Cancer Chemother Pharmacol (2004) 54: 459–468
DOI 10.1007/s00280-004-0808-z

ORIGINAL ARTICLE

**Toshiaki Saeki · Kenji Eguchi · Shigemitu Takashima
Takahiko Sugiura · Toyoaki Hida · Noboru Horikoshi
Keisuke Aiba · Takashi Kuwabara · Makoto Ogawa**

Phase I and pharmacokinetic study of KW-2170, a novel pyrazoloacridone compound, in patients with malignant tumors

Received: 3 October 2003 / Accepted: 1 March 2004 / Published online: 30 July 2004

Abstract *Purpose*: The primary purposes of this study were to determine the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD), to recommend a dose for phase II studies, and to analyze the pharmacokinetics of KW-2170. A secondary purpose was to assess tumor response to KW-2170. *Experimental design*: KW-2170 was given as a 30-min i.v. infusion every 4 weeks. Doses were escalated from 1.0 mg/m^2 according to a modified Fibonacci method. *Results*: A total of 45 cycles of KW-2170 were delivered to 41 patients at doses ranging from 1.0 to 53.0 mg/m^2. The primary DLT was neutropenia which was observed in two of six patients treated at 32.0 mg/m^2 and in two of two patients treated at 53.0 mg/m^2; therefore, the MTD was 53.0 mg/m^2. Although no patients showed a complete response (CR) or partial response (PR), 15 patients were evaluated as having freedom from progression at the 1-month timepoint, with two demonstrating slight tumor shrinkage in their metastatic lesions. None of the patients experienced significant cardiotoxicity. The plasma concentration of KW-2170 declined in a triphasic manner. The half-life, total clearance (CL_{tot}) and volume of distribution (V_{dss}) were nearly constant and independent of dose, and showed a relatively small interpatient variability. A linear relationship was observed between dose and maximum plasma concentration (C_{max}) and area under the concentration–time curve ($AUC_{0-\infty}$). In addition, there was a good correlation between neutropenia and $AUC_{0-\infty}$. This suggests that toxicity may be dependent on systemic exposure to the drug. Two oxidative metabolites were observed in the patients' plasma and urine. *Conclusions*: The primary DLT of KW-2170 in this study was neutropenia, with a MTD of 53 mg/m^2. A significant linear relationship was observed between neutropenia and $AUC_{0-\infty}$. We estimate the recommended dose for phase II studies to be 41.0 mg/m^2.

Keywords KW-2170 · Phase I study · Pharmacokinetics · Pharmacodynamics

T. Saeki · S. Takashima
Department of Surgery,
National Shikoku Cancer Center Hospital,
Matsuyama, Ehime, 790-0007, Japan

T. Saeki (✉)
Department of Clinical Research,
National Shikoku Cancer Center Hospital,
13 Horinouchi, Matsuyama,
790-0007, Japan
E-mail: tsaeki@shikoku-cc.go.jp
Tel.: +81-89-9321111
Fax: +81-89-9312428

K. Eguchi
Division of Pulmonary Medicine,
Tokai University School of Medicine, Isehara,
Kanagawa, 259-1193, Japan

T. Sugiura · T. Hida
Department of Respiratory, Aichi Cancer Center,
Nagoya, Aichi, 464-0021, Japan

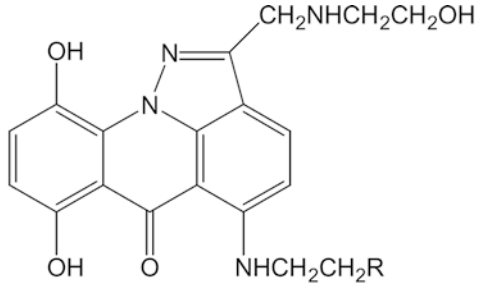
N. Horikoshi · K. Aiba
Department of Medical Oncology,
Cancer Institute Hospital, Toshima-ku, Tokyo,
170-8455, Japan

T. Kuwabara
Kyowa Hakko Kogyo Co. Ltd, Sunto-gun, Shizuoka,
411-8731, Japan

M. Ogawa
Aichi Cancer Center, Nagoya, Aichi,
464-0021, Japan

Introduction

KW-2170 [5-(3-aminopropyl)amino-7,10-dihydroxy-2-[(2-hydroxyethyl)aminomethyl]-6*H*-pyrazolo[4,5,1-*de*]acridin-6-one dihydrochloride; Fig. 1] is a pyrazoloacridone derivative. This compound was designed and chemically synthesized, devising its planar molecular structure and stereospecific interaction with DNA [9]. Its inhibitory effects on DNA synthesis primarily elicit antitumor activity against several cancer cell lines, and DNA intercalation and topoisomerase II-mediated DNA cleavage have been confirmed as the mechanisms [2, 9].



	(A)	(B)	(C)
R	-CH_2NH_2	-COOH	-CH_2OH
salt	2HCl	-	HCl
Molecular weight	470.35	412.41	434.92

Fig. 1 Chemical structures of (*A*) KW-2170 base, (*B*) M1 and (*C*) M2 (KW-2170 is the two hydroxychloride salt form)

In addition, the cytotoxicity of KW-2170 to P-glycoprotein-overexpressing doxorubicin-resistant human cancer cell lines (K562/ADM, A2780/ADM, MCF-7/ADM and KB-A1) and a vincristine-resistant human cancer cell line (KB/VJ-300) is stronger than that of losoxantrone and doxorubicin [2]. Furthermore, two human cancer cell lines (KB/VP-2 and KB/VM-4), which had been selected for their resistance to exposure to topoisomerase II inhibitors, exhibited lower levels of cross-resistance to KW-2170 than to losoxantrone [2]. In toxicologic studies in hamsters and dogs, KW-2170 has been shown to be less cardiotoxic than doxorubicin [2]. A series of nonclinical studies have shown potent antitumor effects of this drug on solid tumors including those in the lung, ovary, and pancreas, indicating that this compound may be an effective antitumor agent against a wide spectrum of solid tumors [2].

