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ORIGINAL ARTICLE

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Phase I study of AG 331, a novel thymidylate synthase inhibitor, in patients with refractory solid tumors

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Abstract *Purpose*: This was a phase I study of AG 331 to determine systemic tolerance and pharmacokinetics following single and multiple escalating intravenous doses. *Methods*: The study was an open-label phase I trial that was divided into two components. In phase IA (single dose), six dose levels from 12.5 to 225 mg/m^2 were administered to 18 patients (3 at each dose level) and serial blood samples were collected for 72 h. Upon achieving satisfactory pharmacologic parameters, the multiple dosing component (phase IB) was initiated. Six dose levels from 50 to 800 mg/m^2 per day were administered for 5 consecutive days to 18 patients. Pre- and postdose blood samples were obtained on days 1–4 and serial blood samples were collected over 24 h following dose 5. Nonhematologic and hepatic toxicities were assessed, serum AG 331 concentrations were measured and pharmacokinetic parameters determined. *Results*: Other than fatigue, no severe toxicities were encountered in phase IA. Liver toxicity was manifested by elevations in transaminase first noted at multiple doses of 200 mg/m^2 per day for 5 days. Fever and malaise but no myelosuppression were noted. The mean terminal $t_{1/2}$ following single doses was significantly shorter than the $t_{1/2}$ following multiple dosing (6.8 vs 9.9 h) and clearance was significantly faster following single doses than following multiple dosing (81.7 vs 30.4 l/h), but no significant difference in V_d was noted. *Conclusions*: The dose-related toxicity profile precludes further clinical development at this time. The pharmacokinetics of AG 331 following single and multiple doses showed significant differences.

Key words AG 331 · TS inhibitor · Toxicity · Pharmacokinetics

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Introduction

Thymidylate synthase (TS), a critical enzyme in the biosynthesis of DNA [1], catalyzes the methylation of deoxyuridine monophosphate (dUMP) to form thymidine monophosphate (TMP) which is converted to the triphosphate (TTP) prior to incorporation into DNA. TTP is the only nucleotide specifically required for DNA synthesis.

5-Fluorouracil (5-FU), the classic TS inhibitor [8], is biotransformed to fluorodeoxyuridine (FdUrd) which is phosphorylated to form fluorodeoxyuridine monophosphate (FdUMP). FdUMP competes with deoxyuridine monophosphate (dUMP) for binding to TS, forming a stable covalent bond which inhibits the enzyme, and consequently, DNA synthesis.

A novel approach in the development of new TS inhibitors is the rational design of drugs based upon the three-dimensional structure of the active site on TS determined from X-ray crystallography [9]. AG 331 [2, 4, 10] is one of several compounds synthesized as a result of this effort. AG 331, *N*6-[4-(morpholinylsulfonyl)ben-

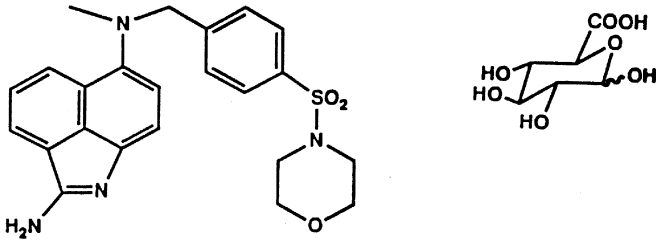

Fig. 1 Chemical structure of AG 331, *N*6-[4-(morpholinylsulfonyl)benzyl]-*N*6-methyl-2,6-diaminobenz[*cd*]indole glucuronate

zyl-*N*6-methyl-2,6-diaminobenz[*cd*]indole glucuronate (Fig. 1), is a novel TS inhibitor that differs from classical antifolates by the lack of a glutamate moiety. This lack of a glutamate obviates the need for specific cellular transport mechanisms for uptake and its greater lipophilicity may facilitate nonspecific cellular uptake and enhance penetration into tumors.

