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Phase I Trial of the Amsacrine Analogue 9-({2-Methoxy-4-[(methylsulfonyl)amino]-phenyl}amino)-*N*,5-dimethyl-4-acridinecarboxamide (CI-921)[1]

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ABSTRACT

CI-921, an analogue of amsacrine with superior activity against *in vivo* and *in vitro* experimental tumor models, has been studied in 16 patients with solid tumors refractory to chemotherapy or for which conventional therapy does not exist. Thirty-nine cycles were given and doses escalated from 39 to 810 mg/m². This total dose was divided over 3 consecutive days and administered by 15-min infusion each day, repeated three times weekly. Neutropenia (Eastern Cooperative Oncology Group) Grade ≥3 occurred at Day 8 (range, 7–13) in 10/13 courses at 648 mg/m² and in 2/2 courses at 810 mg/m² with recovery in 10 (range, 4–20) days. At 810 mg/m² Grade 2 mucositis and phlebitis were noted. Mild nausea and venous irritation occurred in some patients at doses ≥288 mg/m². No objective response was seen. Pharmacokinetics were evaluated following 65 infusions on Days 1 and 3 with plasma concentrations of CI-921 measured by high performance liquid chromatography. Peak plasma concentrations ranged from 3.36 to 85.6 μmol/liter and were significantly correlated with dose. Mean (range) model-independent pharmacokinetic parameters were: distribution half-life, 0.46 h (0.24–1.08); elimination half-life, 2.63 h (1.08–4.98); mean residence time, 2.0 h (1.05–3.35); plasma clearance, 158 ml/h/kg (95–290); and steady-state volume of distribution, 319 ml/kg (219–614) with no significant difference between Day 1 and 3. Toxicity as defined by absolute granulocyte count nadir was significantly correlated with dose, area under concentration-time curve, and peak plasma concentration. The recommended dose for Phase II studies in this schedule is 648 mg/m² (216 mg/m² daily for 3 days) repeated every three weeks.

INTRODUCTION

Amsacrine was developed by the Auckland Cancer Research Laboratory and now has an established role in the treatment of leukemia (1). In an attempt to develop a drug with greater solid tumor activity, a large number of related anilinoacridine compounds were synthesized and evaluated against both human tumor cell lines *in vitro* and mouse solid tumors models *in vivo*. The highly active derivative CI-921[3] (see *structures* in Fig. 1), is more water soluble and less basic than amsacrine. Such properties may change its distributive properties, favoring activity against solid tumors (2). CI-921 is thought to act like amsacrine by poisoning the enzyme DNA topoisomerase II (3).

CI-921 is superior to amsacrine, doxorubicin, and daunorubicin and comparable to cyclophosphamide against both P388 leukemia and Lewis lung carcinoma tumor models in mice (2). It is also active against LC-12 murine squamous cell lung carcinoma, B16 melanoma, several mammary and colon carcinomas, and an Adriamycin-resistant P388 leukemia (4). The major toxicities in preclinical studies include bone marrow hypocellularity, lymphoid depletion, gastrointestinal irritation, and inflammation at injection sites, with no unusual or unexpected toxicities in mice, rats, or dogs.[4] Because of these properties CI-921 was selected for clinical trial.

MATERIALS AND METHODS

Patient Characteristics and Eligibility. 16 patients (12 men and 4 women, age range 21–70 years, age median 57 years) were studied. Tumor types comprised non-small cell lung cancer (six patients), malignant melanoma (four patients), breast cancer (two patients), and small cell lung, gastric, pancreatic, and head and neck cancers (one patient each). At the time of entry into the trial, performance status was 0 in three patients, 1 in 12 patients, and 2 in one patient. Six patients had received no prior treatment while previous therapy included surgery in six patients, radiotherapy in six patients, and chemotherapy in five patients.

All patients had advanced histologically documented cancers for which there was no conventional therapy or which had recurred following such treatment. All patients had recovered from prior chemotherapy or radiotherapy and had adequate pretreatment bone marrow function (AGC $\geq 1.5 \times 10^9$/liter, platelets $\geq 100 \times 10^9$/liter), liver function (alkaline phosphatase, aspartate transferase, bilirubin $\leq 1.5 \times$ upper limit of normal) and renal function (serum creatinine ≤ 0.13 mmol/liter or ≤ 0.16 if creatinine clearance ≥ 1.0 ml/s). Because the cardiac toxicity of amsacrine has been associated with hypokalemia (5), a serum potassium was measured within 24 h of treatment and corrected to within normal limits if necessary. Patients with more than one malignancy (except nonmelanomatous skin cancer or uterine cervical carcinoma *in situ*) were excluded.