Patients and methods

Patient selection

Patients with histologically or cytologically proven malignant tumors in whom standard therapy was ineffective or not available were eligible for this study. Patient entry criteria also included: a life expectancy of at least 8 weeks; Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2; age between 18 and 74 years; adequate function of major organs (white blood cell count 4,000–12,000/mm^3; hemoglobin $\geq$9.5 g/dl; platelet count $\geq$100,000/mm^3; total bilirubin $\leq$ 1.2 mg/dl); aspartate aminotransferase and alanine aminotransferase levels not more than twice the institutional upper limit of normal (ULN); serum creatinine less than or equal to the institutional ULN; creatinine clearance $\geq$60 ml/min (24-h method); electrocardiogram (ECG) within the normal range or not requiring any treatment; left ventricular ejection fraction (LVEF) $\geq$60% (as assessed by radioisotope or ultrasonogram); no influence of prior therapy (washout periods: 4 weeks for surgical treatment, radiation therapy, or anticancer agents; 6 weeks for mitomycin, nitrosoureas and unapproved drugs; 2 weeks for fluoropyrimidine antimetabolites; 2 weeks for immunotherapy or hormone preparations including adrenocorticosteroids; 6 weeks for an LH-RH agonist); prior exposure to anthracyclines (the cumulative doxorubicin-equivalent dose) not greater than 350 mg/m^2; and written informed consent.

Drug administration and study design

KW-2170 and its metabolites were provided by Kyowa Hakko Kogyo (Tokyo, Japan). An intravenous aqueous formulation containing 5 mg KW-2170 per ml was used. The designated dose of KW-2170 was diluted with 100 ml of a sterile saline solution and administered intravenously over a period of 30 min with an infusion pump. The administration of KW-2170 was followed by infusion of a sterile saline solution, which was continued for at least an additional 30 min. The next cycle of administration was commenced after 4 weeks of follow-up.

In the second and subsequent cycles patients, except those with tumor progression identified in the response assessment, were allowed to receive the subsequent cycle only at their own request. They were given the same dose level as that in the first cycle if they had no dose-limiting toxicity (DLT) during that cycle, and one level lower than that in the first cycle if they experienced DLT. The patients were hospitalized during the study period.

The starting dose was set at 1.0 mg/m^2, representing a one-third toxic dose low (TDL) established for single intravenous administration in dogs. The TDL in dogs was considered to be 0.15 mg/kg (3 mg/m^2). Above this dose level, a voltage reduction of the Q-wave and a slight right axis deviation on ECG, myelosuppression, lymphoid depletion in the thymus and lymph nodes, hemorrhage from various organs, pulmonary edema, hepatocyte necrosis, hyaline droplets in the renal tubular epithelium and protein casts, mucosal injury in the digestive tract and an increase in hemosiderin deposition (in the bone marrow only) have been observed (unpublished data). The dose levels of KW-2170 were escalated according to a modified Fibonacci method as shown in Table 1. At least three patients were scheduled to be enrolled at each dose level. The planned cohort size was three to six patients. Dose escalation was to proceed if none of the initial three patients experienced DLT in the first cycle of therapy. If one of the three encountered DLT, three additional patients were treated at the same dose level. The maximum tolerated dose (MTD) was defined as the dose at which DLT occurred in two or more of three patients or in three or more of six patients. The recommended dose was defined as one level lower than the MTD. However, since it was necessary to determine the MTD and recommended dose depending on the toxicities that occurred during the study, final decisions were made by an independent data monitoring committee.

Table 1 Dose escalation

Level	Dose (mg/m^2)	Dose escalation (%)	No. of patients
1	1.0	–	3
2	2.0	100	3
3	3.3	65	3
4	5.0	52	3
5	7.0	40	3
6	9.0	29	3
7	11.5	28	3
8	15	30	3
9	19.5	30	3
10	25	28	3
11	32	28	6
12	41	28	3
13	53	29	2
Total			41

Toxicities were graded according to the National Cancer Institute's Common Toxicity Criteria version 1.0. DLT was defined as follows: grade 4 neutropenia without fever ($>38°C$) or grade 4 leukopenia each lasting for 3 days or longer, febrile neutropenia (fever $>38°C$, neutropenia $<500\ mm^{-3}$) and grade 4 thrombocytopenia; grade 2 cardiotoxicity; grade 2 adverse reactions in the kidneys, circulatory, respiratory and nervous systems; and grade 3 hepatic or gastrointestinal toxicity (excluding nausea, vomiting, and alopecia). Tumor responses were assessed according to the General Rule.

Before study entry, a complete medical history was obtained from all patients, and physical examinations including height, weight, body surface area, ECOG performance status, and clinical staging were performed. Laboratory examinations including a complete blood cell count, blood chemistry profile, electrolyte determination, urinalysis, and ECG were performed before treatment and weekly thereafter, with additional laboratory examinations performed if needed. Before treatment and at the end of each cycle, a chest radiograph, creatinine clearance, measurement of LVEF, and documentation of measurable disease were performed.

During this study, no other anticancer therapies (e.g., anticancer agents, radiation therapy, and immunotherapy) which might have affected the assessment of efficacy and safety were allowed. In addition, patients were not given any concomitant drugs during the study, except that the administration of a 5-HT type 3 antagonist was permitted but only after nausea/vomiting had occurred, and the use of G-CSF was allowed when the event met hematologic DLT criteria. The protocol of this study was approved by the institutional review board of each medical institution.

Pharmacokinetic sampling and analysis

Blood (5 ml) was drawn into a heparinized tube before infusion, and 5 min before the end of infusion, and at 5, 15, and 30 min, and 1, 2, 4, 8, 10, 24, 48, and 72 h after infusion. Plasma samples were obtained by centrifugation and stored at −80°C until analysis. Also, the free fraction in the plasma samples was obtained by ultracentrifugation at 289,000 *g* for 18 h at 4°C and subjected to analysis. Urine was collected before infusion and over the periods of 0–4, 4–8, 8–24, and 24–48 h after infusion. After the total volume was recorded, a 10-ml aliquot was stored at −80°C until analysis. The concentrations of KW-2170 and its two metabolites (M1 and M2; Fig. 1) were measured using a validated HPLC method with an electrochemical detector for plasma samples or with a visual detector at OD 400 nm for urine samples. Both methods had been validated with good precision and reproducibility [6]. A glucuronide of each compound was measured in the urine samples after β-glucuronidase digestion.

