Phase I Study of the Duocarmycin Semisynthetic Derivative KW-2189 Given Daily for Five Days Every Six Weeks[1]

Steven R. Alberts,[2] Charles Erlichman, Joel M. Reid, Jeff A. Sloan, Matthew M. Ames, Ronald L. Richardson, and Richard M. Goldberg

Divisions of Medical Oncology [S. R. A., C. E., R. L. R., R. M. G.] and Developmental Oncology Research [J. M. R., M. M. A.], and Cancer Center Statistics Unit [J. A. S.], Mayo Clinic, Rochester, Minnesota 55905

ABSTRACT

The duocarmycins represent a new group of antitumor antibiotics produced by *Streptomyces* that bind to the minor groove of DNA. KW-2189 is a water-soluble semisynthetic derivative of duocarmycin B_2, with significant activity in murine and human tumor models. We conducted a Phase I trial of KW-2189 in patients who had solid tumors that were refractory to standard chemotherapy or for whom no more effective therapy existed. KW-2189 was administered as a rapid i.v. bolus daily for 5 days every 6 weeks. Twenty-two patients were enrolled and received a total of 31 cycles of KW-2189. Leukopenia, neutropenia, and thrombocytopenia were the dose-limiting toxicities, with nadirs occurring at medians of 36, 38, and 29 days, respectively, at the 0.04 mg/m²/day dose level. Nonhematological toxicities were mild, although one patient developed grade 3 fatigue. Four patients had stable disease over two to four cycles of treatment and showed no cumulative toxicity. The mean $t_{1/2}$, plasma clearance, and steady-state volume of distribution were 13.5 min, 1,287 ml/min/m², and 10,638 ml/m², respectively. Pharmacokinetics were similar on days 1 and 5, with no drug accumulation in plasma. The active metabolite DU-86 was not consistently found in patient plasma. For Phase II trials, when the 5 days every 6 weeks schedule was used, 0.04 mg/m²/day KW-2189 appears to be the maximal tolerated dose, especially for patients who have received prior chemotherapy. At this dose level, the drug was well tolerated, and the toxicities were acceptable.

INTRODUCTION

The DUMs[3] represent a new group of antitumor antibiotics produced by *Streptomyces* (1–4) that exert their antitumor activity through sequence-specific covalent binding to the minor groove of DNA (5). The DNA-alkylating activities of DUM appear to be similar to those of other minor groove-binding agents, including CC-1065 and its analogues (6–8). Following binding to the minor groove of DNA, the DUMs cause DNA fragmentation (9). All seven of the DUMs currently described exhibit antitumor activity *in vitro* (1, 3, 4, 10). However, the clinical use of the DUMs have been limited by their instability in aqueous solutions and in serum (11).

KW-2189, a semisynthetic derivative of DUM B_2, is a water-soluble compound (12) that contains a carbamoyl moiety that is enzymatically cleaved *in vivo* (13) to produce the 1000-fold more active metabolite, DU-86. Recent studies suggest that carboxyl esterase activates KW-2189 by metabolizing its *N*-methyl-piperazine side chain (Fig. 1; Ref. 14). It also appears that KW-2189 itself is able to covalently bind to DNA without the loss of the carbamoyl moiety (15). However, its *in vitro* activity is less than that of DU-86 or DUM B_2 (13, 16). Studies with HeLa S_3 cells have shown that KW-2189 and DU-86 are unique from the other DUMs in that they do not appear to cause DNA fragmentation (17), except with prolonged exposure (18). Instead, their primary action is alkylating adenine in the N3 position, leading to an S-phase arrest (13, 17), in a manner similar to the CC-1065 analogues (19).

In preclinical studies, KW-2189 demonstrated significant antitumor activity (13). KW-2189 produced significant tumor REGR against a variety of murine solid tumors, including B-16 melanoma and M5076 sarcoma. Significant activity was also seen in a series of human tumors xenografts, including lung, stomach, colon, liver, pancreas, and breast cancers, that were inoculated into nude mice, many of which were drug insensitive.