In vitro studies have shown that AG 331 is a potent inhibitor of purified human TS (K_i = 0.4 n*M*) and exhibits an IC_{50} for cellular growth inhibition in the range 0.37–10.55 μ*M* depending on cell type. Antitumor activity has been seen in vivo against a thymidine kinase-deficient variant of murine L5178Y implanted intraperitoneally [6]. Toxicities observed in animals include mild to moderate local vein irritation at the injection site, transient weight loss, and flushing and swelling of extremities [10]. Acute single intravenous (i.v.) dose toxicity studies in mice have resulted in a "no observable adverse effect" dose of 35 mg/kg and a maximum tolerated dose (MTD) of 75 mg/kg.

The pharmacokinetics of AG 331 have been studied in several animal species with terminal $t_{1/2}$ values ranging from 2–4 h in mice to 4–16 h in dogs [7, 10]. The pharmacokinetics in dogs have been determined following administration of a single 60 mg/kg i.v. dose and with repeated doses of 5, 15, and 45 mg/kg per day given over 5 days. The results showed that the volume of distribution, V_d, is substantially greater than the total blood volume, which is not unusual in view of the high lipophilicity of the compound. Single and repeat dose studies in dogs have given an MTD of 60 mg/kg. In a repeat i.v. dose study, the "no observable adverse effect" dose was determined to be 5 mg/kg per day.

The objectives of this phase I trial were to determine systemic and local tolerance following single and multiple doses of AG 331 and to investigate its pharmacokinetics following single and multiple escalating i.v. doses administered as short infusions in patients with refractory solid tumors.

Patients and methods

The study protocol was approved by the Investigational Review Board of the Los Angeles County/USC Medical Center and was conducted in conformance with the ethical standards of the Declaration of Helsinki and its amendments. The investigational nature of the study was discussed with each patient and each was required to provide written informed consent.

Patients

Patients at least 18 years of age with histologically confirmed solid tumors who failed conventional therapy or whose disease was considered refractory to standard chemotherapeutic regimens, or for whom no effective therapy existed, were admitted into the study. Additional inclusion criteria included (1) absolute granulocyte count ≥1500/mm^3 and platelet count ≥100 000/mm^3, (2) adequate renal function with serum creatinine ≤1.5 mg/dl, (3) adequate liver function with serum bilirubin ≤1.5 mg/dl, AST and ALT not more than three times the institutional upper limit of normal, (4) a Karnofsky performance status ≥70, and (5) no radiotherapy or chemotherapy for 3 weeks prior to entering the study, full recovery from acute effects of any prior chemotherapy or radiotherapy, and no nitrosoureas or mitomycin-C for 6 weeks prior to entering the study. Fertile men and women were advised to use adequate contraception, and for women of childbearing potential, a negative pregnancy test was confirmed at the baseline visit.

Patients with an active uncontrolled infection, or history of myocardial infarction within the previous 6 months, those with current clinical evidence of congestive heart failure, or those unable to give informed consent, were excluded from the study.

Drug administration

This was an open-label phase I trial of AG 331 that was structured in two phases. Phase IA (single dose) was conducted to determine the maximum nontoxic dose of AG 331 and the pharmacokinetics following single escalating doses. The results of phase IA were used to determine the starting dose for the multiple dose phase (phase IB). The dose escalation schedules used in the single and multiple dose phases of the study are shown in Table 1. At baseline, a history and physical examination, vital signs, performance status, complete blood count (CBC), differential, platelet count, electrocardiogram, chest radiograph, urinalysis and clinical chemistries, were obtained. Appropriate radiologic examinations were obtained in patients with measurable disease.

