kinetics, as depicted in the scatterplots of C_{max}, and $AUC_{0-\infty}$ as a function of the squalamine dose level (r^2 = 0.5222 and 0.3799, respectively) shown in Fig. 3, *A* and *B*. Although there was significant interpatient variability, using a power model (7), the CI of the slope (β) of the linear fit of the relationship between log ($AUC_{0-\infty}$) and log (dose) includes the value 1 (95% CI for β, 0.89–1.11), which further supports dose proportionality. Squalamine CL did not vary with dose level and was not affected by age, gender, weight, or body surface area. The CL of squalamine averaged 2.67 liters/h (CV, 85%), and the mean $t_{1/2}$ was 4.13 h (CV, 148%). Furthermore, visual inspection of the plasma concentration-time curves suggests that CL remains stable over the duration of the infusion.

Relationships between the principal hepatic toxicities of squalamine during course 1 and pertinent pharmacokinetic parameters were explored, although the small numbers of dose-limiting events and patients at each dose level, as well as the rarity of hepatic effects at squalamine doses below 255 mg/m^2/day, limited the statistical power of such analyses. No definite relationship was evident between increases in bilirubin and hepatic transaminases *versus* dose level, C_{max}, or $AUC_{0-\infty}$. Because elevations in bilirubin and hepatic transaminases were analyzed irrespective of causality, underlying liver metastases may have been a confounding factor. However, percentage elevations in bilirubin, AST, or ALT were not significantly different between patients with and without liver metastases.

DISCUSSION

The importance of angiogenesis in the growth of solid malignancies is increasingly becoming recognized (8–10). With accumulating evidence suggesting that the emergence of angiogenesis is a discrete and necessary step in the development and progression of malignant neoplasms, a burgeoning number of angiogenesis-inhibitory compounds are currently being evaluated for their potential to inhibit tumor growth. Squalamine is a selective angiogenesis inhibitor with a mechanism of action distinct from those of other angiostatic steroids (2). In addition to its unique mechanism of antiangiogenic action, the rationale for developing squalamine includes its prominent tumor growth-inhibitory activity in several types of human tumor xenografts and the agent's predictable and acceptable toxicity profile in preclinical studies. Because preclinical studies have indicated that frequent treatment or protracted exposure to squalamine salts may be superior to less frequent schedules for controlling tumor-induced angiogenesis, the feasibility of administering squalamine on a protracted 5-day CIV schedule was selected for clinical evaluation. This choice is supported by data with other antiangiogenic agents that suggest that more frequent administration is preferable (11, 12), although the optimal schedule for achieving prolonged drug exposure remains to be determined.

As predicted from preclinical toxicology studies, hepatic toxicity, characterized by hyperbilirubinemia and transaminitis, was the principal DLT of squalamine in this study. A similar toxicity profile was observed in another Phase I study of squalamine administered as a 5-day CIV (13). In the present study, an unacceptably high rate of severe episodes of hepatotoxicity, particularly hyperbilirubinemia, occurred in patients treated at the 700 mg/m^2/day dose level, whereas drug-induced elevations in serum bilirubin and transaminases were generally mild to moderate and short-lived at lower dose squalamine levels. Based on these results, the recommended Phase II dose of squalamine should not exceed 500 mg/m^2/day CIV for 5 days every 3 weeks. Although several patients experienced grade 3 elevations in hepatic transaminases at this dose level, these events were not considered dose-limiting because the toxicity resolved soon after discontinuation of treatment and did not persist longer than 7 days. Limited experience in the present trial of four patients treated with at least four courses of squalamine, at doses ranging from 255–500 mg/m^2/day, suggests that hepatotoxicity resolves completely and does not progressively worsen with repetitive treatment. However, further evaluation

Table 4 Noncompartmental pharmacokinetic parameters for squalamine[a]