Given its antitumor activity and water solubility, KW-2189 was selected for clinical trials. Here, we report the results of a Phase I trial involving the administration of KW-2189 daily for 5 days every 6 weeks to patients with solid tumors that were refractory to standard chemotherapy.

PATIENTS AND METHODS

Patient Selection. Patients who had solid tumors that were refractory to standard chemotherapy or for whom no more effective alternative therapy existed were eligible for entry into this trial. The patients had Eastern Cooperative Oncology Group performance scores of ≤2 and life expectancies of >12 weeks. The inclusion criteria included: absolute neutrophil count of ≥1,500/mm³, platelet count of ≥100,000/mm³, bilirubin within

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[1] Supported in part by a grant from Kyowa Pharmaceutical, Inc. (New York, NY).
[2] To whom requests for reprints should be addressed. Phone: (507) 284-8964; Fax: (507) 284-1803.
[3] The abbreviations used are: DUM, duocarmycin; REGR, regression; AST, aspartate aminotransferase; CBC, complete blood count; DLT, dose-limiting toxicity; MTD, maximal tolerated dose; CR, complete response; PR, partial response; PROG, progression; HPLC, high-performance liquid chromatography; MS, mass selection; AUC, area under the plasma concentration *versus* time curve; Cl, plasma clearance; V_{ss}, steady-state volume of distribution.

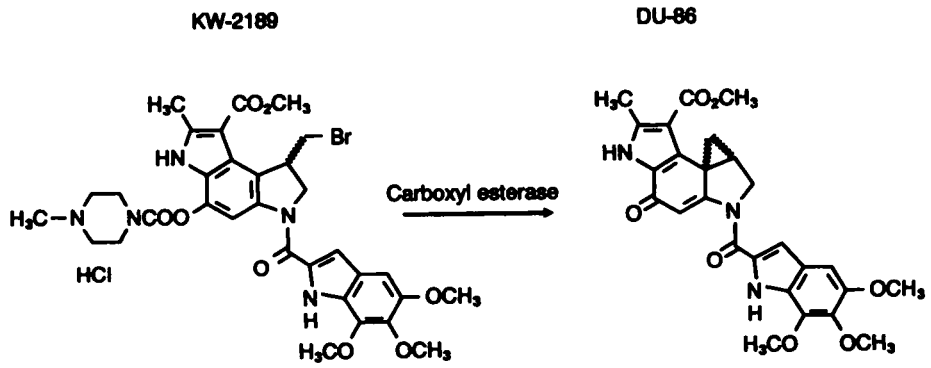

Fig. 1 Conversion of KW-2189 to its major metabolite DU-86 by carboxyl esterase.

the institutional normal limits, AST levels of <3 times the upper limit of normal, creatinine levels of ≤0.3 mg/dl above the upper limit of normal, and negative pregnancy tests for women of childbearing potential. Pregnant or lactating women were restricted from entry into the trial. Patients were excluded if they had undergone major surgery or received immunotherapy, chemotherapy, or radiation therapy within 4 weeks of registration or if >25% of their bone marrow had been irradiated. Patients who had uncontrolled infections, chronic debilitating diseases, New York Heart Association class 3 or 4 heart disease, or central nervous system metastases were also excluded.

Pretreatment Evaluation and Follow-up Studies. A history and physical examination was performed prior to registration and before each subsequent course of treatment. Laboratory studies at the time of registration included: a CBC; tests for levels of sodium, potassium, calcium, phosphorus, total protein, glucose, alkaline phosphatase, AST, total bilirubin, uric acid, creatinine, and albumin; and urinalysis. Electrocardiograms and chest X-rays were also performed at registration. Chemistries and CBCs were repeated prior to each subsequent cycle of therapy. Twice-weekly CBCs and weekly creatinine, AST, and alkaline phosphatase levels were obtained between cycles. Measurements of an indicator lesion were performed within 2 weeks of registration and repeated with each of the subsequent three cycles of therapy. After three cycles of therapy, measurements were performed with alternate cycles.