In both phases of the study, the drug was administered after reconstitition in normal saline, yielding a deep purple solution. In phase IA, patients were administered a single dose of AG 331 delivered as a 10-min i.v. infusion. Six dose levels (three patients at each dose level) were investigated: 12.5, 25, 50, 75, 130, and 225 mg/m^2. Escalation to the next higher level was initiated only after all patients enrolled at the current dose had completed at least one full

Table 1 AG 331 dose escalation schedules

Level	Number of patients enrolled	Dose (mg/m^2)
Phase IA (single dose)		
I	3	12.5
II	3	25.0
III	3	50.0
IV	3	75.0
V	3	130.0
VI	3	225.0
Phase IB (multiple dose[a])		
I	3	50.0
II	3	100.0
III	3	200.0
IV	3	400.0
V	3	600.0
VI	4	800.0

[a] Doses per day for 5 days

course of treatment followed by a 2-week observation period. For pharmacokinetic studies, serial venous blood samples were collected at time O (predose baseline) and at 15 and 30 min, and 1, 2, 3, 4, 5, 6, 7, 8, 12, 18, 24, 48 and 72 h postdose. Total urinary output was collected for 48 h following dosing. During the first dose, vital signs were monitored every 15 min. CBC and differential, platelet count and clinical chemistries were obtained weekly. Prior to each course, a brief physical examination, CBC, differential, platelets, clinical chemistries and urinalysis, were obtained. Tumor measurements were repeated every 4–8 weeks. Toxicities were graded in accordance with the NCI Common Toxicity Grading Criteria and dose modifications initiated in the event of any toxicities greater than grade 2. Any observed grade 3 or 4 toxicity other than fatigue or anemia, would prompt expansion to the entry of three additional patients at that dose level. Dose escalation was halted once the MTD was established. The MTD was defined as the development of grade 4 hematologic toxicity or grade 3 or 4 nonhematologic toxicity in two or more of six patients at a dose level. No dose escalations were carried out within individual patients.

The multiple dose component of the study (phase IB) was initiated at one-fifth of the maximum dose used in the single-dose phase phase IA. Six dose levels (three patients at each dose level) were investigated: 50, 100, 200, 400, 600 and 800 mg/m^2 per day administered as a 60-min i.v. infusion for 5 consecutive days. This was repeated every 4 weeks as long as there was no disease progression or toxicity that failed to return to baseline hematologic and blood chemistry values. Consistent with phase IA, dose escalation to the next higher level was initiated only after all patients enrolled at the current dose level had received at least one full course of treatment followed by a 2-week observation period. Any observed grade 3 or 4 toxicities, other than fatigue or anemia, would prompt expansion to the entry of three additional patients at that dose level. Dose escalation was halted once the MTD was established.

On day 1 through day 4, a venous blood sample was obtained predose (trough) and a second sample immediately after completion of the 60-min infusion (peak). On day 5, serial blood samples were collected at 0 (predose trough) and at 30 min, and 1, 2, 4, 8, 12 and 24 h postdose. Total urinary output was collected over 24 h.

Assay methodology

All collected blood specimens were allowed to clot, centrifuged and the serum transferred to freezer vials. Urine volume was measured and a 100-ml aliquot was saved. Serum and urine specimens were frozen and stored at −70°C until assayed. AG 331 was determined using a previously published HPLC method [5]. Serum and urine samples were extracted with diethyl ether, the organic phase was evaporated to dryness and reconstituted in mobile phase, then injected into the HPLC system. The method was linear over the range 50–2000 ng/ml with a limit of detection of 20 ng/ml and a quantifiable limit of 50 ng/ml. The method was validated for linearity, precision and accuracy.

Pharmacokinetic analysis

Serum concentration-time data were fitted to an appropriate pharmacokinetic model using the polyexponential curve-fitting program, RSTRIP, version 4.05 (MicroMath Scientific Software, Salt Lake City, Utah) and ADAPT II (Biomedical Simulations Resource, University of Southern California, Los Angeles, Calif.). Pharmacokinetic parameters such as the terminal elimination rate constant, β, maximum serum concentration, C_{max}, and area under the serum concentration-time curve, AUC_{0-24} were obtained. In addition, total clearance, Cl_{tot}, and the volume of distribution, V_d, were calculated as:

$$Cl_{tot} = \text{dose}/[\text{AUC}]$$

$$V_d = Cl_{tot}/\beta$$

Results

A group of 37 patients were enrolled in the study receiving a total of 100 courses (range 1 to 11 courses) of AG 331. Patient demographics are shown in Table 2. The majority of patients (78%) had received prior chemotherapy, primarily 5-fluorouracil (5-FU), since tumors of gastrointestinal origin predominated. Those patients who had received more than one prior chemotherapeutic regimen had been treated with 5-FU alone and subsequently in combination with other agents including leucovorin, cisplatin, mitomycin or levamisole. Five patients were removed from the study (because of symptomatic progression or refusal) prior to any assessment leaving 32 evaluable patients.

