A Phase I and Pharmacokinetic Study of Squalamine, a Novel Antiangiogenic Agent, in Patients with Advanced Cancers[1]

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ABSTRACT

Purpose: **A Phase I study of squalamine, a novel antiangiogenic agent originally isolated from the dogfish shark *Squalus acanthias*, was conducted in patients with advanced cancers to: (*a*) determine the maximum tolerated dose (MTD), dose-limiting toxicity (DLT) and pharmacokinetics of squalamine lactate when given as a 120-h continuous i.v. infusion every two weeks; and (*b*) to obtain information on prolonged (>120-h) continuous i.v. infusions in patients who have tolerated 120-h infusions.**

Experimental Design: **A rapid dose escalation scheme was used that permitted intrapatient dose escalation. Three or more patients were treated at each dose, of which at least one patient started treatment *de novo* at that dose. Once DLT was encountered, the dose was decreased by one dose level, and the duration of infusion was prolonged from 10 up to 30 days in 5-day increments.**

Results: **Nineteen patients were treated at eight squalamine dose levels; the number of patients/dose level who received 120-h infusions were [expressed as dose in mg/m^2/day (number of patients initiated *de novo* at that dose/total number of patients treated at that dose)]: 6 (3/3), 12 (3/6), 24 (1/5), 48 (2/6), 96 (4/10), 192 (2/6), 384 (3/8), and 538 (1/5). DLT was encountered at 384 mg/m^2/day (1/3 *de novo* patients, 5/8 total patients) and 538 mg/m^2/day (1/1 *de novo* patients, 4/5 total patients) and consisted of hepatotoxicity, characterized by grade 3 transaminase elevations that resolved 3–11 days after ceasing squalamine infusion. Three patients did not experience hepatotoxicity when first treated at 384 mg/m^2/day but developed DLT at the same dose when de-escalated from 538 mg/m^2/day. Other toxicities included grade 1–3 fatigue, grade 1–2 nausea, anorexia, and neuromuscular symptoms. The maximum duration of continuous i.v. infusion was 20 days at a dose rate of 192 mg/m^2/day in one patient without adverse effects. Pharmacokinetic calculations revealed a linear relationship between area under the curve or *Cmax* and squalamine dose rate up to 384 mg/m^2/day, with a prolonged terminal squalamine persistence in patient plasma (median $t_{1/2}$ = 18 h; range, 8–48 h). Transient tumor responses were observed in a patient with synovial cell sarcoma and a patient with breast carcinoma with cutaneous metastases.**

Conclusions: **The best tolerated dose rate of squalamine when administered as a 120-h continuous i.v. infusion was 192 mg/m^2/day; however, patients without prior exposure to squalamine appeared to tolerate a dose rate of 384 mg/m^2/day without DLT. On the basis of preclinical evidence of synergy with cytotoxic agents and demonstration of human safety from this trial, additional clinical trials have been initiated with squalamine in combination with chemotherapy for patients with late stage lung cancer and ovarian cancer.**

INTRODUCTION

Angiogenesis is a critical process during tumor growth and metastasis, and drugs that suppress angiogenesis carry therapeutic potential in the treatment of cancer (1). Specific targeting of the neovasculature in tumors can be expected to have few systemic toxicities, because angiogenesis in the adult is largely confined to sites of wound healing, reproductive organs, and tumors (2). Moreover, because endothelial cells are genetically stable, a low rate of resistance can be expected to antiangiogenic treatment (3). Angiogenesis is regulated by a multitude of growth factors, and dysregulated angiogenesis, as seen in tumors, results from an imbalance between the positive and negative regulators of angiogenesis (4). Because multiple growth factors are involved in this pathway, targeting a single growth factor such as vascular endothelial growth factor may not be an optimal antiangiogenic strategy. An alternative approach is the direct inhibition of proliferating endothelial cells with angiogenesis inhibitors, an approach that is presently being pursued in several clinical trials.

Squalamine is a novel aminosterol that was originally isolated from the tissues of the dogfish shark, *Squalus acanthias*, and is chemically synthesized for clinical use (5). Squalamine is a 7,24-dihydroxylated, 24-sulfated cholestane steroid conjugated to a spermidine at C3 (Fig. 1; Ref. 6). Squalamine has been shown to inhibit mitogen-induced proliferation and migration of endothelial cells *in vitro* and caused significant *in vivo* inhibition of angiogenesis in the rabbit cornea micropocket assay (7). A unique effect seen after the direct application of squalamine to chick embryo vasculature consisted

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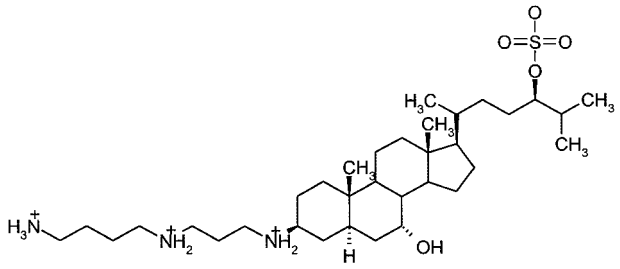