Drug Administration. KW-2189 (Kyowa Pharmaceutical, Inc., New York, NY) was stored as a freeze-dried preparation. Each vial, containing 1 mg of KW-2189, was reconstituted with 10 ml of D_5W prior to administration. Reconstituted drug was stored at 10°C until administration to reduce hydrolysis, which occurs at a rate of 4%/h at room temperature. Following completion of the preregistration tests and evaluations and after informed consent was obtained, KW-2189 was administered daily as a rapid i.v. bolus over 30–60 s via a side port of a free-flowing 5% dextrose in water infusion for 5 days. The dose escalation scheme is shown in Table 1. Cycles of treatment were repeated every 6 weeks if patients met the criteria for retreatment.

Study Design and Toxicity Criteria. Three patients were entered at each predetermined dose level. Dose escalation was not permitted until all three patients at each predetermined dose level had completed a minimum of 6 weeks of follow-up.

Table 1 Dose escalation scheme for the Phase I trial of KW-2189

Level	Dose (mg/m²/day × 5 days)	No. of patients	No. of cycles
−1	0.03	3	3
1	0.04	9	16[a]
2	0.06	10	12[b]

[a] One patient received three cycles at 0.036 mg/m²/day following a dose reduction after the first cycle.

[b] One patient received one cycle at 0.03 mg/m²/day following a dose reduction after the first cycle.

Dose escalation was not permitted in any one patient. DLTs were defined using the Mayo/National Cancer Institute Common Toxicity Criteria as reversible grade 4 hematological toxicity, whereas the nonhematological toxicities were considered dose limiting if their grades were ≥3, with the exception of nausea and vomiting (grade of 4 was dose limiting) and neurological toxicity (grade of ≥2 was dose limiting). If DLT was seen in two of three patients at a given dose level, then that dose level was considered the MTD. If DLT was seen in one of three patients at a given dose level, then three additional patients were enrolled at that dose level. If two or more of the six patients showed DLT, then that dose level was considered the MTD. If the above criteria were not met, then three patients were enrolled in the next higher dose level. Dose de-escalation was also permitted to define the MTD. Once the MTD was reached, three additional patients were enrolled at that dose level to better define the frequency and severity of toxicities. If a patient failed to complete all 5 days of treatment or the subsequent 37 days of observation, they were replaced with another patient for the purposes of determining the MTD. If a patient developed DLT but showed evidence of response to KW-2189 or had stable disease, repeat treatment was permitted after a dose reduction. If clinically significant toxicities persisted for >3 weeks after the scheduled time of retreatment, the patient was removed from the study.

Response to treatment with KW-2189 was defined as CR, PR, REGR, stable, or PROG. A CR was defined as total disappearance of all evidence of tumor for at least one cycle of therapy. A PR was defined as a ≥50% reduction in the bidimensional measurements of the indicator lesion or a ≥30% reduction in palpable hepatomegaly. A REGR was defined as

definite decrease in the size of an evaluable lesion not meeting the criteria for a CR or PR. A PROG was defined as ≥25% increase in the bidimensional measurements of a tumor after a cycle of therapy or the appearance of new lesions. Patients had stable disease if they did not meet the criteria for CR, PR, REGR, or PROG. Patients were also removed from treatment if they had a significant clinical deterioration that could not be attributed to treatment or other medical conditions.

Pharmacokinetic Studies. Blood samples (7 ml) were drawn through a heparin lock in a peripheral vein of the arm opposite that used to administer the KW-2189. To remove heparin from the tubing, 2 ml of blood were withdrawn prior to sample collection. A prestudy blood sample was obtained prior to the drug administration. Samples were obtained on days 1 and 5 at 0, 2, 5, 10, 15, 30, 60, 90, and 120 min after completion of the infusion. On days 2–5, samples were obtained prior to treatment. Samples were cooled in an ice-water bath for 15 s and immediately centrifuged (5 min at 2500 × *g*). The plasma was transferred to an amber polypropylene tube, capped, and immediately frozen by immersion in a dry ice/ethanol bath and stored at −70°C for subsequent drug analysis.