The pharmacokinetic parameters of KW-2170 in each patient were calculated by a three-compartment open model using the computer program WinNonlin Standard version 3.1 (Pharsight Corporation, Mountain View, Calif.). The following parameters were determined: rapid distribution half-life ($t_{1/2\alpha}$), slow distribution half-life ($t_{1/2\beta}$), elimination half-life ($t_{1/2\gamma}$), total clearance (CL_{tot}), volume of distribution (V_{dss}), and protein unbound fraction (f_u). For comparison of KW-2170 pharmacokinetics with those of its metabolites, the following parameters were calculated based on a non-compartmental method. The terminal half-life ($t_{1/2}$), maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were obtained based on observed values. The area under the concentration–time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule from time 0 to the last measurable KW-2170 concentration and by extrapolation to infinity using the elimination rate constant of the terminal phase calculated by the log-linear regression of plasma concentration and time. T_{max}, C_{max} and $AUC_{0-\infty}$ for the metabolites were determined in an identical manner to those for KW-2170.

A linear regression of C_{max} and $AUC_{0-\infty}$ against dose was performed. When 0 was included in the 95% confidence interval of the *y*-intercept according to SAS analysis (release 6.12; SAS Institute, Cary, N.C.), the pharmacokinetics of KW-2170 were defined as linear. A correlation between KW-2170 AUC and leukocyte counts and neutrophil counts were analyzed by linear regression with a significance level of 0.05.

Results

Patients

All 41 patients enrolled were treated with KW-2170. The dose levels, dose escalation, and number of patients at each level are shown in Table 1. Two cycles were given to one patient at level 1 and one patient at level 3, and three cycles were given to one patient at level 4. The patient characteristics are summarized in Table 2.

Table 2 Patient characteristics

Number of patients enrolled	41
Male/female	26/15
Age (years)	
Median	55
Range	33–73
ECOG performance status	
0	13
1	27
2	1
Prior treatment with anthracyclines	
Yes/no	15/26
Tumor type	
Non-small-cell lung cancer	17
Breast	8
Colorectal	3
Cholangiocarcinoma	2
Small-cell lung cancer	2
Head and neck	2
Other	7

Toxicity

DLT was observed in four patients. Febrile neutropenia occurred in two patients at level 11 and one patient at level 13, and grade 4 neutropenia lasted for 3 days in one patient at level 13. Since one of the initial three patients at level 11 experienced DLT, three additional patients were enrolled at this level. DLT was observed in two of these six patients, and the dose level was escalated to level 12. As none of the three patients at level 12 experienced DLT, the dose was escalated to level 13, at which the first two patients had DLT. Therefore, the MTD was determined to be level 13 (53.0 mg/m^2).

Table 3 shows the number of patients who experienced hematologic toxicity during the first cycle at all dose levels. Hematologic toxicity occurred frequently at level 8 or higher, and all such adverse reactions tended to increase in severity with increasing dose level. Treatment was given to only four patients who experienced DLT, and all recovered with 4-day to 6-day G-CSF administration. None of the patients developed infection. Table 4 details the neutropenia observed during the first cycle at levels 8–13. The number of days to recovery was further classified into patients treated with G-CSF or not. The median lowest values of absolute neutrophil count (ANC) tended to decrease as dose level increased. The median time to nadir ranged from 11.5 to 17 days, and was independent of dose level. Patients not given G-CSF took a median of 7–9.0 days to recover from

Table 3 Hematologic toxicity by dose level

	Grade	Level													Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	
No. of patients		3	3	3	3	3	3	3	3	3	3	6	3	2	41
Leukopenia	1				2		1	1	1	1	1				7
	2								1	1	2	3	1		8
	3								1			2	2	1	6
	4											1		1	2
Thrombocytopenia	1											2		2	4
Anemia	1			1		1				1	2	1		1	7
	2								1				2		3
	3											2		1	3
Neutropenia	1				1	1	1			1	1				5
	2								1	1	1	1	1		5
	3								1			2	2		5
	4											3[a]		2[b]	5
Lymphopenia	1			1						1					2
	2							1			1	1			3
	3	1				1	1	1	1	2		2	1	1	11
	4								1		1	1	2	1	6

[a]Two of three patients had DLT (febrile neutropenia).
[b]Both had DLT (febrile neutropenia and grade 4 neutropenia of 3 days duration.

Table 4 Neutropenia

Level	No. of patients	Median lowest ANC ($\times 10^2$ mm^{-3})	Median days to nadir ($\times 10^2$ mm^{-3})	No. of patients with nadir < 2000 mm^{-3}	Median days to recovery	
					Without G-CSF	With G-CSF
8	3	11.4	17.0	2	5.5	–
9	3	18.7	17.0	2	7.0	–
10	3	15.8	15.0	2	9.0	–
11	6[a]	6.1	14.5	6[a]	9.0	3.5
12	3	6.6	17.0	3	8.0	–
13	2	2.0	11.5	2	–	5.0

[a]Without G-CSF: four patients; with G-CSF: two patients.

neutropenia, whereas ANC rapidly normalized with a median recovery time of 3.5–5 days in G-CSF-treated patients. Table 5 shows the nonhematologic toxicity that occurred in three or more patients during the first cycle at each dose level.

Nonhematologic toxicity was generally mild. Fever (in absence of infection), nausea, vomiting, and alopecia were slightly more frequent at higher dose levels, but none were grade 3 or higher. For the treatment of nausea/vomiting, a 5-HT type 3 antagonist was given to three patients in total, one patient each at levels 5, 12, and 13, all of whom recovered in 3–5 days after the worst symptoms.

The adverse reactions related to cardiovascular toxicity predicted to occur based on nonclinical studies (unpublished data) were hypotension in three patients, arrhythmia detected only by palpation (with no ECG abnormalities) in one patient, and decreased LVEF in one patient, all of which were assessed as grade 1. Only grade 1 hypotension was seen among patients previously treated with anthracyclines. The adverse reactions that occurred during the second or third cycle in the three patients who received continued administration were arrhythmia (sinus tachycardia), cardiac ischemia, hemoglobin decrease, hypertension, hypotension, and hematuria, all of which were grade 1. Repeated administration did not aggravate any adverse reaction in any of the patients. During this study, there were no treatment-related deaths or early deaths within 30 days after completion of the study treatment.

Response

No patients achieved a complete response (CR) or partial response (PR). Progressive disease (PD) was documented in 26, and 15 were free from progression at the 1-month time-point. Tumor shrinkage was identified in 2 of the 15 patients who remained free from progression at the 1-month time-point. In one patient (level 3–2; cholangiocarcinoma), a computer tomography scan on day 28 of the first cycle showed shrinkage of an abdominal lymph node. In the other patient (level 4–2; oral cavity carcinoma), the metastatic lesions in the left axillary lymph node and left cervical lymph node (both measurable) had shrunk by up to 83.3% and 50.0%, respectively. However, this patient was not evaluated as PR from a comprehensive point of view, because the metastatic lesion in the mediastinal lymph node failed to shrink.