Fig. 1 Structure of squalamine.

of transient constriction of small capillaries with occlusion of blood flow (7). Mechanistic studies have revealed that squalamine inhibits the sodium-hydrogen exchanger (isoform NHE3), causing changes in intracellular pH that lead to alterations in the shape and volume of endothelial cells (8). This mechanism is believed to contribute to the angiogenesis inhibition observed with squalamine. More recent studies show that squalamine is taken up specifically by endothelial cells and stays intracellular for at least 5 days. Intracellular uptake is accompanied by avid binding of squalamine to calmodulin, causing intracellular redistribution of calmodulin in endothelial cells that down-regulates cellular signaling pathways and may also contribute to the antiangiogenic effects of squalamine (9).

Angiogenesis inhibition by squalamine is paralleled by antitumor activity as demonstrated in several tumor xenograft models. In an animal model of breast cancer, administration of 10 or 20 mg/kg/day of squalamine after cyclophosphamide chemotherapy caused a statistically significant increase in time to progression in nude mice (10). In a glioma xenograft model in rats, squalamine as a single agent at a dose of 20 mg/kg/day decreased tumor growth by 36–43% (11). In combination studies, squalamine strongly potentiated the antitumor activity of cytotoxic agents (cyclophosphamide, cisplatin, paclitaxel, or 5-fluorouracil) in treatment of both a rat mammary carcinoma and a murine Lewis lung carcinoma (12). Other studies have also shown significant enhancement of antitumor activity combining squalamine with cytotoxic agents in human lung (13), ovarian (14), prostate (15), and neuroblastoma (16) cancer models at doses as low as 2 mg/kg/day (equivalent to 9 mg/m^2/day) in mice.

On the basis of these preclinical antiangiogenic and antitumor data, squalamine was selected for clinical development as a therapeutic agent for the treatment of human malignancies. Unpublished pharmacokinetic experiments in rats, dogs, and mice suggest a relatively rapid elimination of squalamine from the plasma of animals administered drug i.v. (initial elimination half-lives in all species were $<$90 min[3]). We therefore chose to conduct the first Phase I study of squalamine as a 120-h continuous i.v. infusion in patients with advanced cancers.

PATIENTS AND METHODS

Study Population. Adults ($\geq$18 years of age) with a histologically confirmed diagnosis of nonleukemic malignancy refractory to the best available therapy were eligible for this study. Patients were required to have an ECOG[4] performance status of 2 or less and an anticipated survival of at least 12 weeks. Patients with metastasis to the central nervous system were allowed only if the metastases were clinically stable off therapy. Laboratory criteria for eligibility included a WBC count $\geq$3,000/mm^3, absolute granulocyte count $\geq$1,500/mm^3, platelet count $\geq$100,000/mm^3, hemoglobin $\geq$9 g/dl, bilirubin $\leq$1.5 mg/dl, transaminases (ALT and AST) $\leq 2 \times$ ULN or $\leq 3 \times$ ULN for patients with liver metastasis, alkaline phosphatase $\leq 2 \times$ ULN or $\leq 4 \times$ ULN for patients with liver metastasis, normal prothrombin time (unless taking anticoagulants), and serum creatinine $\leq$1.5 mg/dl or a creatinine clearance $\geq$60 ml/min. Patients with a clinically significant atrial or ventricular arrhythmia, recent myocardial infarction ($\leq$6 months), congestive heart failure, or greater than first-degree heart block were excluded from the study. Patients were required not to have received cytotoxic therapy within 3 weeks of treatment (6 weeks in case of mitomycin C or nitrosourea) and to have recovered from the toxicities of prior cancer therapies. All patients were informed of the investigational nature of the study drug and signed an informed consent. The study was approved by the Institutional Review Board of Georgetown University.

Study Design. Before beginning treatment, patients had a complete medical history and physical examination including an electrocardiogram. Complete blood counts; serum chemistries; renal, hepatic, and coagulation parameters; urinalysis; and chest X-ray also were obtained. Patients underwent a preenrollment history and physical examination, and laboratory tests were performed weekly during study participation. Bidimensional tumor measurements (using radiological studies) or appropriate tumor markers were obtained at baseline (within 4 weeks of starting treatment) and every 2 months during study participation. For patients with measurable disease, standard response criteria were used (17).