Dose level (mg/m^2/day)	No. of patients	C_{max} (μg/ml)	C_{ss} (μg/ml)	CL (liters/h/m^2)	V_z (liters/m^2)	$t_{½}$ (h)	$AUC_{0-\infty}$ (μg·h/ml)
6	1	0.76	0.35	0.88	1.19	0.94	35.04
12	1	0.77	0.35	1.27	26.84	14.63	47.17
24	3	0.64 (32%)	0.48 (20%)	2.23 (18%)	8.65 (66%)	2.85 (67%)	54.89 (18%)
34	1	0.91	0.90	1.80	17.38	6.69	94.07
47.6	1	4.97	1.22	0.84	42.25	34.77	224.89
66.64	1	2.03	1.54	1.60	14.37	6.23	208.13
93	1	2.17	2.08	2.03	19.87	6.77	229.18
130	1	11.80	3.45	1.28	24.46	13.25	508.15
182	1	6.56	5.21	1.42	33.95	16.60	641.83
255	6	8.02 (28%)	6.20 (45%)	3.65 (99%)	60.56 (158%)	7.49 (80%)	747.18 (34%)
357	6	10.22 (34%)	8.63 (43%)	4.65 (66%)	54.08 (103%)	7.19 (72%)	1,109.20 (33%)
500	7	20.65 (26%)	17.72 (33%)	2.72 (95%)	42.39 (87%)	11.04 (62%)	2,153.79 (26%)
700	3	16.10	14.43	2.13	38.57	12.57	1645.32
Mean	33			2.67 (85%)	36.84 (124%)	9.46 (81%)	

[a] Values represent mean [CV (%)] for all patients who completed the entire 5-day squalamine infusion ($n = 26$). V_z, terminal volume; $AUC_{0-\infty}$, area under the time-concentration curve from zero extrapolated to infinity.

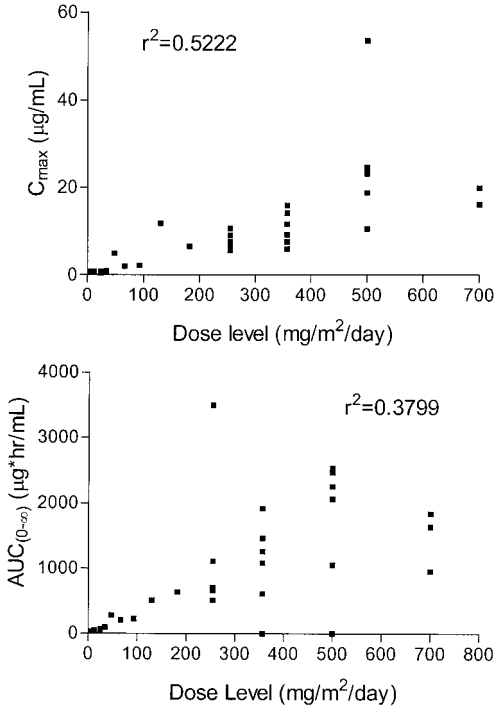

Fig. 3 *A* and *B*, scatterplots of C_{max} and $AUC_{0-\infty}$ values as a function of the squalamine dose level.

will be required to better characterize the tolerability of repetitive dosing because the inhibition of angiogenesis is likely to necessitate chronic administration of antiangiogenic agents.

Two main differences were apparent in the present study in comparison with a previously published Phase I trial of squalamine using a similar schedule of administration (13). First, squalamine exhibited dose-proportional kinetics in the current study, whereas Bhargava *et al.* (13) concluded that squalamine $AUC_{(0-t)}$ and C_{max} *versus* dose were nonlinear at the highest dose level. The difference in PK profile may be attributed to a difference in sample size. In the earlier study (13), only one patient was evaluated at the 500 mg/m^2 dose level, whereas the present study evaluated a larger number of patients in the 357–700 mg/m^2 dose range ($n = 17$). Given the considerable interpatient PK variability observed with squalamine, experience with more patients suggests that the kinetics of squalamine are dose-proportional. The interpatient PK variability may reflect interindividual variation in hepatic transport and metabolizing enzymes because preliminary preclinical studies suggest that hepatic metabolism and biliary excretion were the principal modes of drug disposition.[4]

Second, the recommended Phase II dose for squalamine was higher in the current study because of a difference in the definition of DLT. In the present study, only grade 4 transaminase elevations or grade 3 transaminase elevations lasting >7 days were considered dose-limiting, thus permitting dose escalation beyond the level reached in the previous trial (13).