Pharmacokinetics

The mean plasma concentration–time profiles of KW-2170 at different dose levels after a 30-min infusion are shown in Fig. 2. The plasma concentration of KW-2170 showed a C_{max} at 5 min before the completion of infusion, declined rapidly soon thereafter, and then declined slowly in a biphasic manner. These profiles were well-fitted to a three-compartment open model. The pharmacokinetic parameters are summarized in Table 6. The half-life, CL_{tot}, and V_{dss} were virtually constant and independent of dose, and showed relatively small interpatient variability. The overall mean values of $t_{1/2\alpha}$, $t_{1/2\beta}$, $t_{1/2\gamma}$, CL_{tot}, and V_{dss} were 0.0665 h, 1.31 h, 31.6 h, 1.10 l/h/kg, and 11.8 l/kg, respectively. The free fractions of KW-2170 ranged from 1.97% to 6.45%, and were independent of dose.

At doses of 7 mg/m^2 and higher, the concentrations of two metabolites (M1 and M2) were also determined. Figure 3 shows the plasma concentration–time profiles of KW-2170, M1 (carboxylated form, Fig. 1) and M2 (hydroxylated form, Fig. 1) at the dose of 41 mg/m^2 as an example. The concentration of M1 was much higher and that of M2 was lower than that of KW-2170. As shown in Table 7, the half-lives of M1 and M2 were 21.6 and 18.3 h, respectively, which were comparable to that of KW-2170. The $AUC_{0-\infty}$ ratios of M1 and M2 to KW-2170 were 5.19 and 0.274, respectively. These metabolite

Table 5 Nonhematologic toxicity by dose level

	Grade	Level													Total
		1	2	3	4	5	6	7	8	9	10	11	12	13	
No. of patients		3	3	3	3	3	3	3	3	3	3	6	3	2	41
Nausea	1		1						1	1		3	1	1	8
	2					1							1		2
Vomiting	1					1			1			1			3
	2				1								1		1
Stomatitis	1	2					1	1			1				5
Hematuria	1			1	1			1	2		1	1	1	1	9
Alopecia	1											2		2	4
Hypotension	1					1			1					1	3
Headache	1				1						1	1			3
Constipation	1					1		1				1			3
Skin disorder	1							1				1			2
	2		1									1			2
Fever in absence of infection	1	1	1			1			2		1	1			7
	2											2		1	3