Plasma concentrations of KW-2189 and its metabolite DU-86 were determined by a sensitive HPLC assay with MS/MS detection. Reverse-phase HPLC separations were performed on a Develosil ODS-HG-5 column (150 mm × 2.0 mm inside diameter, 5-μm particle size; Nomura Chemical) using a mobile phase that consisted of 10% 20 mM ammonium acetate (pH 6) and 90% acetonitrile delivered at a flow rate of 0.2 ml/min. The HPLC column was connected in tandem to a Fisons VG Quatro II mass spectrometer (Micromass) with a positive ion electrospray ionization source and cone voltage, dwell time, and collision energy set at 50 V, 0.2 s, and 22 eV, respectively. The mass pairs for KW-2189, DU-86, and DU-149 (internal standard) were 698/491, 492/234, and 643/436, respectively. Preparation of samples for HPLC/MS analysis involved extraction of the analytes into methyl-*t*-butyl ether from human plasma made basic by the addition of pH 9.0 phosphate buffer. After evaporation of the organic solvent, the residue was reconstituted in 100 mg/ml ascorbic acid in 9:1 acetonitrile:water. The reconstituted samples were injected (25 ml) directly into the chromatographic system. Calibration curves for KW-2189 and DU-86 were linear in the concentration range of 0.2–20 ng/ml, with correlation coefficients of >0.99 for all curves. The assay precision ranged from 3 to 10.6% for KW-2189 and DU-86. The lower limit of quantitation for the assay was 0.2 ng/ml for KW-2189 and DU-86.

Statistical Considerations. The pharmacokinetic parameters of KW-2189 were calculated by noncompartmental analysis using the program PCNONLIN Version 4.2 (Statistical Consultants, Inc.). The AUC was determined by trapezoidal approximation from the start of treatment to the last detectable plasma concentration (C_{last}), with residual area (AUC_r) after C_{last} calculated by $AUC_r = C_{last}/k_{el}$. Values of k_{el} (the terminal elimination rate constant) were calculated by linear least squares regression of the last two to five time points in the plasma concentration-time profiles. The elimination half-life was calculated by $t_{1/2} = 0.693/k_{el}$. Cl of KW-2189 was calculated by Cl = D/AUC, and V_{ss} was calculated by V_{ss} = [D·(area under the first moment/AUC^2)] − [(D·T)/(2·AUC)], where D is the dose and T is the length of infusion.

Table 2 Characteristics of the 22 patients enrolled in the Phase I trial with KW-2189

Characteristic	No. of patients
Average age	
59 yr (range, 33–72 yr)	
Sex	
Male	11
Female	11
Tumor type	
Colorectal	13
Melanoma	2
Biliary tract	1
Breast	1
Nasal cavity	1
Ovary	2
Pancreas	1
Renal	1
Prior chemotherapy (no. of regimens)	
1	7
2	3
3	5
4+	7
Prior radiation	
Yes	6
No	16
Performance status	
0	7
1	13
2	2

Data monitoring and baseline analysis were carried out via a standardized set of computerized routines developed by the Mayo Cancer Center Statistics Unit specifically for Phase I studies. These routines produced a series of standard summary reports, graphical representations, and inferential output. Descriptive statistics formed the primary basis of analysis.

RESULTS

Twenty-two patients were enrolled in the study, all of whom were evaluable for safety. One patient failed to complete all of his interim blood tests but was otherwise evaluable for safety. This group of patients received a total of 31 cycles of KW-2189. The number of patients at each dose level and the number of cycles of treatment given by dose level are shown in Table 1, and the patients' characteristics are shown in Table 2. The most common tumor type was colorectal. Of the initial three patients enrolled in the study, DLT was seen in two patients. Following an initial dose reduction (level −1) in the next three patients, it was then possible to escalate the dose of KW-2189 beyond the initial dose level by restricting entry to patients who had received three or fewer prior chemotherapy regimens. Toxicities that were potentially related to treatment with KW-2189 are outlined in Table 3.