Pretreatment evaluation included history and physical examination, tumor measurement, biochemical profile, hematological profile, urinalysis, chest X-ray, electrocardiogram, and echocardiogram. Additional imaging studies were performed as appropriate for assessment of disease status. Prior to entry, all patients were informed of the experimental nature of the treatment, advised of the possible side-effects, risks, and limitations of the drug, and were required to sign a consent form, which, together with the protocol, had been approved by the local institutional ethical committee. During treatment, patients were evaluated weekly, biochemical and hematological screens were obtained twice weekly, and chest X-ray, electrocardiogram, and urinalysis were performed prior to each course of therapy. Echocardiograms were repeated after every alternate course and at the end of the study period. Standard Eastern Cooperative Oncology Group criteria were used for evaluation of response and toxicity with the exception of anemia. Grade 1 hemoglobin toxicity was defined as a fall of 1.5–3 g/dl, Grade 2 as 3–3.9 g/dl, Grade 3 as 4–4.9 g/dl, and Grade 4 as ≥5 g/dl. All patients were evaluated for toxicity. Patients were evaluable for response if they completed one planned treatment cycle of three consecutive daily doses of CI-921.

Treatment. CI-921 as the hydroxyethanesulfonate salt was reconstituted in sterile water and dissolved in 200 ml 5% dextrose. Each cycle of therapy was divided over 3 days, and cycles were repeated at 3-week

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[3] The abbreviations and trivial name used are: CI-921, 9-[{2-methoxy-4-[(methylsulfonyl)amino]phenyl}amino)-*N*,5-dimethyl-4-acridinecarboxamide; AGC, absolute granulocyte count.

[4] Investigator's Brochure. Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan.

intervals. Each dose was given by constant infusion via an i.v. cannula over 15 min, followed by a 50-ml flush of 5% dextrose. A total of 39 treatment courses were given (one to six courses per patient, median 2). Although the planned starting dose (Table 1) was 12 mg/m^2 based on the preclinical animal toxicity data,[4] results available from another trial center (6) documenting the absence of toxicity at this level enabled the starting dose to be advanced to 39 mg/m^2 (13 mg/m^2 daily for 3 days). Subsequently, two other levels were omitted because of clinical information from the other study. Otherwise, at least three patients were treated at each dose level before escalation. Escalation within patients was permitted. Dose limiting toxicity was defined as: AGC nadir $<0.5 \times 10^9$/liter (or a platelet nadir of $<50 \times 10^9$/liter), recovery to $<1.5 \times 10^9$/liter AGC (or $<100 \times 10^9$/liter platelets) by day 35 after the first day of treatment, or ≥Grade 2 nonhematological toxicity (excluding alopecia). The maximum tolerated dose was that dose which produced dose limiting toxicity in at least 50% of patients initially treated at that dose level.

Sample Collection, Analysis, and Pharmacokinetics. Blood samples (5 ml) were taken at 0, 7, 15, 20, 30, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 12, 18, and 24 h following infusion on Days 1 and 3, with pretreatment and end of infusion samples (time 0 and 15 min) only on Day 2. Further samples were taken at 36, 48, 72, and 96 h after the third infusion. All blood samples were centrifuged immediately following collection and the plasma separated and frozen at −80°C until assay. Solutions of CI-921 in plasma were found to be stable for at least 9 months when stored at −80°C. Concentrations of CI-921 were assayed by high performance liquid chromatography (7). Samples were acidified, washed with hexane, readjusted to pH 9.0 and extracted with diethyl ether. Calculated recoveries were 98–107% for concentrations of 0.05–20 μmol/ml. Evaporated extracts were chromatographed on a Waters Radial-Pak C18 column using acetonitrile-water containing 0.01 M triethylamine phosphate as the mobile phase. A UV detector (254 nm) enabled concentrations ≥50 nmol/liter to be measured with acceptable accuracy and precision (coefficient of variation <10%). Lower values were disregarded. Model-independent pharmacokinetics parameters were calculated by the usual methods (8, 9), correlations assessed by Pearson's correlation coefficient (r), and pharmacokinetic parameters compared by Student's t test.

RESULTS

Toxicity. Neutropenia was the dose-limiting hematological toxicity (Table 2). No consistent myelosuppression was noted at doses ≤288 mg/m^2 (96 mg/m^2 × 3). Of four patients treated at 432 mg/m^2 (144 mg/m^2 × 3), one heavily pretreated patient developed Grade 3 neutropenia. At doses ≥648 mg/m^2 (≥216 mg/m^2 × 3), neutropenia was seen in 15 courses (Table 2), becoming evident from Days 7 to 13 (median, Day 8) with recovery by Days 13 to 28 (median, Day 18). There was no instance of treatment-related death and no episode of severe sepsis. Thrombocytopenia did not occur.