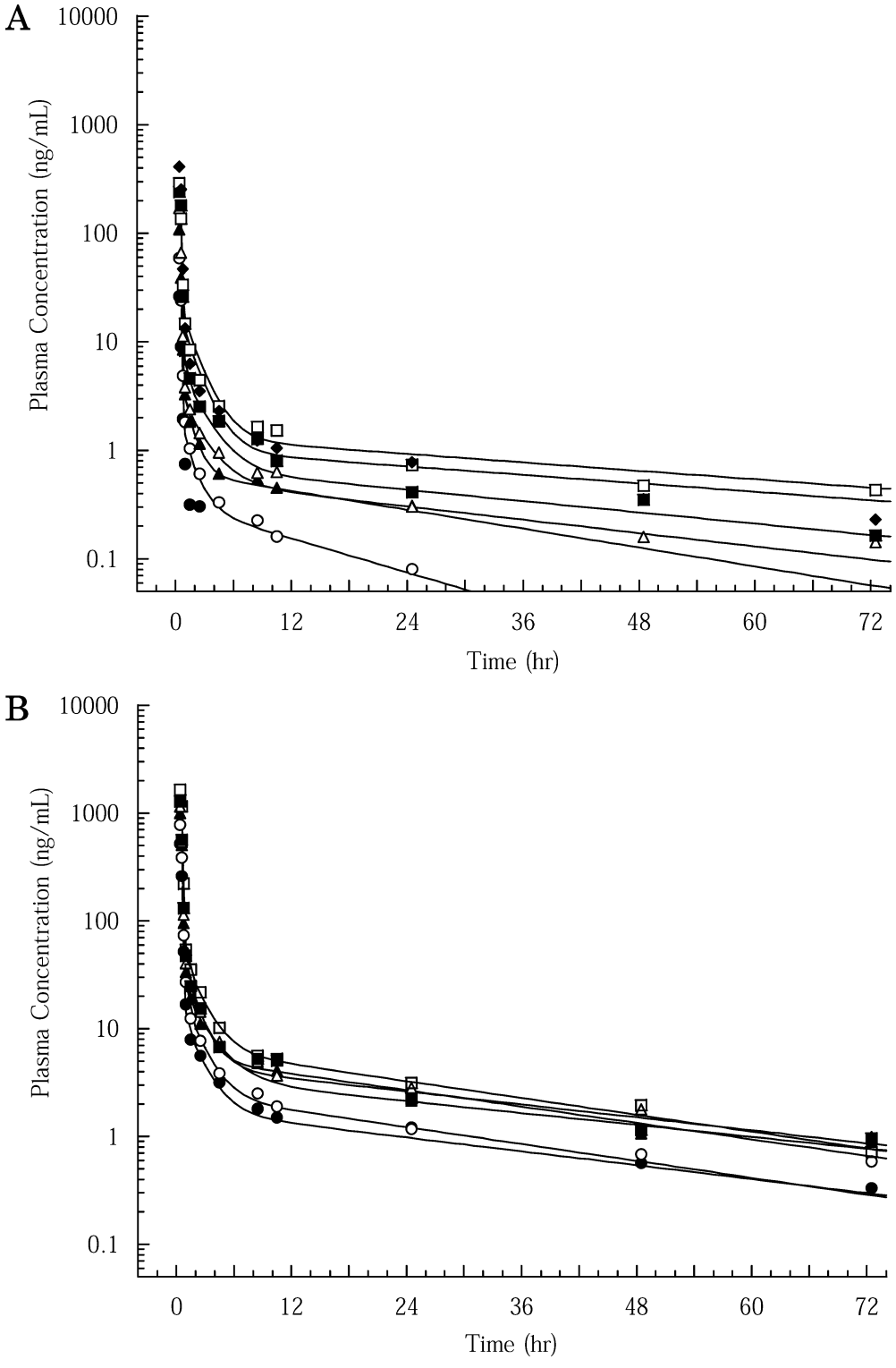


Fig. 2a, b Mean plasma concentration–time profiles of KW-2170 during and after 30-min i.v. infusion of KW-2170 at doses of 1–53 mg/m^2. **a** (1–11.5 mg/m^2) *filled circles* 1 mg/m^2, $n=3$, *open circles* 2 mg/m^2, $n=3$; *filled triangles* 3.3 mg/m^2, $n=3$; *open triangles* 5 mg/m^2, $n=3$; *filled squares* 7 mg/m^2; $n=3$; *open squares* 9 mg/m^2, $n=3$; *filled diamonds* 11.5 mg/m^2, $n=3$. **b** (15–53 mg/m^2) *filled circles* 15 mg/m^2, $n=3$; *open circles* 19.5 mg/m^2, $n=3$; *filled triangles* 25 mg/m^2, $n=3$; *open triangles* 32 mg/m^2, $n=6$; *filled squares* 41 mg/m^2, $n=3$;*open squares* 53 mg/m^2, $n=2$. Each point represents the mean. Each line represents the simulated plasma concentrations using the parameters obtained by the three-compartment model analysis

parameters were almost constant across all doses (data not shown). There was no tendency toward the saturation of metabolite formation.

The relationship between C_{max} and $AUC_{0-\infty}$ and dose is plotted in Fig. 4a and Fig. 4b, respectively. Significant correlations were observed, and increased proportionally to the dose.

Urinary excretion

The urinary excretion of KW-2170 for 48 h was as low as 0.09–0.33% of the dose over the entire dose range. M1 was the main metabolite in the urine, accounting for 9.30–15.58% of the dose. No glucuronide of KW-2170 or M1 was detected in the urine. M2 and M2

Table 6 Pharmacokinetic parameters of KW-2170 determined by a three-compartment open model (means ± SD)

Dose (mg/m^2)	*n*	T_{max} (h)	C_{max} (ng/ml)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$t_{1/2\gamma}$ (h)	CL_{tot} (l/h/kg)	V_{dss} (l/kg)	f_u (%)
1	3	0.350 ± 0.000	26.2 ± 1.9	–[a]	–	–	–	–	1.97 ± 0.39
2	3	0.344 ± 0.010	59.3 ± 8.9	0.0606 ± 0.0108	0.869 ± 0.170	15.2 ± 8.3	1.47 ± 0.24	5.67 ± 3.29	2.92 ± 1.61
3.3	3	0.350 ± 0.000	109 ± 20	0.0556 ± 0.0079	0.682 ± 0.025	23.8 ± 11.5	1.24 ± 0.30	12.0 ± 9.2	2.36 ± 0.36
5	3	0.350 ± 0.000	171 ± 28	0.0583 ± 0.0121	1.58 ± 0.66	31.0 ± 8.8	1.28 ± 0.34	11.8 ± 1.5	3.39 ± 0.54
7	3	0.361 ± 0.019	242 ± 66	0.0707 ± 0.0075	2.18 ± 1.84	44.0 ± 28.8	0.985 ± 0.209	16.5 ± 16.5	2.82 ± 1.09
9	3	0.378 ± 0.035	288 ± 35	0.0683 ± 0.0260	1.90 ± 1.19	65.3 ± 41.1	0.911 ± 0.179	29.8 ± 24.2	3.10 ± 0.26
11.5	3	0.422 ± 0.075	410 ± 7	0.0685 ± 0.0119	1.10 ± 0.08	26.6 ± 5.1	1.08 ± 0.05	7.91 ± 1.91	2.49 ± 1.50
15	3	0.350 ± 0.000	522 ± 53	0.0679 ± 0.0009	1.35 ± 0.59	28.5 ± 4.4	1.21 ± 0.07	9.86 ± 2.07	1.64 ± 0.53
19.5	3	0.350 ± 0.000	779 ± 158	0.0807 ± 0.0220	1.22 ± 0.31	30.0 ± 20.8	0.923 ± 0.123	8.65 ± 10.10	3.70 ± 2.38
25	3	0.350 ± 0.000	999 ± 62	0.0693 ± 0.0015	1.89 ± 0.98	33.2 ± 4.8	1.00 ± 0.13	11.0 ± 1.1	2.50 ± 1.02
32	6	0.353 ± 0.007	1150 ± 210	0.0643 ± 0.0099	1.10 ± 0.29	31.8 ± 8.0	0.957 ± 0.153	11.6 ± 5.7	2.90 ± 1.59
41	3	0.350 ± 0.000	1290 ± 80	0.0666 ± 0.0048	0.870 ± 0.202	24.3 ± 4.7	1.26 ± 0.16	9.02 ± 2.71	2.68 ± 0.60
53	2	0.350	1650	0.0717	1.19	23.2	1.06	6.34	6.45
Overall mean[b]				0.0665	1.31	31.6	1.10	11.8	

[a]Three-compartment model analysis. Data from level 1 were not fitted to three-compartment model because concentrations were measurable at few time points.
[b]Mean of each parameter of all patients at doses of 2–53 mg/m^2 ($n = 38$).