Hematological Toxicities. Thrombocytopenia and neutropenia were the most common DLTs (Table 3). At the 0.06 mg/m²/day dose level, 3 of 10 patients developed grade 4 thrombocytopenia with their first cycle of KW-2189. The thrombocytopenia nadir at this dose level occurred at a median of 28 days (range, 25–35 days), with a median platelet nadir of

Table 3 Summary of the number of evaluable patients developing toxicities thought to be related to KW-2189, by dose level

	0.03 mg/m²/day (n = 3)				0.04 mg/m²/day (n = 9)				0.06 mg/m²/day (n = 10)			
Toxicity	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 1	Grade 2	Grade 3	Grade 4
Hematological												
Anemia	1				3	1	1		1			
Neutropenia					1	1		2	2		1	2
Thrombocytopenia					4	3	1		5	1	1	3
Nonhematological												
Gastrointestinal												
Nausea					5				2			
Vomiting					2	1						
Diarrhea	1				1	2			1			
Other												
Lethargy		2			1	2	1		2	2		
Fatigue	1					2	1					
Anorexia		1			3	1	1		2			

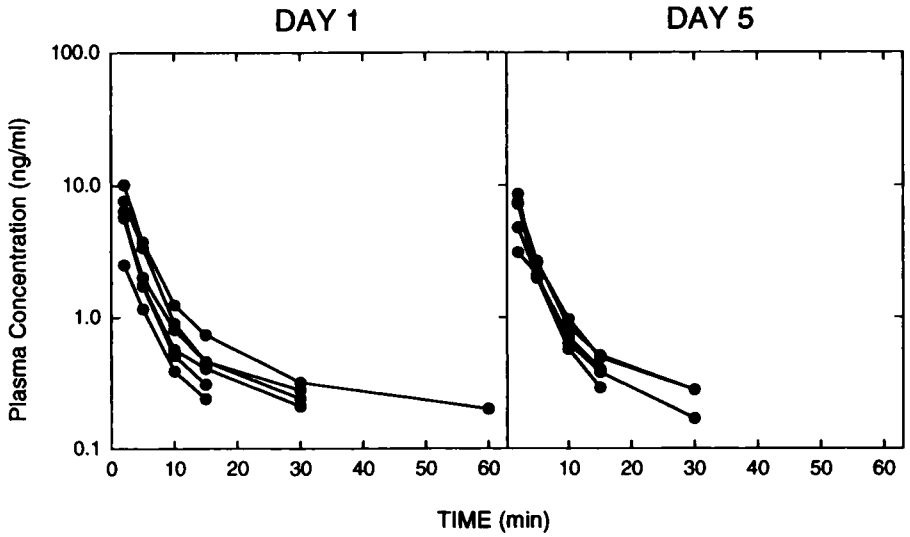

Fig. 2 Pharmacokinetic profiles on days 1 and 5 for six patients treated with 0.04 mg/m² KW-2189.

88,000 (range, 7,000–132,000). For patients who developed thrombocytopenia of grade ≥3 (5 patients), the mean episode duration was 8 days (range, 7–24 days). No episodes of bleeding occurred in this group of patients.

An absolute neutrophil count of $<0.5 \times 10^3/\mu l$ occurred in 2 of 10 patients at the 0.06 mg/m²/day dose level and in 2 of 9 patients at the 0.04 mg/m²/day dose level (Table 3). The onsets of grade 4 neutropenia occurred at means of 33 and 40 days at these two dose levels, respectively, with onsets ranging from 29 to 43 days and a median duration of 4.5 days. A total of five patients developed thrombocytopenia of grade ≥3, of which three developed neutropenia of grade ≥3. None of the patients with grade 4 neutropenia developed neutropenic fevers.