Mild nausea/vomiting and anorexia were seen in eight patients at doses ≥192 mg/m^2. Nausea was generally well controlled with low dose oral metoclopramide, with no episode of intractable vomiting. Most patients complained of mild irritation at the infusion site during drug infusion. Pain in the infusion arm occurred in six patients at ≥288 mg/m^2 necessitating an increase in the infusion volume to 250 ml followed by a 300-ml flush of 5% dextrose. Grade 2 thrombophlebitis with painful swelling and erythema in the infusion arm developed in one patient each at 432 mg/m^2 (one of four treatments), 648 mg/m^2 (one of 13 treatments), and 810 mg/m^2 (one of two treatments) but no skin ulceration occurred. In two patients inadvertent extravasation of CI-921 occurred during infusion, but this was not painful and did not result in soft tissue damage. Painful mouth ulcers (Grade 2) occurred in one of two patients treated at 810 mg/m^2.

One patient who was subsequently shown to have liver metastases developed a Grade 1 increase in alkaline phosphatase at 432 mg/m^2 and a Grade 2 increase in alkaline phosphatase and aspartate transferase in a following course at 288 mg/m^2. Five patients developed a transient increase in alkaline phosphatase at 648 mg/m^2 (Table 2) with recovery by Days 7–10. The patient with a Grade 2 elevation of alkaline phosphatase also showed an increase in aspartate transferase (Grade 1) in each of three courses. One patient at 810 mg/m^2 showed a transient Grade 1 elevation of serum creatinine with complete recovery within 4 days. No alopecia, cardiac, central nervous system, or pulmonary toxicity occurred.

Response. No patient achieved an objective response.

Pharmacokinetics. The kinetics of CI-921 were studied after the first and third infusions of 32 cycles of treatment (and in one case following Day 1 infusion only) over a dose range of 13 to 270 mg/m^2 (Fig. 2). Peak plasma concentrations of CI-921 at the end of the infusion ranged from 3.36 to 85.6 μmol/liter. The area under the concentration-time curve ranged from 2.4 to 99 μmol·h/liter. Mean (range) model-independent kinetic parameters were as follows: distribution half-life ($t_{½}\alpha$) 0.46 h (0.24–1.08), elimination half-life ($t_{½}\beta$) 2.63 h (1.08–4.98), mean residence time 2.0 h (1.05–3.35), plasma clearance 158 ml/h/kg (95–290), and steady-state volume of distribution 319 ml/kg (219–614). There was no significant difference between parameters for the first and third infusion. Toxicity, as defined by logarithm of the absolute granulocyte count nadir, was significantly correlated with dose ($r = -0.87$; Fig. 3), area under the concentration-time curve ($r = -0.84$), and maximum concentration achieved in plasma ($r = -0.86$). More detailed pharmacokinetic data will be published elsewhere (9).

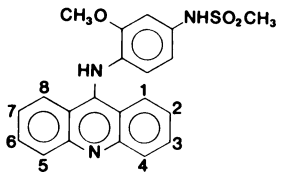

Fig. 1. Structure of amsacrine; CI-921 is the 4-CH_3, 5-$CONHCH_3$ derivative.

Table 1 *Dose escalation scheme*

Total dose[a] (mg/m^2)	Number of patients (new/total)	Number of courses
12	Omitted	
24	Omitted	
39	2/2	2
60	Omitted	
84	Omitted	
108	2/3	3
144	3/6	6
192	1/4	4
288	1/3	4
432	1/4	4
540[b]	0/1	1
648	4/8	13
810	2/2	2

[a] Total dose divided over 3 days.
[b] One patient received a 25% dose escalation from 432 mg/m^2.

DISCUSSION

CI-921 has proven to be a relatively nontoxic drug which is well tolerated by patients. When given according to this protocol, predictable and rapidly reversible neutropenia was the dose-limiting toxicity. Myelosuppression developed at 7–13 days posttreatment with rapid recovery. The effect on hemoglobin was minor and there was no fall in platelet count. Venous

Table 2 *Frequency and grade of toxicity of CI-921 at dose levels ≥192 mg/m² (total dose)*

Dose[a] (mg/m²)	No. of courses	Toxicity[b] grade	AGC	Hb	Nausea/vomiting	Phlebitis	Mouth ulcer	Alkaline phosphatase	Aspartate transferase	Renal
192	4	1			3					
288	4	1		1	2	1				
		2						1	1	
432	4	1	2	3	3	2		1		
		2				1				
		3	1							
648	13	1	1	8	5	5		5	3	
		2	2	1		1		1		
		3	9							
		4	1							
810	2	1		1	1	1				1
		2		1		1	1			
		3	1							
		4	1							

[a] Total dose over 3 days.
[b] Eastern Cooperative Oncology Group toxicity criteria.