Three or more patients were treated/dose level. Of these, at least one patient started treatment *de novo* at each dose, whereas the remainder could be escalated from lower dose levels. In the absence of toxicity during the first treatment cycle, patients were allowed to have their dose escalated during subsequent treatment cycles. This method permitted rapid escalation of dose and reduced the time required to define a squalamine MTD. If one patient developed a DLT, the cohort at that dose was expanded to treat six patients. The squalamine MTD was defined as the highest dose tested at which fewer than two of six patients experienced DLT. Patients who tolerated therapy could continue squalamine treatment as long as their tumors showed no evidence of progression. All patients were evaluated for toxicity, and patients who completed at least four treatment cycles were evaluated for response.

Criteria for terminating treatment included disease progression, patient noncompliance, intercurrent medical illness (unre-

[3] J. Williams, personal communication.

[4] The abbreviations used are: ECOG, Eastern Cooperative Oncology Group; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; ULN, upper limit of normal.

lated to the cancer), a request to withdraw, or the development of unacceptable toxicity. The National Cancer Institute Common Toxicity Criteria[5] (version 2.0) were used to grade toxicities.

Treatment Regimen and Dose Escalation. Squalamine lactate was provided by Magainin Pharmaceuticals, Inc. (currently Genaeva Corporation). The drug was reconstituted with sterile water for injection, USP, and the appropriate dose was further diluted in 5% dextrose in water, USP, to yield a final concentration of 1 mg/ml squalamine lactate for injection. Squalamine was administered via a centrally placed infusion line as a 120-h continuous i.v. infusion using a portable pump. Each infusion was followed by a rest period of at least 9 days. Treatments were repeated every 14 days, which constituted one treatment cycle, unless resolution of any toxicities required a brief delay.

The starting squalamine dose for clinical studies was selected by the pharmacokinetically guided dose escalation method as 1/10th of the MTD in the most sensitive toxicology species (rats; Ref. 18), or 6 mg/m^2/day. Dose rates of squalamine were increased in 100% increments up to a dose of 384 mg/m^2/day, after which dose escalation proceeded in 40% increments until DLT was encountered. Once DLT was encountered, the dose was decreased by one dose level; if this dose was well tolerated, then the duration of successive infusions was prolonged from 10 up to 30 days in 5-day increments.

Pharmacokinetic Sampling. Blood samples were obtained during the first cycle of squalamine treatment to study the plasma pharmacokinetics of squalamine. Samples were taken immediately prior to the start of drug infusion: 30 min and 1, 2, 4, 8, 12, 16, 24, 48, 72, 96, and 120 h after starting the infusion; and 7.5, 15, 30, 45 min and 2, 4, 12, 24, and 48 h following the end of infusion. Three ml blood samples were collected at each time point in Vacutainer tubes containing heparin and immediately placed on ice. Plasma was harvested by centrifugation at 4°C and stored frozen at −20°C until analysis.

Liquid Chromatography Mass Spectrometric Methods. The total quantity of squalamine in patient plasma diluted 1:1 with 0.1 N NaOH was determined after doping all samples with deuterated squalamine reference standard (d^6-squalamine) and using OASIS HLB solid phase extraction cartridges (Waters Corp., Milford, MA) as prescribed by the manufacturer. Extracted sample volumes of 20 μl were analyzed by liquid chromatography mass spectrometry using a Series II 1090 liquid chromatograph (Hewlett Packard, Palo Alto, CA) linked in tandem with a Finnigan TSQ mass spectrometer (Finnigan Corp., San Jose, CA) that was equipped with a standard ESI source and operated in selected ion monitoring mode, scanning ions 626.5 and 632.5 ± 0.4 amu.

Pharmacokinetic Analysis. The pharmacokinetics of squalamine after a single 120-h continuous i.v. infusion were determined for 13 patients at the seven highest squalamine dose rates using both compartmental and noncompartmental methods. Occasional unavailable data values were interpolated by equating them to the next observed nonmissing data point value.

[5] See the National Cancer Institute website at http://ctep.info.nih.gov/CTC3/default.htm for details.

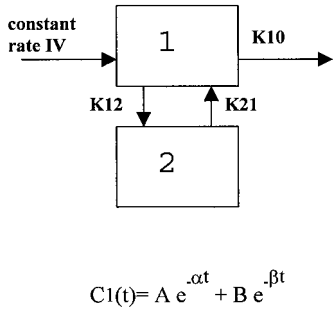

Fig. 2 Two-compartment continuous i.v. infusion pharmacokinetic model for squalamine disposition with assumed no lag time and first-order elimination rate constant K10.

Plasma concentration *versus* time curves were fit either to a two-compartment, first-order elimination model using weighted, nonlinear least squares fit analysis (Fig. 2) or to a noncompartmental model. The model of best fit was estimated by visual examination of variances between the curve of best fit predicted by each pharmacokinetic model and actual data values. Determinations of maximum concentration (*Cmax*) and time to *Cmax* (*Tmax*) were taken from the range of data values on or before the end of the 120 h continuous i.v. infusion. The following pharmacokinetic parameters were estimated from the two-compartment model, assuming plasma was represented by compartment 1: area under the concentration-time curve ($AUC_{0\text{-}t}$, t = 168 h after the start of infusion) using the trapezoid rule; rate constants K12, K21, and K10 (rate constant for squalamine removal from plasma); and α and β for the equation $C_{\text{compartment }1}(t) = A\ e^{-\alpha t} + B\ e^{-\beta t}$ describing the concentration C of squalamine in compartment 1 at time t, with $\beta \gg \alpha$. All analyses were carried out using WinNonLin software (Pharsight Corp., Mountain View, CA).