Toxicities

In the single-dose component (phase IA) of the study, the results shown in Table 3 indicate that AG 331 was well tolerated up to and including a dose of 225 mg/m^2. No myelosupression nor grade 3 toxicity was observed. However, nausea, vomiting and a metallic taste emerged at 75 mg/m^2. Other toxicities included purplish urine (one patient), and skin rash (three patients) that was accompanied by edema and erythema at the infusion site (first noted at 130 mg/m^2).

Table 4 and 5 show the toxicities that were observed during the multiple dose component (phase IB). The most frequently encountered toxicities were fatigue, nausea and vomiting, and elevation of liver function

Table 2 Demographics of study patients

Number of Patients	
Total	37
Male/female	20/17
Age (years)	
Mean	57.9
SD	11.3
Weight	
Mean	69.5
SD	17.5
Ethnic origin	
Caucasian	23
Hispanic	5
African American	4
Asian	4
Other	1
Primary tumor	
Colorectal	23
Pancreas	4
Stomach	2
Unknown primary	2
Gallbladder	2
Esophagus	2
Larynx	1
Soft tissue sarcoma	1

Table 3 Frequency and severity of nonhematologic toxicity (NCI Common Toxicity Grading Criteria) following single doses (phase IA) of AG 331 (three patients at each dose level)

Single dose (mg/m^2)	Fatigue				Nausea				Vomiting				Fever				Diarrhoea			
	Grade				Grade				Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
12.5	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0
25	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
50	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
75	0	0	0	0	2	0	0	0	2	0	0	0	1	0	0	0	1	0	0	0
130	1	0	0	0	0	0	1	0	0	1	1	0	2	0	0	0	0	1	0	0
225	1	0	0	0	2	0	0	0	2	0	0	0	1	0	0	0	1	0	0	0

Table 4 Frequency and severity of nonhematologic toxicity (NCI Common Toxicity Grading Criteria) following multiple doses (phase IB) of AG 331 administered as a 60-min i.v. infusion daily for 5 days

Dose (mg/m^2)	No. of patients	Nausea/vomiting				Fatigue				Fever				Anemia			
		Grade				Grade				Grade				Grade			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
50	3	0	0	1	0	2	0	0	0	1	0	0	0	0	0	3	0
100	3	0	1	1	0	0	0	1	0	2	0	0	0	1	1	1	0
200	3	1	1	0	0	0	1	1	0	1	0	0	0	2	0	0	0
400	3	2	1	0	0	0	1	0	0	1	0	0	0	1	2	0	0
600	3	0	3	0	0	1	1	0	0	0	0	0	0	1	2	0	0
800	4	0	2	2	0	0	2	2	0	0	1	2	0	0	2	1	0

Table 5 Frequency and severity of liver toxicity (NCI Common Toxicity Grading Criteria) following multiple doses (phase IB) of AG 331 administered as a 60-min i.v. infusion daily for 5 days

Dose (mg/m^2)	Bilirubin				AST (SGOT)				ALT(SGPT)				Alkaline phosphatase			
	Grade				Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
200	0	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0
400	0	0	0	0	0	0	0	0	1	0	0	0	2	1	0	0
600	0	0	0	0	1	0	0	0	0	1	1	0	1	2	0	0
800	0	1	0	1	1	0	0	0	1	1	0	0	1	1	1	0

tests. The first hepatotoxic event occurred at a dose of 200 $mg/m^2 \times 5$ in a patient with metastatic laryngeal carcinoma and chronic hepatitis-C with abnormal baseline liver function tests. The patient had been previously treated with liposomal doxorubicin which was tolerated with minimal elevations in transaminase. Other cases of transaminase elevation but of lesser severity were observed at higher doses (Table 5). Other toxicities include reports of purplish urine (six patients) and metallic taste (five patients). No patients experienced myelosuppression.