Of the patients who received three or more prior chemotherapy regimens, DLT (grade 4 neutropenia) was seen in two patients at the 0.04 mg/m²/day dose level. No DLT was observed in the patients at this dose level who had received fewer than three prior chemotherapy regimens.

Four patients completed more than one cycle of treatment with KW-2189. This included one patient at the 0.04 mg/m²/day level (five cycles) and one patient at the 0.06 mg/m²/day level (two cycles), neither of whom required a dose reduction. A third patient completed four cycles of treatment after an initial 10% dose reduction for hematological toxicities with cycle 1 and a fourth patient completed two cycles after a 50% dose reduction with the second cycle. No apparent cumulative toxicities were seen in the two patients receiving more than two cycles of treatment.

Nonhematological Toxicities. The nonhematological toxicities were generally mild (Table 3). One patient at the 0.04 mg/m²/day dose level developed grade 3 anorexia and grade 3 fatigue that necessitated removing him from the study. Lethargy was the most prominent nonhematological toxicity, occurring in nine patients. However, only one patient developed grade 3 lethargy. Nausea and vomiting were mild or absent, and none of the patients required antiemetics prior to or immediately after the administration of KW-2189. No significant (grade ≥3) hepatic or renal toxicity was observed.

Pharmacokinetics. The pharmacokinetics of KW-2189 were characterized for 22 patients on days 1 and 5 of treatment,

Table 4 Summary of KW-2189 pharmacokinetic parameters for days 1 and 5 by dose level[a]

	Dose level −1: 0.03 mg/m² (*n* = 3)		Dose level 1: 0.04 mg/m² (*n* = 6)		Dose level 2: 0.06 mg/m² (*n* = 9)	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
$t_{1/2\beta}$ (min)	4.09 (1.76)	4.77 (1.60)	18.7 (13.8)	10.5 (6.3)	9.88 (9.88)	7.65 (3.28)
AUC (ng/ml · min)	42.1 (23.8)	23.1 (5.2)	45.8 (17.5)	36.5 (11.1)	39.8 (10.8)	40.9 (9.9)
Cl (ml/min/m²)	913 (560)	1,347 (339)	980 (363)	1,185 (358)	1,617 (480)	1,546 (379)
V_{SS} (liters/m²)	3,536 (2,728)	7,028 (1,008)	11,979 (3,844)	10,463 (3,111)	12,110 (9,682)	9,668 (6,573)

[a] Values represent means (SDs).

with 0.03–0.06 mg/m² given as a rapid i.v. infusion over 30–60 s once daily for 5 days. Plasma concentrations of KW-2189 and DU-86 were determined with a sensitive, carefully validated LC-MS assay. Plasma profiles for six patients who received 0.04 mg/m² KW-2189 are illustrated in Fig. 2. The disappearance of KW-2189 from plasma was rapid, with concentrations falling below the assay detection limit (0.2 ng/ml) within 10–30 min of drug administration for 19 of 22 patients. Three patients had measurable plasma concentrations of KW-2189 for 60–120 min. For several patients, the KW-2189 plasma concentration at the end of the infusion was lower than the plasma concentration found 2 min after the end of the infusion, possibly as a result of incomplete distribution of KW-2189 after the short infusion.

The plasma profiles of KW-2189 appear to be multiphasic, but the assay detection limit was at the transition between the distribution and elimination phases, and characterization of the pharmacokinetics by compartmental methods was not possible. Thus, the pharmacokinetic parameters were estimated by noncompartmental analysis. The mean pharmacokinetic parameters for days 1 and 5 are shown in Table 4. The means do not include data for four patients that had differences in their plasma profiles compared to the rest of the patient cohort. Three patients did not have a 2-min specimen obtained. The fourth patient had detectable KW-2189 concentrations for the entire sample collection period on days 1 and 5, which may be artifactual due to sampling from a central used for drug administration. Individual AUCs and Cls are illustrated in Fig. 3, *A* and *B*, respectively. We did not observe dose proportionality for AUCs (Fig. 3*A*) because DLT led to evaluation of only three dose levels over a narrow range (0.03–0.06 mg/m²/day for 5 days), and substantial (3-fold) interpatient variability was found for AUCs at each dose level. As a result, a modest difference was observed for the Cls (Table 4 and Fig. 3*B*) between the lower (0.03 and 0.04 mg/m²) and higher (0.06 mg/m²) doses of KW-2189. There was a broad range of k_{el}s and $t_{1/2}$s, which were dependent on the length of time that KW-2189 was detected in plasma. The mean $t_{1/2}$ was 13.5 min, with a broad range of 2.13–69.3 min for day 1. These trends need to be evaluated further by additional data at higher doses of KW-2189.