RESULTS

Patient Characteristics

Nineteen patients were treated in this study between January 1998 and September 1999. The characteristics of patients who received treatment are listed in Table 1. One patient elected to discontinue treatment after two cycles and was therefore not evaluated for response. There were more females (74%) than males among the study population, and most patients (68%) had an ECOG performance status of 0 or 1. All patients had had prior surgery, 18 patients had received prior chemotherapy regimens for their cancers, 13 had prior radiation treatment, 6 had prior biological or immunotherapy, and 4 had prior hormonal therapy (Table 1).

Squalamine Dose Escalation to MTD

Nineteen patients received a total of 70 infusion cycles of squalamine. Eight dose levels were evaluated ranging from 6 to 538 mg/m^2/day (Table 2). Thirteen patients underwent intrapatient dose escalation. DLT was encountered in 4 of 5 patients treated at a dose rate of 538 mg/m^2/day; the fifth patient received only 3 days of treatment at this dose and did not experience DLT. Of these four patients, one was initiated at 538

Table 1 Patient characteristics

No. of patients assessable	19
Toxicity	19
Response	18
Median age (range)	54 (21–85)
Gender	5M/14F
ECOG PS[a]	
0	2
1	11
2	6
Prior therapy	
Chemotherapy	18
Hormonal therapy	4
Bio-/Immunotherapy	6
Radiotherapy	13
Surgery	19
Primary malignancy	
Lung and bronchus	3
Breast	3
Ovarian	3
Sarcoma	3
Melanoma	2
Colon	1
Anal	1
Islet cell pancreatic	1
Cholangiocarcinoma	1
Renal cell	1

[a] PS, performance status.

Table 2 Dose escalation scheme

Dose (mg/m^2/day, 120-h infusion)	No. of patients started at this dose	Total no. of patients treated	Total no. of infusions
6	3	3	3
12	3	6	9
24	1	5	6[a]
48	2	6	6
96	4	10	14
192	2	6	10[b]
384	3	8	17[c]
538	1	5	5[d]

[a] Maximum duration of continuous i.v. infusion, 10 days ($n = 1$).
[b] Maximum duration of continuous i.v. infusion, 20 days ($n = 1$).
[c] Maximum duration of continuous i.v. infusion, 7 days ($n = 1$).
[d] Treatment was stopped in one patient, and the dose was decreased by two dose levels in another patient, because of DLT. Both patients received 3 days of treatment at this dose. A third patient had his dose decreased after 3 days of treatment because of a limited supply of study drug.

mg/m^2/day, whereas the remaining three were escalated from lower dose levels. These three patients did not experience DLT when first treated at a dose rate of 384 mg/m^2/day (during intrapatient dose escalation) but experienced DLT at the same dose when their dose was lowered from 538 mg/m^2/day because of toxicity (Table 3). Additionally, 1 patient of 3 who were initiated *de novo* at 384 mg/m^2/day also experienced a DLT. Therefore, a dose of 192 mg/m^2/day was the best tolerated dose of squalamine when administered as a 120-h infusion, although a dose of 384 mg/m^2/day appeared to be well tolerated in patients without prior exposure to squalamine.

Toxicity

The median duration of treatment with squalamine was 6 weeks (range, 3–26 weeks), with three patients receiving treatment for >3 months and 2 of these patients receiving treatment for 4.5 months. The principal DLT encountered at a dose rate of 538 mg/m^2/day consisted of hepatotoxicity. A comprehensive listing of toxicities seen at all doses is provided in Table 4.

Hepatotoxicity. No abnormalities of liver function tests were seen up to a dose of 192 mg/m^2/day. Four patients (1 *de novo*, 3 escalated from lower dose) developed asymptomatic elevations of hepatic transaminases (AST and ALT) during treatment at a dose of 538 mg/m^2/day. These abnormalities were noted 2–5 days after starting treatment and consisted of grade 3 elevations (AST and ALT, each 5.1–20 × ULN) in 3 patients and grade 4 elevations (AST and ALT, each ≥20 × ULN) in 1 patient. The AST values returned to baseline sooner (range, 6–19 days) than ALT values (range, 11–23 days). All patients had accompanying rises in levels of alkaline phosphatase and lactate dehydrogenase (toxicity grades 1–2), whereas a moderate elevation in bilirubin was observed in one patient. All four patients who developed DLT at 538 mg/m^2/day were subsequently retreated at a lower dose level (384 mg/m^2/day) and developed a similar DLT (grade 3 elevation of AST and/or ALT). Of these, 1 patient was able to continue prolonged treatment at a further reduced dose rate of 192 mg/m^2/day without significant toxicities. Additionally, 1 patient who began treatment at 384 mg/m^2/day developed DLT (grade 3 elevation of ALT) two days after starting treatment, which lasted for 2 days.