Although no clinical responses were observed in either phase of the trial, seven patients achieved stable disease with a median duration of 5.5 months (range 3–11 months).

Pharmacokinetics

The mean AG 331 serum concentrations versus time following single escalating doses are shown in Fig. 2. Individual patient pharmacokinetic parameters were determined and are summarized in Table 6. The mean terminal $t_{1/2}$ (0–24 h) for all doses of AG 331 was 6.8 ± 1.9 h (range 3.7–10.4 h), and the mean clearance was 81.7 ± 25.0 l/h (range 8.4–119.0 l/h). The mean V_d was 8.1 ± 2.7 l/kg (range 3.7–12.9 l/kg). Only 3.2 ± 1.2% of the dose of AG 331 was recovered from the urine unchanged over a 48-h period.

Mean serum concentration-time profiles for patients at each multiple dose level are presented in Fig. 3. At each dosing level, the first four doses of the five-dose

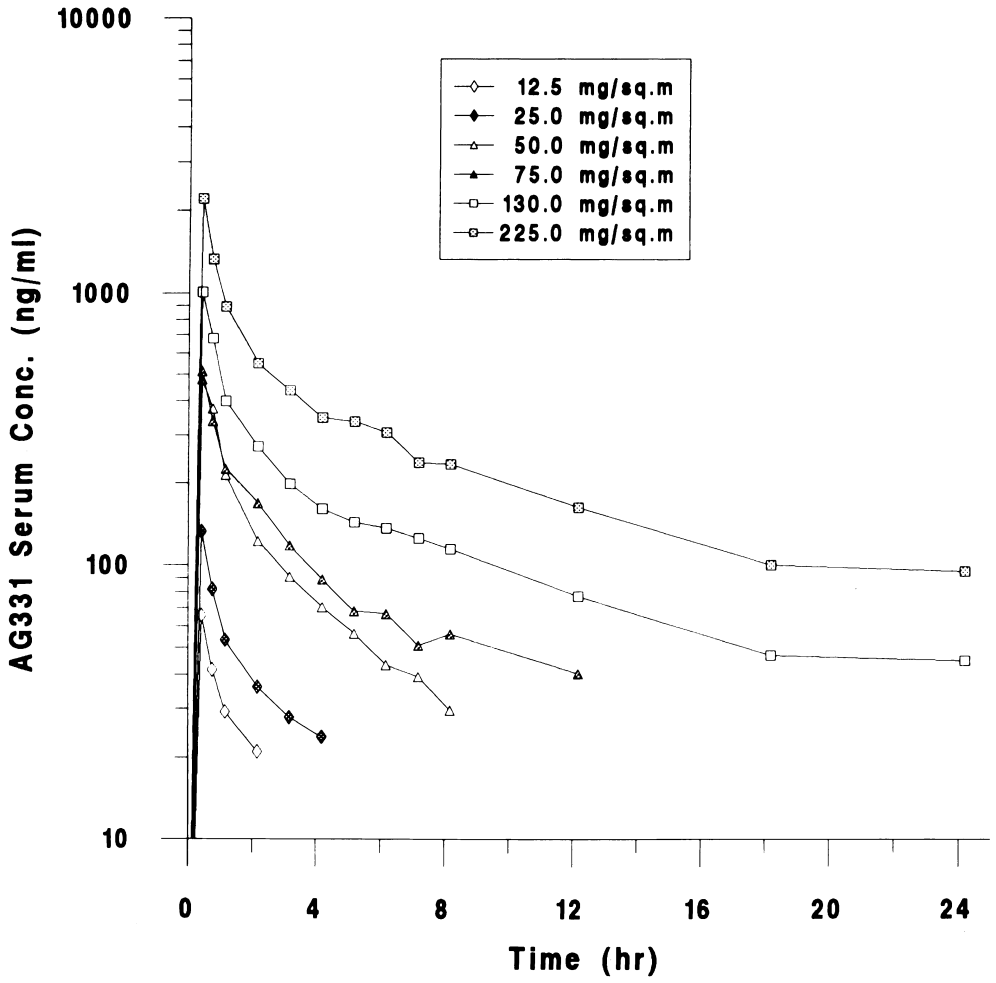

Fig. 2 Mean serum concentration-time profiles for AG 331 following single i.v. doses