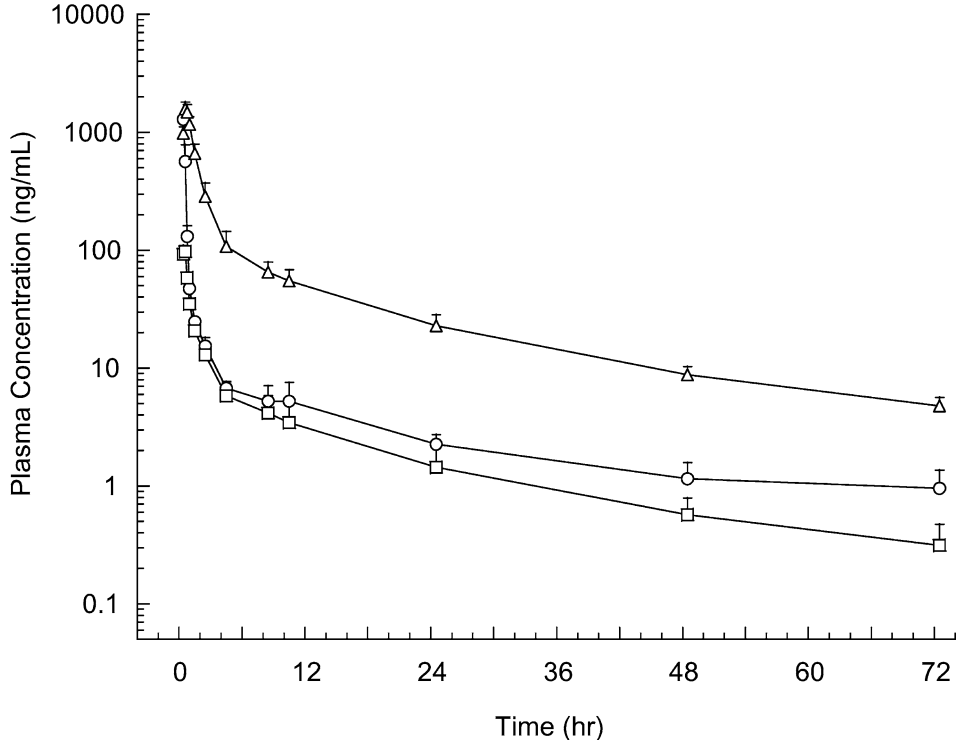


Fig. 3 Mean plasma concentration–time profiles of KW-2170 (*circles*), M1 (*triangles*) and M2 (*squares*) during and after a 30-min intravenous infusion of KW-2170 at a dose of 41 mg/m^2

glucuronide were detected in the urine, with excretion rates of 0.47–0.68% and 1.86–2.90%, respectively.

Pharmacokinetics/pharmacodynamics (PK/PD) relationship

Relationships between neutropenia and the $AUC_{0-\infty}$ of KW-2170 were investigated. A significant linear relationship was observed between leukopenia and $AUC_{0-\infty}$ (Fig. 5a; $r = 0.859$; $P < 0.001$). A significant relationship was also observed between neutropenia and $AUC_{0-\infty}$ (Fig. 5b; $r = 0.873$; $P < 0.001$).

Discussion

KW-2170 is a novel pyrazoloacridone compound that exhibits, like anthracyclines, antitumor activity primarily through DNA intercalation, and also induces

Table 7 KW-2170 and its two metabolites determined by non-compartment analysis at the dose of 41 mg/m^2 (means ± SD)

Compound	T_{max} (h)	C_{max} (ng/ml)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng h/ml)	AUC ratio[a]	Urinary excretion[b] (% of dose)
KW-2170	0.350 ± 0.000	1290 ± 80	26.0 ± 3.3	787 ± 49		0.32 ± 0.05
M1	0.611 ± 0.035	1590 ± 210	21.6 ± 2.3	4090 ± 790	5.19 ± 0.98	15.58 ± 2.54
M2	0.511 ± 0.140	101 ± 9	18.3 ± 1.0	218 ± 58	0.274 ± 0.055	0.68 ± 0.10

[a]Ratio to $AUC_{0-\infty}$ of KW-2170.
[b]Excretion for 48 h.

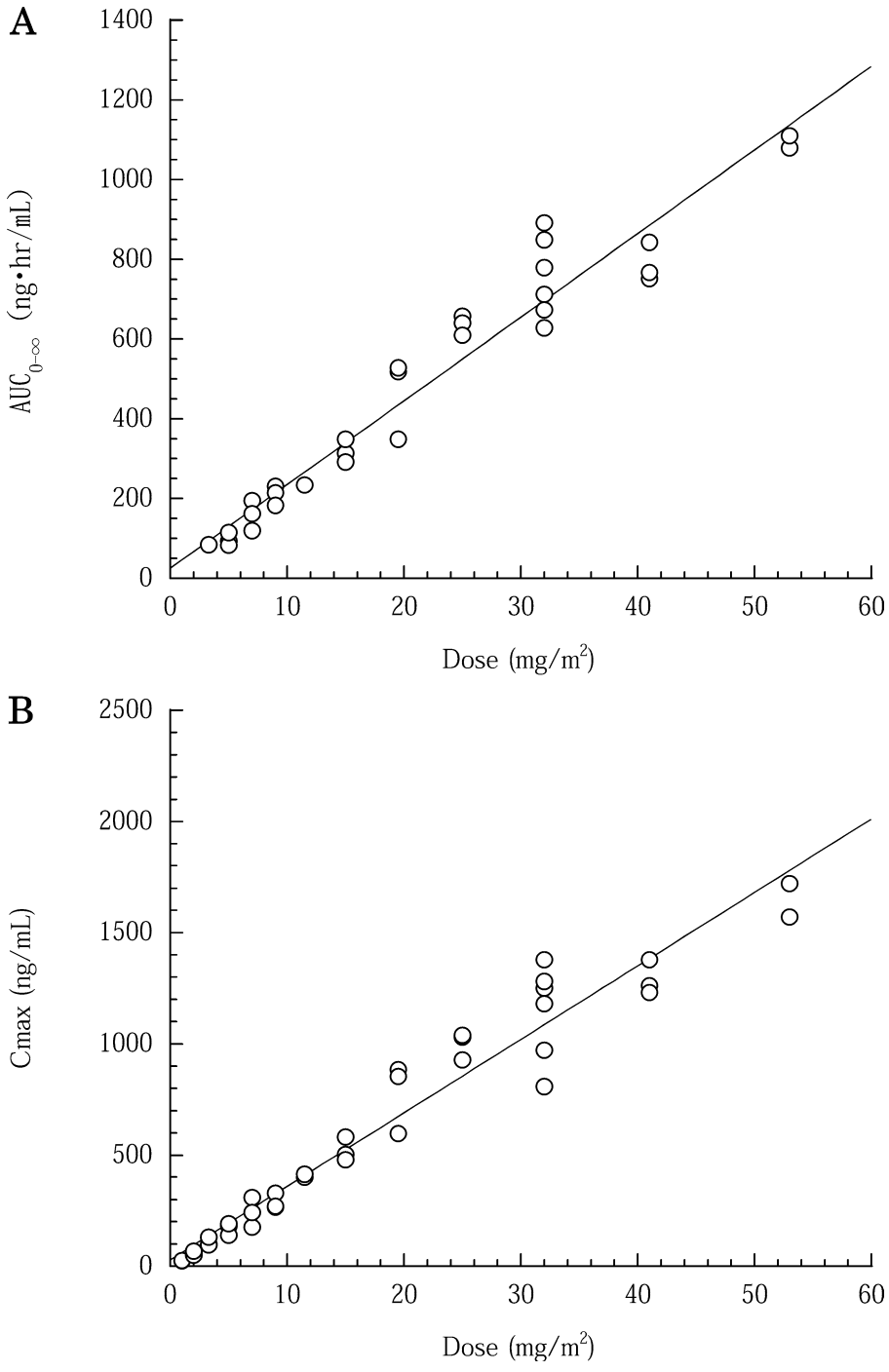


Fig. 4a, b Relationship between KW-2170 dose and (**a**) $AUC_{0-\infty}$ and (**b**) C_{max}. Each symbol represents an observed value. The line represents the regression line by a linear least square method. The obtained formulas were: (**a**) $y = 21.6x + 25.5$ (dose 3.3–53 mg/m^2, $r = 0.971$, $P < 0.001$, and (**b**) $y = 33.0x + 29.3$ (dose 1–53 mg/m^2, $r = 0.974$, $P < 0.001$). The 95% confidence intervals were −22.6–73.5 for $AUC_{0-\infty}$ and −27.6–86.2 for C_{max}