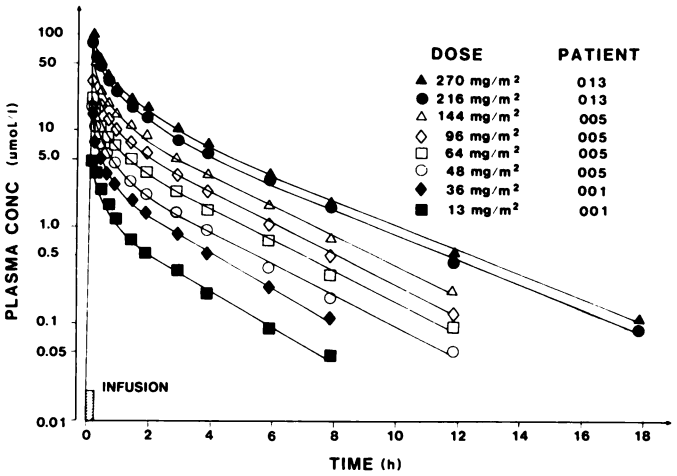

Fig. 2. Plasma CI-921 concentrations after the first day infusion in three patients over the dose range 13 to 270 mg/m² (daily dose).

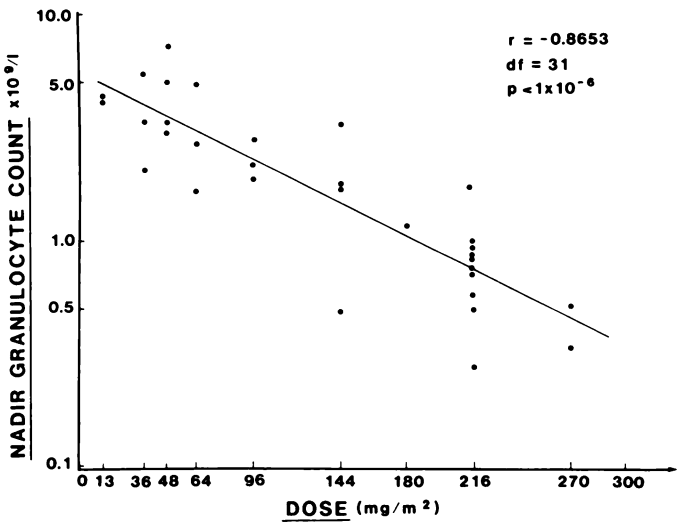

Fig. 3. The relationship between toxicity, as defined by AGC nadir, and daily dose.

irritation was common, but was overcome to some extent by the use of larger infusion volumes. Although three patients developed thrombophlebitis at the higher doses, ulceration did not result and resolution was rapid. No sequelae resulted in two patients with inadvertent extravasation of drug during infusion, suggesting that CI-921 is not a vesicant. There was minimal toxicity in other organ systems at the doses used.

The number of patients required to determine the maximum tolerated dose and the duration of the trial were minimized firstly by escalating drug dose in the same patient and secondly by omitting dose levels already proven to be nontoxic at another Phase I trial center (6, 10). The maximum tolerated dose of 810 mg/m² was determined by the development of neutropenia, thrombophlebitis, and oral toxicity. In contrast, in a parallel Phase I study at another center when CI-921 was given in a single dose repeated every three weeks, the dose-limiting toxicity was reversible renal toxicity and myelosuppression at 648 mg/m² (6).

CI-921 is easier to administer than amsacrine and does not require the use of organic solvents. It also appears to be less toxic than amsacrine when administered at the same dose and schedule (11). This may be related to differences in physicochemical and pharmacokinetic properties. Both drugs are highly protein bound in plasma (12), but the proportion of unbound drug is significantly less for CI-921 (0.3%) than for amsacrine (3.0%). CI-921 has a greater volume of distribution than was determined for amsacrine in a Phase III trial (8). Its clearance is also proportionately greater, giving a terminal half-life about one-half that of amsacrine (8). However, the limit of detection of CI-921 in this assay (50 nmol/liter) may have obscured additional components to the pharmacokinetic profile at later time points. The use of a more sensitive assay would not contribute significantly to the area under the concentration-time curve, but could alter the determination of the terminal half-life.

Toxicity as defined by bone marrow suppression (nadir granulocyte count) correlated well with pharmacokinetic parameters of drug exposure (Fig. 3). A total dose of 648 mg/m² (216 mg/m² × 3) is recommended for Phase II studies in this schedule. Such a study is currently being undertaken in non-small cell lung cancer.

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