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Phase I and pharmacokinetic study of a novel mitomycin C analog KW-2149

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KW-2149 is a new, semisynthetic, C-7-*N*-substituted, mitomycin C (MMC) analog showing equal or superior antitumor activity in both *in vitro* and *in vivo* assays The preclinical activity profile combined with the hematological toxicity data in rodents and the water solubility of the compound compare favorably with MMC. The aim of this phase I study was to determine the toxicity profile and the optimal dosage of KW-2149. In this phase I study 37 patients received 97 courses of KW-2149 administered as an i.v. bolus injection every 21 days at sequential dose levels: 5, 10, 17, 25, 35, 47, 60, 75, 90 and 100 mg/m^2. Hematological toxicity was moderate even at the 100 mg/m^2 dose level. Grade IV leucopenia and thrombocytopenia were observed in one of three patients at the 100 mg/m^2 dose level. There was some evidence of a delayed-type bone marrow toxicity. Pulmonary toxicity was dose limiting, with grade III toxicity occuring in all three patients treated at a dose of 100 mg/m^2. The type of lung toxicity was similar to the one observed with other antitumor antibiotics. No renal or cardiac toxicity was observed. Other toxicities were generally mild. Antitumor activity was observed in four patients. Data of drug monitoring demonstrated rapid metabolism and/or distribution of KW-2149 with a short half-life and the emergence of the cytotoxic metabolites M-16 and M-18. The dose-limiting toxicity of KW-2149 is pulmonary toxicity.

Key words: Analog, KW-2149, mitomycin C, pharmacokinetics, phase I.

Introduction

The mitomycins constitute a class of alkylating antibiotics derived from *Streptomyces caespitosus*. Further research by Wakaki *et al.* led to the discovery of mitomycin C (MMC) in 1958.[1] This compound underwent clinical testing both in Japan and the Western world, but wide-scale application of the drug was initially hampered by the severe myelotoxicity with different daily low-dose schedules. Intermittent dosing once every 4–8 weeks resulted in manageable hematological toxicity.[2] MMC has a very broad range of activity against solid tumors.[3] Mitomycins have a unique chemical structure in which a quinone, aziridine and carbamate function are arranged around a pyrrolo(1,2-*a*)indole nucleus. A striking property of MMC is its reducibility due to the presence of a quinone ring. Reduction of the quinone system of MMC triggers a series of spontaneous reactions within the molecule, giving rise to a reactive species which alkylates DNA at the C-1 and C-10 positions. MMC is believed to operate as an alkylating agent once it has undergone reduction, hence the name of bioreductive alkylation.[4,5] Studies on the mechanisms of action have demonstrated that DNA is the main target of MMC, whereby DNA synthesis is inhibited in proliferating cells.[5] The formation of covalent cross-links between DNA and MMC *in vivo* was demonstrated by Tomasz *et al.*[6] The generation of oxygen radicals could well contribute to the overall cytotoxicity of MMC.[7]

The observation that the quinone ring of MMC is easily reduced by a thiol group suggests that the intramolecular reductive activation could be accomplished by a substituent at the C-7-*N* position.[8–10]

The drug with a 2-((2-(γ-L-glutamylamino) ethyl)dithio)ethyl group at the C-7-*N* position, KW-2149 (Figure 1), was selected for clinical testing because of its promising *in vitro* and *in vivo* activity, its less pronounced myelotoxicity compared to MMC, and its superior water solubility.[11–14] In animal tumor models KW-2149 was at least as active as MMC, and against P388 leukemia, M5076 sarcoma and B16 melanoma it was found more active than MMC.

In these models, KW-2149 proved also to be active against the MMC-resistant leukemias P388 and L1210. In human tumor xenografts the activity of MMC and KW-2149 was compared by a single

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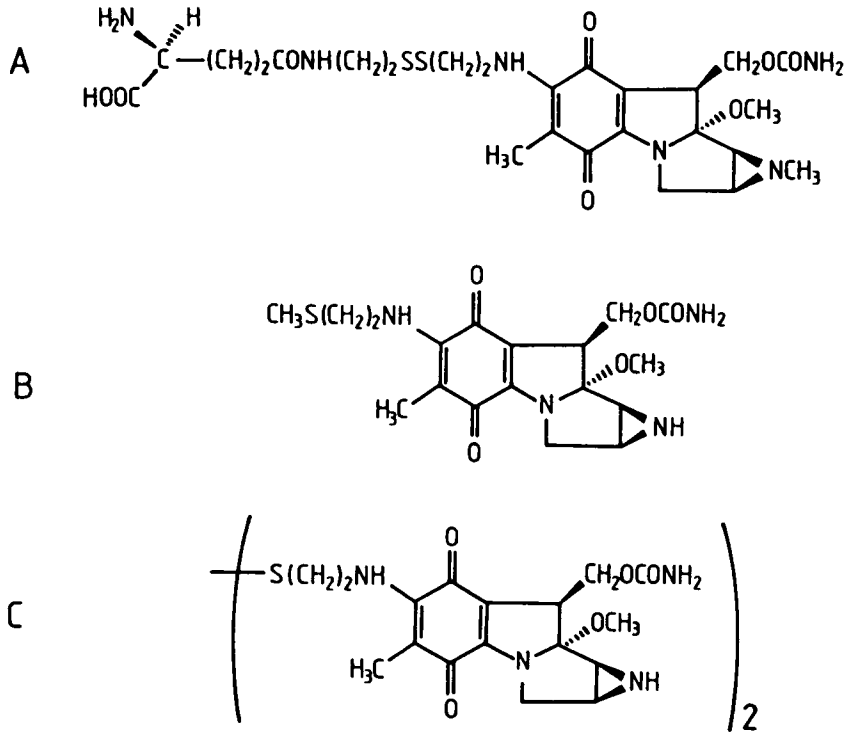