The pleiotropic effects of squalamine on endothelial cells, which are not readily quantifiable, precluded the use of a specific molecular target to measure drug effect. Although circulating angiogenic factors, such as VEGF, have served as surrogate markers of antiangiogenic activity or pharmacodynamic indicators of effect for other agents, preclinical studies have demonstrated that the antiangiogenic effects of squalamine do not alter VEGF levels (3). Achieving and maintaining squalamine C_{ss} plasma values in patients that were at least 1 order of magnitude greater than the drug concentrations required to reduce vessel caliber in preclinical models (>0.1 μg/ml) provides some reassurance that relevant biological doses were attained (3). However, in the chorioallantoic membrane model, some degree of reversibility in capillary constriction was noted approximately 100 min after squalamine treatment (3). Likewise, preclinical studies of squalamine plus cisplatin have demonstrated that although CD31 immunostaining of vascular endothelium diminishes soon after treatment, the effect dissipated by 48 h posttreatment (14). Therefore, more protracted or more frequent administration schedules may be needed to optimize the antiangiogenic activity of squalamine.

The lack of major tumor regressions in this study does not necessarily imply that squalamine is devoid of relevant clinical activity because a wide dose range was evaluated in a preponderance of heavily pretreated patients with advanced bulky malignancies, as is typically represented in Phase I trials. Furthermore, the predominant beneficial effect of squalamine in preclinical models was tumor growth delay, which might translate into increased time to progression and survival that will only be evident in sufficiently powered randomized studies. Based on preclinical models, the therapeutic effects of squalamine are also likely to be more apparent in earlier disease stages because the transition to an angiogenic phenotype occurs much earlier in tumor development and may even precede the appearance of solid tumors (10). For advanced stages of malignant disease, in which cytoreduction of tumor is acutely required, antiangiogenic agents may be more effective in combination with cytotoxic agents rather than as monotherapy. In studies with the murine Lewis lung carcinoma and rat mammary carcinoma, squalamine demonstrated additive antitumor activity when administered in combination with various cytotoxic agents (15). Similarly, in H460, Calu-6, NL20-TA, and chemoresistant MV522 human lung xenograft models, squalamine substantially increased the antitumor efficacy of carboplatin, cisplatin, and the combination of paclitaxel and carboplatin (14, 16). Squalamine also significantly enhanced the activity of cyclophosphamide against the human MX-1 breast carcinoma xenograft (17) and improved control of tumor growth mediated by hormonal blockade in the LNCaP prostate tumor xenograft model (18). Based on preclinical studies demonstrating synergy with cytotoxic agents, a Phase I/IIa study of escalating doses of squalamine in combination with carboplatin and paclitaxel in non-small cell lung cancer has recently been completed that shows that the regimen was well tolerated, and in the first 18 patients, an objective response rate of 27% was observed (19).

Administration of squalamine on a protracted 5-day CIV schedule every 3 weeks is feasible in patients with advanced solid malignancies. At the recommended dose of 500 mg/m^2/day for 5 days, most patients experienced brief, inconsequential elevations of hepatic transaminases, and plasma concentrations exceeded those that are biologically relevant. Further disease-directed evaluations of squalamine in combination with cytotoxic agents in patients with advanced disease, as well as studies in early disease states, are warranted based on the results of preclinical studies that suggest that squalamine is more efficacious in such settings.

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