Table 6 AG 331 pharmacokinetic parameters following single escalating doses

Patient	Dose (mg/m^2)	Actual dose (mg)	$t_{1/2}\beta$ (h)	AUC_{0-24} ($\mu g \cdot h/l$)	V_d (l/kg)	Cl_{tot} (l/h)
GL	12.5	21.5	7.3	297	12.3	72.4
LS	12.5	23.5	8.1	237	12.9	99.0
RA	12.5	24.3	5.6	310	7.4	8.4[a]
WB	25.0	46.5	5.4	423	8.3	110.0
CG	25.0	48.0	6.7	444	11.2	108.0
EM	25.0	52.1	8.2	476	11.6	109.5
IM	50.0	92.0	6.6	2031	3.7	45.3
MN	50.0	91.5	3.7	878	6.0	104.2
CB	50.0	94.0	7.5	1042	8.8	90.2
RD	75.0	125.0	3.9	1660	5.9	75.3
MR	75.0	139.0	4.0	1463	6.2	95.7
MY	75.0	165.0	9.1	1542	4.9	92.7
RS	130.0	226.0	5.7	3504	4.8	60.5
BO	130.0	273.0	10.4	3602	9.0	64.5
KD	130.0	286.0	6.9	2328	9.5	119.0
HM	225.0	340.0	9.3	7166	9.4	41.0
AP	225.0	337.5	6.7	6554	7.5	50.4
CH	225.0	350.0	6.7	6839	6.4	51.8
Mean ± SD			6.8 ± 1.9		8.1 ± 2.7	81.7 ± 25.0