Fatigue. Fatigue was encountered in patients at all dose levels and was not related to the dose rate, total dose, or duration of treatment. Most patients complained of mild to moderate fatigue (grades 1–2) beginning after 72–96 h of infusion, which resolved within 5–16 days of stopping infusion. One patient developed grade 3 fatigue consistent with a DLT during treatment at a dose rate of 12 mg/m^2/day, which resolved completely within 4 days of stopping treatment. Because the patient was receiving concomitant radiation to lung nodules and several patients had been treated at higher doses without grade 3 fatigue, it was decided to escalate the dose after the patient completed radiation. The patient did not have recurrent fatigue for the next two dose levels (24 and 48 mg/m^2/day) but again encountered grade 3 fatigue at 96 mg/m^2/day, which was reproducible with treatment and lasted for 3–8 days. The patient's dose rate was reduced to 24 mg/m^2/day, and the duration of infusion was prolonged to 10 days. The patient had recurrence of grade 3 fatigue 5 days after stopping treatment, which lasted for 3 days. Further dose reduction could not be accomplished in this patient because of progressive disease. This was the only patient in the study who received concomitant radiation, and the initial episode of grade 3 fatigue was thought to be partly related to radiation. Because none of the other patients treated at these dose levels developed grade 3 fatigue, dose escalation was continued as planned. In all patients, fatigue resolved completely prior to subsequent treatment, and no cumulative fatigue was observed with repeated squalamine doses. No patient discontinued treatment because of fatigue.

Table 3 Characteristics of hepatotoxicity (DLT) seen with squalamine

Patient no. and dose (mg/m^2/day)	Treatment cycle (C)	Toxicity/Grade	Time to onset (days)	Time to resolution (days)
010				
538	C4	AST/3	3	7
		ALT/3	3	12
384	C6	AST/3	7	14
015				
538	C3	AST/3,	3	4
		ALT/3	3	12
384	C4	ALT/3	6	4
017				
538	C2	AST/3,	4	6
		ALT/3	4	6
384	C3	AST/3,	6	2[a]
		ALT/3	6	2[a]
019				
538	C1	AST/4,	6	19[b]
		ALT/4	6	23[b]
384	C2	ALT/3,	9	10
		AST/3	9	5
021				
384	C3	AST/3	3	5

[a] Reduced to grade 2; did not fully resolve until 70 days later.
[b] Reduced to grade 1; did not fully resolve until 19–23 days later.

Table 4 Summary of toxicities seen at all dose levels (number of patients)

	Dose level (mg/m^2)															
	6 (n = 3)		12 (n = 6)		24 (n = 5)		48 (n = 6)		96 (n = 10)		192 (n = 6)		384 (n = 8)		538 (n = 5)	
Toxicity	I–II[a]	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV	I–II	III–IV
Fatigue	2	0	3	1	2	1	4	0	5	1	2	0	3	2	4	0
Nausea/Vomiting	0	0	4	0	2	0	0	0	3	0	1	0	2	0	1	0
Anorexia	0	0	2	0	0	0	0	0	3	0	1	0	2	0	0	0
Muscle cramps	0	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0
Myalgia	0	0	0	0	0	0	0	0	1	0	2	0	1	0	1	0
Parasthesias	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
Hepatotoxicity	0	0	0	0	0	0	0	0	0	0	0	0	1	5[b]	0	4[b]

[a] I–II and III–IV represent grades of toxicity (using the National Cancer Institute Common Toxicity Criteria).
[b] DLT.

Gastrointestinal Symptoms. Mild to moderate (grades 1–2) nausea and vomiting were encountered at dose rates ranging from 12 to 384 mg/m^2/day. Nausea was not dose related and could be controlled with standard antiemetics. Mild anorexia was randomly encountered across dose levels and was associated with <10% loss in body weight. No diarrhea or stomatitis was observed.

Neuromuscular Symptoms Grade 1 myalgias and muscle cramps were observed during treatment at dose rates >96 mg/m^2/day. Myalgias occurred during or shortly after squalamine infusions and resolved in 2–3 days, whereas muscle cramps were transient and appeared unrelated to the timing of the infusion. No electrolyte abnormalities were associated with these symptoms, and they were unrelated to the dose of squalamine administered.