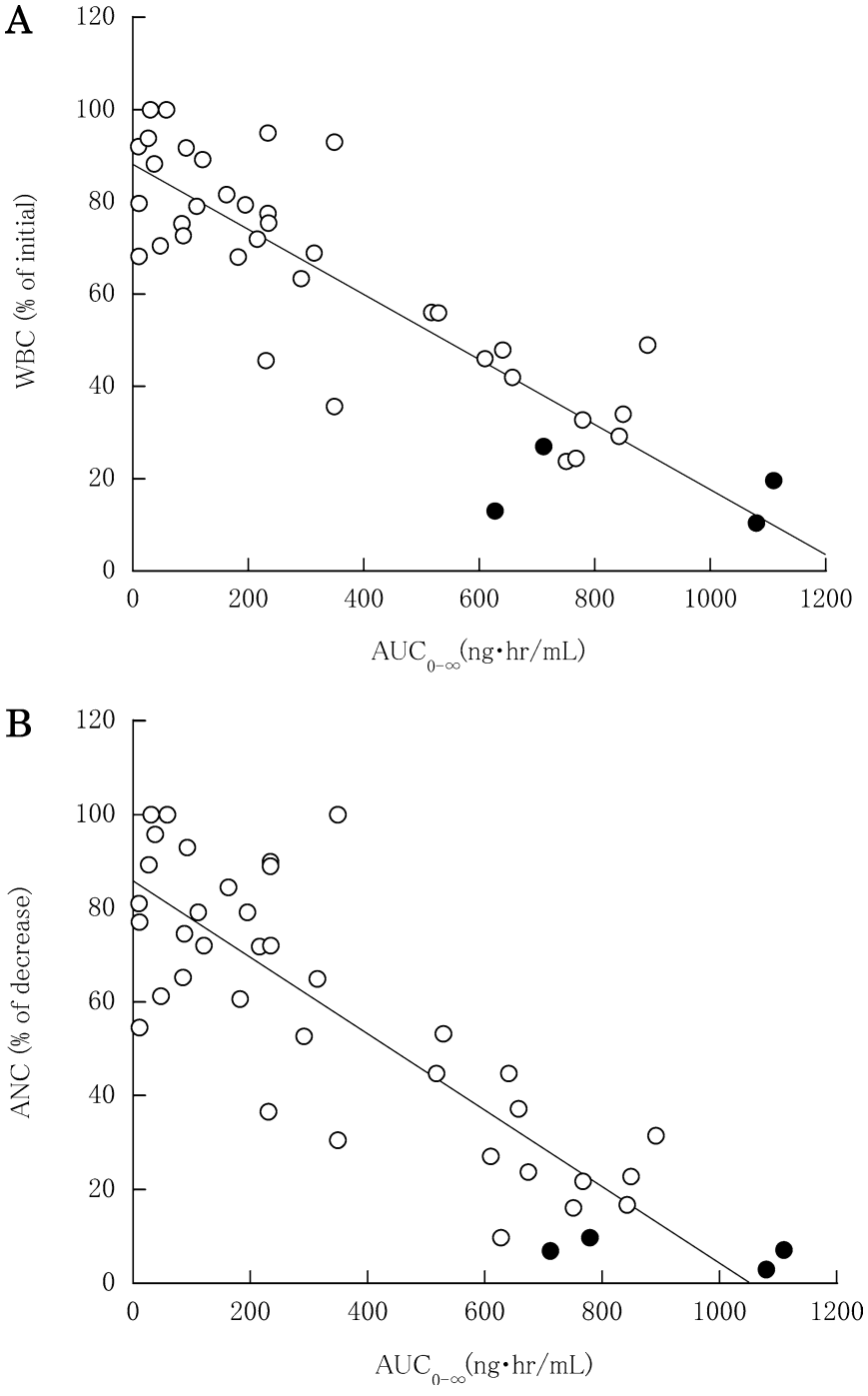


Fig. 5a, b Relationship between KW-2170 $AUC_{0-\infty}$ and (**a**) leukocyte counts and (**b**) neutrophil counts at doses of 3.3–53 mg/m^2. Each symbol represents an observed value of each patient and the closed symbol represents the patients with DLT. The line represents the regression line by a linear least square method. The obtained formulas were: (**a**) $y = -0.0703x + 88.1$ ($r = 0.859$, $P < 0.001$), and (**b**) $y = -0.0806x + 85.9$ ($r = 0.873$, $P < 0.001$)

topoisomerase-mediated DNA cleavage [9]. Nonclinical data have shown that KW-2170 is effective against a variety of cancer cell lines that are resistant to drugs including doxorubicin, demonstrating that its efficacy profile is different from that of doxorubicin [2]. In addition, results from toxicologic and pharmacologic studies in various experimental animals including dogs suggest that KW-2170 is associated with a reduced severity of cardiotoxicity compared with doxorubicin.

The cardiotoxicities identified during the phase I study were a patient with grade 1 arrhythmia detected by palpation on day 1 of administration and a grade 1 decreased LVEF (75–61%) on day 28. Both were of minimal severity and clinically insignificant, with no impact of prior exposure to doxorubicin. In this study, the cumulative toxicity could not be evaluated due to the small number of cycles of continued administration; however, these findings at least suggest that a single dose of KW-2170 causes no acute cardiotoxicity.

The most common adverse reaction was hematologic toxicity as predicted from nonclinical studies. The DLT identified in this study was neutropenia, which was

comparable in severity to that with standard anticancer agents and promptly resolved with G-CSF treatment. Neutrophil nadirs closely correlated with dose level, C_{max} and $AUC_{0-\infty}$, with relatively small interpatient variability. Four patients receiving high doses close to the MTD experienced thrombocytopenia, but all were grade 1.