The pharmacokinetics of KW-2189 were not altered by repeated daily administration for 5 days. The mean AUCs, Cls, and V_{ss}s were similar on days 1 and 5 (Table 4). Overall, the mean ± SD for Cl and V_{ss} were 1,287 ± 548 ml/min/m² and 10,638 ± 7,747 ml/m², respectively, on day 1 and 1,393 ± 383 ml/min/m² and 9,493 ± 4,972 ml/m², respectively, on day 5.

The active metabolite DU-86 was detected in three patients who received 0.06 mg/m² KW-2189. However, the plasma concentrations of DU-86 approached the assay's lower detection limit (0.2 ng/ml). Thus, it was not possible to describe the pharmacokinetics of DU-86 in this population of patients who were administered low doses of KW-2189.

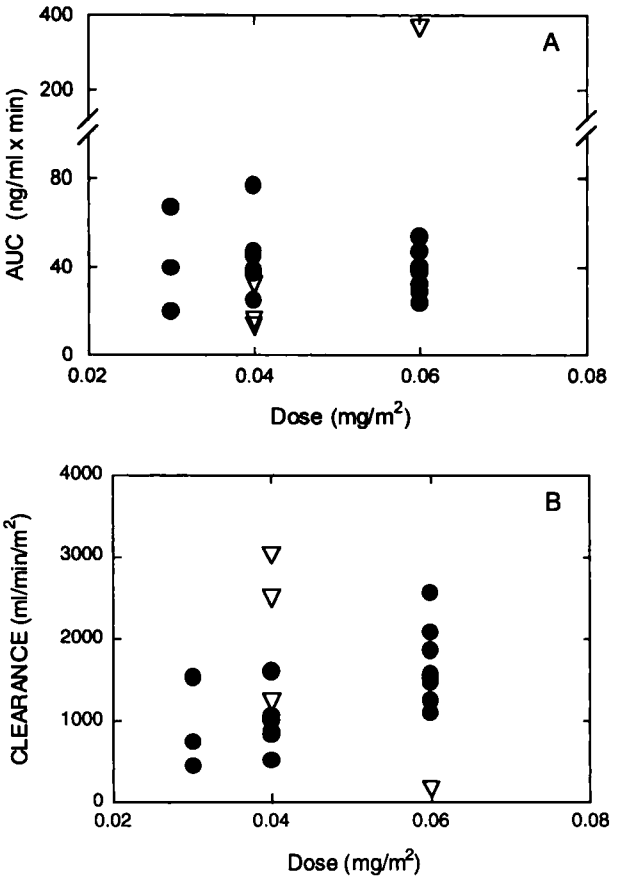

Fig. 3 Scatterplots for KW-2189 AUC *versus* dose (*A*) and KW-2189 Cl *versus* dose (*B*) following rapid i.v. bolus of KW-2189 on day 1 of a 5-day administration schedule. Values are shown for the 18 patients (●) used to calculate the mean values reported in Table 3 and for the 4 patients (▽) excluded from these calculations.

Tumor Response. Four patients had stable disease for at least one cycle of treatment. One patient had stable disease for four cycles of treatment, and another had stable disease for three cycles. No objective responses were seen.