[a] Outlier; not used in computation of mean

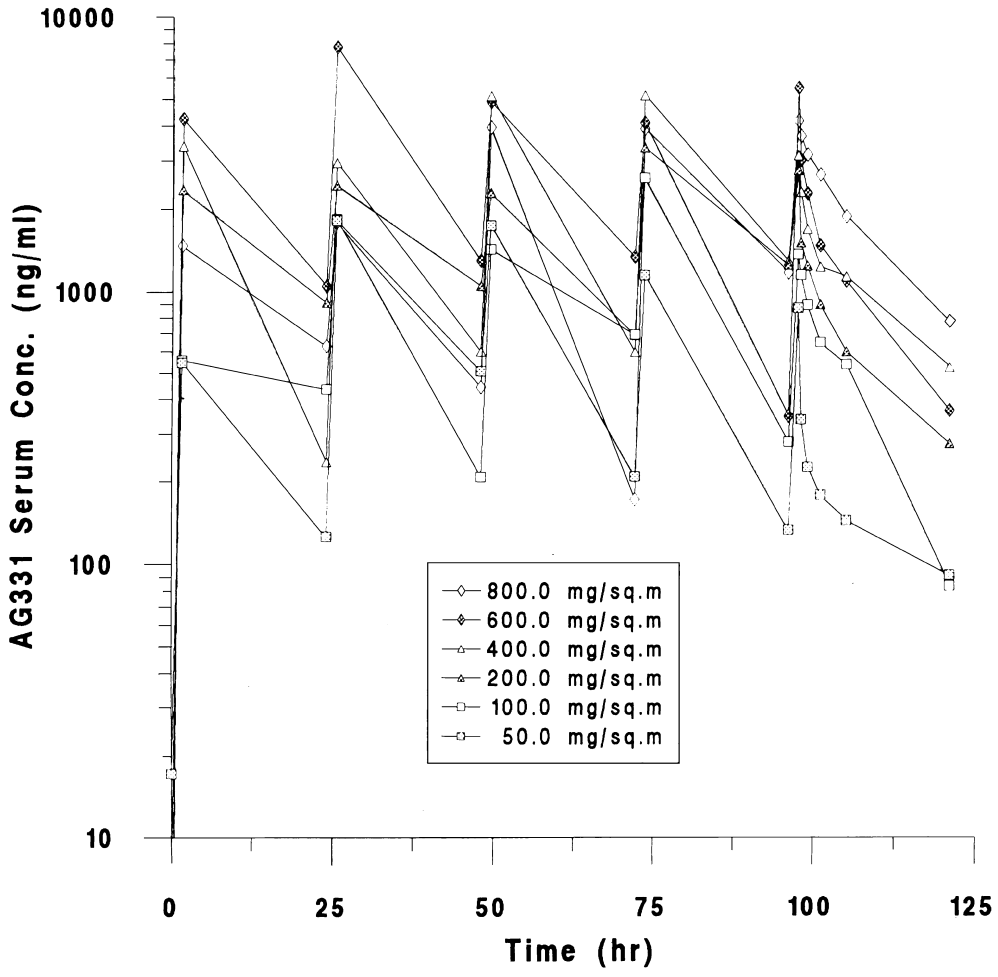

Fig. 3 Mean serum concentration-time profiles for AG 331 following multiple doses

Table 7 AG 331 pharmacokinetic parameters following multiple escalating doses (day 5)

Patient	Dose (mg/m^2)	Actual dose (mg)	$t_{1/2},\beta$ (h)	$AUC_{0-24\ h}$ ($\mu g \cdot h/l$)	V_d (l/kg)	Cl_{Tot} (l/h)
KF	50	110.0	13.3	2986	7.6	36.8
HS	50	80.5	12.1	2869	8.5	28.1
RM	50	103.0	15.0	5148	5.2	20.0
WS	100	150.0	7.5	2970	11.2	50.5
LS	100	186.0	5.0	25469	0.7[a]	7.3[a]
MG	100	179.0	4.5	1372	11.3	130.5[a]
PC	200	316.0	7.7	12370	5.3	25.6
SP	200	264.0	11.2	11930	8.6	22.1
TD	200	342.0	9.2	11207	6.1	30.5
BB	400	800.0	12.5	25156	6.3	31.8
WE	400	656.0	9.5	19081	8.3	34.4
BD	400	640.0	12.0	35698	5.4	17.9
RJ	600	960.0	8.7	62651	3.5	15.3
WW	600	1350.0	12.8	37771	6.2	35.7
RP	600	1056.0	8.3	19757	10.3	53.4
MG	800	1100.0	6.4	31296	7.1	35.1
MC	800	1112.0	14.6	66029	8.2	16.8
WL	800	1232.0	7.4	34847	6.3	33.0
Mean ± SD			9.9 ± 3.2		7.4 ± 2.2	30.4 ± 11.0

[a] Outlier; not used in computation of means

regimen are shown as predose troughs and postinfusion peaks. Figure 3 also shows the 0–24-h serum level-time profile following the fifth (final) dose. Individual patient and mean pharmacokinetic parameter values are shown in Table 7. The day-5 mean $t_{1/2}$ (0–24 h) for all doses administered in the multiple dosing component was 9.9 ± 3.2 h (range 4.5–15.0 h). The mean clearance was 30.4 ± 11.0 l/h (range 7.3–130.5 l/h) and the mean V_d was 7.4 ± 2.2 l/kg (range 0.7–11.3 l/kg).