Other Toxicities. One patient developed ataxia and dysmetria during treatment at a dose rate of 384 mg/m^2/day, which lasted for 7 days. These symptoms did not recur during retreatment with squalamine at the same dose rate. Another patient had extravasation of squalamine around the Mediport site on the chest during infusion, which led to swelling, erythema, and tenderness lasting for 5 days. There was no skin or tissue breakdown at the site of extravasation, and no residual scarring or neuromuscular deficit was detectable.

Duration of Infusion

The duration of squalamine infusion was prolonged beyond 120 h in 2 patients. The first patient received a continuous i.v. infusion of squalamine for 10 days at a dose rate of 24 mg/m^2/day and developed grade 3 fatigue, as mentioned previously. The second patient received one cycle of continuous i.v. squalamine infusion for 10 days at a dose rate of 384 mg/m^2/day, which was well tolerated. However, during the second cycle of prolonged infusion at the same dose, the patient developed grade 3 transaminase elevations, requiring discontinuation of treatment after 7 days. This patient's squalamine dose rate was further reduced to 192 mg/m^2/day, and she subsequently

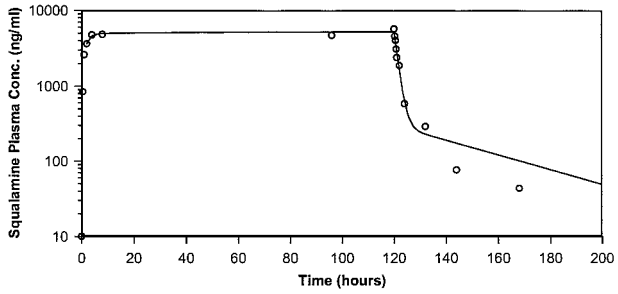

Fig. 3 Plasma concentration-time profile of a patient treated with squalamine at a dose rate of 192 mg/m^2/day for 5 days. The observed concentrations (○) are plotted along with the fitted concentrations from a two-compartment pharmacokinetic model (*plotted line*).

tolerated 15 and 20 days of continuous i.v. squalamine infusion without significant toxicities.

Objective Tumor Responses

Transient tumor regressions were observed during treatment with squalamine as a single agent. A patient with cutaneous metastases from breast cancer had a partial response (>50% reduction in size of skin nodules) with breakdown of the skin overlying the tumor nodules and concomitant decrease in tumor marker (20% decrease in CA 15-3 levels) after 6.5 weeks of treatment. This patient started treatment at a dose of 6 mg/m^2/day and was receiving treatment at 24 mg/m^2/day when the response was noted. The response lasted for 6 weeks, during which her dose was escalated to 96 mg/m^2/day, followed by progression of systemic metastases. The skin ulceration persisted during treatment, did not worsen with dose escalation, and resolved within 1 month of stopping squalamine infusions. A patient with a high-grade synovial sarcoma metastatic to the lungs received palliative radiation (4400 cGy) to large lung nodules concurrently with squalamine treatments. There was >50% reduction in the size of lung nodules within the radiation port, which could be related to radiation, squalamine, or the combination. This patient received treatment for 14 weeks before systemic progression of disease. All patients in the study had progression of their disease after variable periods of treatment.

Pharmacokinetics

Pharmacokinetic samples were drawn during the first cycle of treatment in 19 patients. Of these, 13 patients (with dose rates ranging from 12 to 538 mg/m^2/day) had data adequate for estimating pharmacokinetic parameters using a two-compartment, first-order elimination model (Fig. 2). Fig. 3 shows a representative squalamine plasma concentration *versus* time curve.

A summary of calculated pharmacokinetic parameters as a function of squalamine dose rate is provided in Table 5. Squalamine $AUC_{0\text{-}t}$ and *Cmax* values were dose and dose rate proportional over the dose rate span 12–384 mg/m^2/day, with evidence of nonlinearity at 538 mg/m^2/day (Fig. 4 and Table 5). The $AUC_{0\text{-}t}$ at lower doses (6–24 mg/m^2/day) was estimated using noncompartmental methods. The median time to maximal

Table 5 Estimates of pharmacokinetic parameters obtained from 13 patients treated at the seven highest squalamine dose rate levels

Errors for treatment groups with >1 patient are expressed as the SE. The compartment volumes V1 and V2 were used in the calculation of $AUC_{0\text{-}t}$ and the initial and terminal squalamine half-lives.