DISCUSSION

In this Phase I trial with KW-2189, given as a daily rapid i.v. infusion for 5 days every 6 weeks, we found neutropenia and thrombocytopenia to be the DLTs. At the highest dose level achieved (0.06 mg/m^2/day), thrombocytopenia was more frequent and more prolonged than neutropenia. The pattern of toxicity is similar to that observed in Phase I trials of the minor groove-binding drugs adozelesin and carzelesin (20, 21). No cumulative myelotoxicity was seen in two patients who received more than two cycles of KW-2189, including one patient who received five cycles of KW-2189. The onset of thrombocytopenia and neutropenia was delayed in a manner similar to that observed with the nitrosoureas (22), as well as that with adozelesin (20). Thrombocytopenia and neutropenia nadirs occurred at medians of 29 and 33 days, respectively, after treatment with KW-2189. The nonhematological toxicities were, in general, mild with lethargy being the most prominent of these toxicities. However, one patient was removed from the trial because of grade 3 fatigue.

KW-2189 is a potent drug requiring administration of doses that are ~1000-fold lower than those of standard chemotherapy agents, such as doxorubicin. Characteristics of KW-2189 pharmacokinetics required the availability of a sensitive and specific HPLC assay with MS/MS detection. The distribution and Cl of KW-2189 were rapid and were not altered by repeated administration over 5 days. Consistent with rapid Cl, KW-2189 did not accumulate in plasma. Because a low dose of KW-2189 was administered in this trial, we did not consistently detect the active metabolite DU-86 in patient plasma. The rapid Cl of KW-2189, inability to consistently detect DU-86, and interpatient variability for AUCs prevented any meaningful pharmacodynamic analysis.

Two other Phase I trials of KW-2189 have been reported. Niitani *et al.* (23), in Japan, gave KW-2189 as a single bolus injection and found myelosuppression to be the DLT at a dose of 0.4 mg/m^2. Other toxicities in this study, including anorexia, fatigue, nausea/vomiting, anemia, and elevation of AST, were mild. Abbruzzese *et al.* (24), at the M. D. Anderson Cancer Center, recently presented interim results of their trial, in which KW-2189 was given as a single i.v. bolus every 42 days. Potential DLT (grade 4 thrombocytopenia) was seen in two of six patients entered at the 0.2 mg/m^2 dose level. An additional patient entered at a dose level of 0.25 mg/m^2 experienced only grade 2 thrombocytopenia. When compared to these two trials, our trial, using a multiday administration schedule, appears to be potentially more myelosuppressive.

The antitumor activity of KW-2189 has been demonstrated in murine and human tumor models (13). Significant activity was seen in a number of tumors known to be insensitive to a variety of other chemotherapeutic agents, including Adriamycin, cisplatin, cyclophosphamide, and mitomycin-C. The superior activity of KW-2189 could, in part, be due to its ability to overcome multidrug resistance. In cell culture, P-glycoprotein and Na^+, K^+-ATPase transporter were not found to have a role in the efflux of KW-2189 (14). Instead, decreased activity of KW-2189 appears to occur in cells with a decreased level of intracellular carboxyl esterase (14). Similar observations have been made with camptothecin and paclitaxel (25, 26). Carboxyl esterase levels in human tumors have not been well studied. In one reported series of 179 tumors, representing 18 tumor types, high carboxyl esterase levels correlated positively with CPT-11 activity *in vitro* (27). The role of carboxyl esterase in the development of resistance to KW-2189 and other potential mechanisms of resistance are not fully characterized.

On the basis of this study, the recommended dose of KW-2189 for Phase II studies, in patients who have received prior chemotherapy, is 0.04 mg/m^2/day, given daily for 5 days every 6 weeks. At this dose level, the drug was well tolerated and the toxicities appeared acceptable. Patient who have not received prior chemotherapy may tolerate the higher dose of 0.06 mg/m^2/day. Other trials are underway to assess the MTD for a 1-day administration schedule of KW-2189. Given its unique mechanism of action and favorable preclinical results, KW-2189 is currently being used in several Phase II trials, including two Mayo/North Central Cancer Treatment Group (NCCTG) trials for patients with hepatocellular carcinoma or melanoma.

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