Discussion

AG 331 is a novel lipophilic (log P = 3.8) TS inhibitor that has shown potent human TS inhibition in vitro (K_{is} = 1.2 n*M*). This phase I study was initiated with a single 12.5 mg/m^2 dose administered as a 10-min i.v. infusion and escalated to 225 mg/m^2 without demonstrating greater than grade 2 systemic toxicities. Local irritation at the injection site necessitated administration through a central venous access line for single doses of ≥100 mg/m^2 and for all multiple doses. In addition, the infusion period was increased from 10 min to 30 min for multiple doses. The central line was also used to obtain blood samples for pharmacokinetic studies to reduce the trauma of obtaining blood samples from a peripheral vein over a 5-day period. Although the line was carefully flushed with saline, some residual drug may have remained in the line when blood samples were obtained resulting in analytical results that could be unexpectedly high and variable. This made fitting of the serum level-time profile to obtain pharmacokinetic parameters more difficult.

Although the single-dose MTD was not achieved at a single dose level 6 (225 mg/m^2), plasma levels were judged to be sufficient for TS inhibitory effects and the multiple dosing phase (phase IB) was initiated starting at approximately one-fifth the maximum dose used in phase IA and were escalated in six steps to a dose of 800 mg/m^2 per day for 5 days. Hepatic toxicity first became evident at a dose of 200 mg/m^2 per day for 5 days and was again noted at 600 and 800 mg/m^2 per day. Hepatotoxicity was also the dose-limiting toxicity of AG 331 in a study conducted at Fox Chase Cancer Center using a 5-day continuous infusion schedule [3]. In that study, 22 patients were treated at doses ranging from 25 to 800 mg/m^2 per day. Reversible elevations of bilirubin and transaminases with accompanying fever and malaise were observed at doses greater than 400 mg/m^2 in most patients.

In the single-dose component (phase IA), blood specimens were collected over a 72-h period following dosing. At doses of ≤75 mg/m^2, the serum concentration could be determined for only 2–12 h after dosing because serum levels were below the lower limit of the analytical method. However, at higher doses, serum levels could be monitored for at least 24 h postdose. Even at the highest single dose (225 mg/m^2), the peak serum level was only 2.2 μg/ml. This suggests that AG 331 is rapidly distributed outside the vascular compartment or bound to vascular tissue or may be rapidly metabolized. The individual patient serum levels as well as the derived pharmacokinetic parameters showed substantial variability. This is not surprising considering that the study was conducted in patients with varying degrees of disease progression.

Individual patient serum concentration-time data could be adequately fitted to a two-compartment pharmacokinetic model. Model selection was based upon the number of postinfusion data points available and using the Akaike Information Criterion. Thus, the $t_{1/2}$ values that were obtained may not reflect the terminal $t_{1/2}$ of AG 331, but may be an intermediate or hybrid $t_{1/2}$. The $t_{1/2}$ of (0–24 h) from the single-dose phase was significantly different ($P < 0.05$) from the $t_{1/2}$ obtained from the multiple-dose phase. This difference may be attributed to differences in sampling time and the inability to measure serum concentrations for longer periods following lower single doses.

The mean clearance of 81.7 ± 25.0 l/h following single doses was significantly different ($P < 0.05$) than the mean day-5 clearance of 30.4 ± 11.0 l/h following the multiple dosing regimen. The difference between the mean apparent volume of distribution 8.1 ± 2.7 l/kg) following single doses was not statistically significant ($P > 0.05$) from the mean volume of distribution (7.4 ± 2.2 l/kg) obtained following multiple doses. AG 331 is highly lipophilic and may be sequestered in tissues outside the vascular space. With repeated doses, peripheral binding sites may become saturated, which may explain the higher clearance rate and volume of distribution following single doses.

Some drug accumulation occurred with repeated doses reaching a maximum level on day 3 of the 5-day regimen, then either plateauing or, in some cases, decreasing slightly. No trend or changes in pharmacokinetic parameters were observed with dose escalation in either the single- or multiple-dosing regimen. Only a small fraction of the administered dose was recovered from the urine, indicating that AG 331 may undergo extensive metabolism.

The results of this phase I trial provide some interesting information on the pharmacokinetics of AG 331 following single and multiple escalating doses. However, its toxicity profile coupled with practical issues requiring central venous access make AG 331 unattractive for further clinical development at this time.

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