Squalamine dose level (mg/m^2/day)	K12 half-life (min)	K21 half-life (hr)	K10 half-life (min)	Initial squalamine plasma half-life, ln2/α (min)	Terminal squalamine plasma half-life, ln2/β (hrs)	*Cmax* (ng/ml)	*Tmax* (hr)	$AUC_{0\text{-}t}$ (ng·h/ml)	V1 (ml)	V2 (ml)
12 (n = 1)	1.2	0.06	29.9	0.8	2.0	291	72	29,182	3065	8781
24 (n = 1)	Ind.[a]	1.2	84.6	72.6	1.4	640	48	50,528	7111	0.2
48 (n = 1)	365.4	3.25	61.5	50.4	4.0	891	96	89,410	7495	3987
96 (n = 4)	46.6 ± 9.7	11.2 ± 8.7	48.6 ± 7.6	37.5 ± 12.3	6.2 ± 6.2	1,863.5 ± 740.2	55 ± 48	183,834 ± 79,864	5111 ± 1791	3290 ± 2431
192 (n = 2)	48 ± 21.2	28.2 ± 11.5	57.6 ± 12.7	55.8 ± 29.4	24.7 ± 22.3	5,580 ± 197.9	108 ± 16.9	555,777 ± 103,995	3789 ± 11	5761 ± 1696
384 (n = 3)	360.8 ± 288.1	12.8 ± 8.0	67.2 ± 27.0	47.4 ± 20.3	16.4 ± 9.7	11,317 ± 3,778	73 ± 61	1,027,376 ± 207,051	5241 ± 610	12027 ± 6374
538 (n = 1)	65.4	11.6	103.8	85.2	15.8	32,200	72	2,975,660	3840	7279
Median value	NC[b]	7.1	55.2	49.8	7.1		96		4427	4065

[a] Ind., indeterminate.
[b] NC, not calculated.

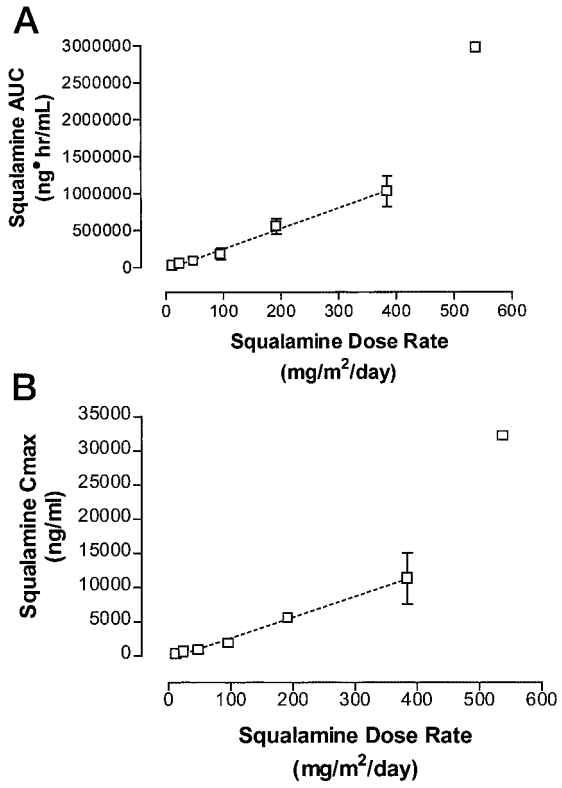

Fig. 4 *A*, squalamine pharmacokinetic parameter *AUC* as a function of squalamine dose rate. *Broken line*, fit of linear regression model to the data up to a dose rate of 384 mg/m^2/day ($r^2 = 0.99$; $P < 0.001$). *B*, squalamine pharmacokinetic parameter *Cmax* as a function of squalamine dose rate. *Broken line*, fit of linear regression model to the data up to a dose rate of 384 mg/m^2/day ($r^2 = 0.99$; $P < 0.001$). *Bars*, SE.

concentration (*Tmax*) was 96 h and ranged from 4 to 120 h across all dose rate levels. Squalamine was initially cleared rapidly from the circulation (clearance, 3704 ± 1816 ml/min), with a calculated median half-life for squalamine removal from plasma (ln2/K10) of 49.2 min (range, 38.4 to 103.8 min) and a calculated initial plasma half-life ($t_{1/2\alpha} = \ln 2/\alpha$) of 49.8 min (range, 0.8–85.2 min). The terminal squalamine plasma half-life ($t_{1/2\beta} = \ln 2/\beta$) was calculated to be much longer, a median of 7.2 h (range, 0.9–40.5 h) by a two-compartment model (Table 5). Both of these latter values suggest persistence outside the circulatory system of a residual amount of squalamine that slowly leaches back into the patient plasma.

DISCUSSION

Squalamine was selected for clinical development based on its potent antiangiogenic and antitumor activities in preclinical experiments. Pharmacology studies in rodents and dogs revealed a rapid elimination after i.v. injection. Therefore, a 120-h continuous infusion schedule was selected to maximize the exposure of tumor to drug. This study represents the first clinical trial of squalamine in humans using this schedule of administration (19). Another Phase I trial using squalamine in a slightly different schedule (120-h infusion once every 3 weeks) is currently being conducted (20).