It is noteworthy that no patient experienced grade 3 or 4 nonhematologic toxicity, and nausea/vomiting associated with KW-2170 appeared to be less frequent and less severe than with standard chemotherapy. Only four patients experienced grade 1 alopecia even at dose levels close to the recommended dose; therefore, KW-2170 appears to be unique in that it is associated with a lower frequency and severity of alopecia compared with structurally similar doxorubicin and mitoxantrone, which cause moderate to severe alopecia in 71–80% [1, 3] and 12–22% [1, 3] of patients, respectively.

The pharmacokinetics of KW-2170 in patients was linear over the dose range of 1–53 mg/m^2. This was supported by dose-proportional increases in C_{max} and $AUC_{0-\infty}$, constant metabolic ratios and constant protein bindings across the doses. KW-2170 exhibited a rapid distribution ($t_{1/2\alpha} = 0.0665$ h), slow elimination ($t_{1/2\gamma} = 31.6$ h), and large distribution volume ($V_{dss} = 11.8$ l/kg or 431 l/m^2 or 653 l) [6]. These features are similar to the structure-related compounds mitoxantrone ($t_{1/2\alpha} =$ 0.05–0.2 h, $t_{1/2\gamma} = 3.9$–215 h, $V_{dss} = 213$–2248 l/m) [2, 4] and doxorubicin ($t_{1/2\alpha} = 0.05$–0.78 h, $t_{1/2\gamma} = 13$–50 h, $V_{dss} = 9$–66 l/kg) [8]. However, pyroxantrone has faster elimination and smaller distribution volumes than KW-2170 ($t_{1/2\alpha} = 0.025$–0.0833 h, $t_{1/2\beta} = 0.268$–0.608 h, $V_{dss} = 19.3$–26 l, calculated by a two-compartment model) [5].

The interpatient variability in the pharmacokinetics of KW-2170 was relatively small. At 41 mg/m^2, the CV% of CL_{tot} and V_{dss} were 12.7% and 30.0%, whereas those of mitoxantrone are 51.2% and 58.8%, respectively [7].

Two metabolites (M1 and M2) were detected in the patient plasma. The antiproliferative activities of KW-2170 and its metabolites against DU145 cells after 1 h of treatment have been compared. The IC_{50} values of KW-2170, M1 and M2 are 1.5 μmol/l (705 ng/ml), > 100 μmol/l (> 41,400 ng/ml), and 12.2 μmol/l (5,306 ng/ml), respectively (unpublished data). The activities of the two metabolites are lower than that of KW-2170. The enzymes metabolizing KW-2170 to M1 and/or M2 have not been identified yet. Some amino-oxidases may contribute to this metabolism. Further studies are planned to investigate this enzyme system.

There was good correlation between neutropenia and KW-2170 $AUC_{0-\infty}$, suggesting that the toxicity may be dependent on systemic exposure to the drug. This is consistent with the result of an in vitro experiment demonstrating that the cytotoxicity of KW-2170 is AUC-dependent but not time-dependent (unpublished data).

There were no responders in this study involving patients with malignant tumors in whom standard therapy was ineffective or not available. However, despite the fact that the patients had received a variety of prior therapies, two patients achieved shrinkage of metastatic lesions. These results encourage us to perform further trials for the evaluation of this compound.

The MTD of KW-2170 given as a 30-min intravenous infusion was determined to be 53.0 mg/m^2, and the recommended dose for phase II studies was estimated at 41.0 mg/m^2. This study showed that the DLT was manageable neutropenia, that there was a significant correlation between pharmacokinetics and pharmacodynamics, and that the interpatient variability in pharmacokinetics was relatively small. These findings suggest that KW-2170 may be clinically useful. We believe that it is necessary to reassess the recommended dose in a future study, given that the number of patients receiving the recommended dose was too small and that G-CSF support might potentially enable treatment at higher doses. Furthermore, other dosing schedules should be studied in the future to maximize the potential of KW-2170.

Acknowledgements We thank Hiroaki Shimizu, Takako Arai, Masashi Katayama, Tomoko Kuramitsu, Mayumi Mukai and Yoshiko Maeda for their skillful technical assistance. We also thank Maya Kurita, Isuzu Matsushita, Mami Matsuda, Miyuki Horiuchi and Hiroe Nozawa for their support in clinical research management.

References

1. Allegra JC, Woodcock T, Woolf S, Henderson IC, Bryan S, Raisman A, Dukart G (1985) A randomized trial comparing mitoxantrone with doxorubicin in patients with stage IV breast cancer. Invest New Drugs 3:153
2. Ashizawa T, Shimizu M, Gomi K, Okabe M (1998) Antitumor activity of KW-2170, a novel pyrazoloacridone derivative. Anticancer Drugs 9:263
3. Cowan JD, Osborne CK, Neidhart JA, Von Hoff DD, Constanzi JJ, Vaughn CB (1985) A randomized trial of doxorubicin, mitoxantrone and bisantrene in advanced breast cancer (A South West Oncology Group Study). Invest New Drugs 3:149
4. Faulds D, Balfour JA, Chrisp P, Langtry HD (1991) Mitoxantrone: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in the chemotherapy of cancer. Drugs 41:400
5. Hantel A, Donehower RC, Rowinsky EK, Vance E, Clarke BV, McGuire WP, Ettinger DS, Noe DA, Grochow LB (1990) Phase I study and pharmacodynamics of piroxantrone (NSC 349174), a new anthrapyrazole. Cancer Res 50:3284
6. Kuramitsu T, Takai K, Ohashi R, Kuwabara T (2002) Determination of the anticancer drug KW-2170, a pyrazoloacridone derivative, and its metabolites in human and dog plasma by high-performance liquid chromatography using an electrochemical detector. J Chromatogr B 768:231
7. Savaraj N, Lu K, Manuel V, Loo TL (1982) Pharmacology of mitoxantrone in cancer patients. Cancer Chemother Pharmacol 8:113
8. Speth PAJ, van Hoesel QGCM, Haanen C (1988) Clinical pharmacokinetics of doxorubicin. Drug Dispos 15:15
9. Sugaya T, Miura Y, Shida Y, Ozawa Y, Matsukuma I, Ikeda S, Akinaga S, Morimoto M, Ashizawa T, Okabe M, Ohno H, Gomi K, Kasai M (1994) 6*H*-Pyrazolo[4,5,1-*de*]acridin-6-ones as a novel class of antitumor agents. Synthesis and biological activity. J Med Chem 37:1028