The dose escalation scheme for this study was based on doubling of dose method, and intrapatient dose escalation was permitted in the absence of toxicity. This method has two advantages: (*a*) it provides an opportunity to patients starting at low dose levels to escalate to higher (and possibly therapeutic) doses of the drug; and (*b*) if no toxicities are encountered at higher doses during intrapatient dose escalation, fewer *de novo* patients can be started at lower dose levels, thus reducing the number of patients required to define MTD. Therefore, using the conventional (3 patients/cohort) design, this study would have required at least 27 patients to define the MTD (*i.e.*, 42% more patients). The disadvantage to this design is that limited pharmacokinetic data are available for the lower dose levels, which may obscure interpatient variability.

Consistent with animal toxicology studies, the principal DLT of squalamine was hepatotoxicity, characterized by transient plasma elevations of hepatic transaminases accompanied by mild elevations of bilirubin and lactate dehydrogenase. Hepatotoxicity was dose related and resolved completely upon discontinuation of treatment. For the four patients who experienced hepatotoxicity at the highest dose rate of squalamine (538 mg/m^2/day), all had hepatotoxicity when treated at the next lower dose rate level (384 mg/m^2/day), including those treated previously without incident at 384 mg/m^2/day. However, one of these patients was able to tolerate a further reduced dose rate of 192 mg/m^2/day and had no hepatotoxicity, even when the period of continuous i.v. infusion was lengthened to 20 days. The mechanism of hepatotoxicity remains unclear at this time.

Animal toxicology studies had predicted the possibility of gastrointestinal and renal toxicities and possible cardiac conduction disturbances. However, none of these toxicities were encountered in humans. Fatigue was a prominent toxicity across dose levels and was mild to moderate in most cases. One patient developed grade 3 (severe) fatigue at three different dose levels (12, 24, and 96 mg/m^2/day), but grade 3 fatigue was not encountered at higher dose rates. Because the incidence or severity of fatigue was not related to the dose rate, it may be an idiosyncratic toxicity associated with squalamine. However, fatigue was clinically manageable and did not require discontinuation of treatment in any patient. The duration of fatigue was generally brief, and no cumulative fatigue was observed with treatment. Mild neuromuscular toxicities consisting of myalgia, muscle cramps, and parasthesias were observed at dose rates ≥96 mg/m^2/day.

Plasma pharmacokinetics revealed a relatively short initial elimination half-life and a high plasma clearance, which are consistent with unpublished animal studies. The *AUC* and *Cmax* increased linearly with dose and dose rate up to 384 mg/m^2/day, whereas clearance appeared unchanged across dose levels. Prior studies in mice revealed that daily i.v. dosing with 20 mg/kg squalamine was highly effective in reducing primary and metastatic tumor growth (12). Unpublished pharmacokinetic data for mice administered 20 mg/kg squalamine i.v. established an *AUC* of 215,000 ng-h/ml as inhibitory for tumor growth.[3] Therefore, as shown in Table 5, biologically relevant concentrations of squalamine were achievable in plasma of patients treated at dose rates ≥96 mg/m^2/day. Limited experience was

obtained on prolonged (beyond 120 h) continuous i.v. infusions of squalamine in this trial. It appears that squalamine may be tolerated as a continuous infusion for up to 20 days. However, a larger patient accrual will be required to confirm the safety of this schedule of administration.

Although transient tumor regressions were observed during treatment, no long-term antitumor activity could be demonstrated with single-agent squalamine. This may not represent lack of clinical activity, because all patients in this study had advanced disease, and antiangiogenic agents such as squalamine are thought to work best in settings of minimal residual disease or low tumor burden as single agents (1). Recent studies have shown synergistic activity of squalamine with cisplatin or carboplatin using human lung cancer xenografts (13) and with cisplatin using ovarian cancer (14) or neuroblastoma xenografts (16), with squalamine strongly enhancing the cytotoxic effects of chemotherapeutic agents while having moderate activity on its own. On the basis of these results, clinical trials of squalamine in combination with cisplatin and carboplatin have been initiated for treatment of patients with non-small cell lung cancer or ovarian cancer.

In conclusion, this study demonstrates that squalamine, a novel antiangiogenic agent, can be given safely to patients as a 120-h continuous i.v. infusion up to a dose rate of 192 mg/m^2/day. Patients without prior exposure to squalamine may be able to tolerate a dose rate of 384 mg/m^2/day without toxicity. Indeed, in an ongoing Phase I study using a slightly different schedule of administration (20), patients have been treated at squalamine dose rates $>$300 mg/m^2/day without significant toxicities.[6] The predominant toxicity from squalamine is hepatotoxicity, which occurs in a dose-dependent manner and is fully reversible upon stopping treatment. Fatigue also appears to be a prominent toxicity in some patients but is unrelated to squalamine dose. The absence of sustained antitumor activity with single-agent squalamine and preclinical evidence of synergy with chemotherapeutic agents support the future study of squalamine in combination with chemotherapy regimens. On the basis of this data, combination trials have begun in non-small cell lung cancer and ovarian cancer, and preliminary results should be available soon.

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