Figure 1. Molecular structure of KW-2149 (A), M-16 (B) and M-18 (C).

i.v. injection at the LD_{10}. Their activity proved to be comparable. No clear dose-schedule dependence was observed in these preclinical studies. The antitumor effect of KW-2149 has also been tested against a variety of cell lines and compared with MMC, again confirming the similar spectrum of antitumor effect. The cytotoxic activity of KW-2149 and its two active metabolites, M-16 and M-18, has recently been described in cell lines with different resistance patterns.[14,15]

Preclinical toxicology of KW-2149 showed that changes in heart, kidney and lung were produced similar to those known to be caused by MMC. In animal studies, again compared with MMC, reduced myelotoxicity was observed.[16] In toxicology studies in mice, LD_{10} and LD_{50} values of KW-2149 were 4–5 times higher as are those of MMC. The LD_{10} was 16.3 mg/kg (48.9 mg/m^2) in male mice and 16.7 mg/kg (50.1 mg/m^2) in female mice.

Materials and methods

Eligibility

All patients entered in this study had histologically confirmed solid tumors for which no established form of therapy was available. Prior to entry, all patients had a complete history taken and a physical examination performed. Height, weight, performance status and tumor measurements were recorded. Further assessment included a complete blood count, coagulation tests, serum electrolytes and kidney function, liver tests, and urine analysis. An ECG, a chest radiograph, lung function tests including carbon monoxide diffusion capacity and a radionucleotide left ventricular ejection fraction were done prior to entry. Eligibility criteria included (i) age between 18 and 70 years, (ii) performance status < 3, (iii) a life expectancy of at least 3 months, (iv) 4 weeks beyond prior radiotherapy or chemotherapy (6 weeks for nitrosoureas or extensive radiotherapy), (v) adequate kidney function (creatinine < 1.4 mg/dl), liver function (bilirubin < 1.5 mg/dl, AST/ALT < 2 ULN) and bone marrow reserve (white cell count $> 4000/mm^3$, platelets $> 100\,000/mm^3$), (vi) no other medical problems which would make compliance to the protocol unlikely, and (vii) no prior treatment with MMC and/or cisplatin. Approval for this study was given by the Ethical Committees in both institutions in Antwerp and Lyon. All patients gave written informed consent.

Dosage and formulation

KW-2149 was supplied by Kyowa Hakko Kogyo (Tokyo, Japan) as a lyophilized gray–green colored

powder consisting of 10 mg KW-2149, 200 mg lactose and NaOH to adjust pH to about 7. Lactose was added as excipient to increase stability. Each vial was reconstituted with 2.0 ml of sterile water and used immediately afterwards.

This phase I study used as initial dose 5 mg/m^2, being 1/10th of the LD_{10} in mice. Since in animal models no schedule dependency had been detected, an intermittent, once 3 weekly, regimen was selected. This dose was given as an intravenous injection over 10 min every 3 weeks or after full recovery. Doses were escalated using a modified Fibonacci progression scheme. No dose escalation within an individual patient was permitted. At least three patients with a total of at least four evaluable courses had to be entered at a non-toxic dose level before escalation was permitted. If toxicity occurred, more patients had to be entered at a given dose level. The maximum tolerated dose was defined as the dose producing grade III toxicity in at least 30% of patients treated at that dose.

Follow-up scheme, toxicity and response evaluation

Patients were seen weekly in the outpatient clinic, and medical history, physical examination and routine blood and urine tests were done on each occasion. Prior to each course, kidney function was measured by a creatinine clearance, and an ECG and chest X-ray were done. After two cycles a tumor response evaluation was performed and toxicity studies were completed.

Clinical toxicity grading using the WHO grading system was done weekly.[17] Patients underwent weekly cardiovascular examinations, 3 weekly ECG controls and 6 weekly quantitative left ventricular function measurements. They also underwent lung function tests prior to entry and after every two courses or more frequent if indicated. The response was evaluated after every two courses using standards WHO criteria.[17]

Pharmacokinetic study

Pharmacokinetic studies were initiated from the 10 mg/m^2 dose level in every patient during the first two courses. In some patients at the higher dose levels, sampling was performed during every course. Blood samples were drawn from the contralateral arm of the infusion in heparinized polypropylene tubes containing oxidized L-glutathione (200 mg/80 µl, 2.5%) to prevent interaction with sulfydryl groups of the biological matrix. Samples were collected before treatment, at 5, 10, 15, 30 and 60 min, and then at 2, 4, 6, 9, 12, 24, 36 and 48 h after the start of the infusion. After mixing, each sample was immediately centrifuged at 4000 *g* for 15 min and the plasma was transferred to polypropylene tubes. Samples were then stored at −80°C until analysis.

Analytical methodology

KW-2149, M-16 and M-18 were kindly supplied by Kyowa Hakko Kogio (Tokyo, Japan). KW-2149, M-16 and, in five patients, M-18 were measured in the plasma by previously reported HPLC with some minor modifications.[18] Plasma samples were thawed and centrifugated for 10 min at 2000 *g*. One milliliter aliquots were subjected to solid-phase extraction using XAD-2 columns. Columns were preconditioned with 4 × 1.0 ml methanol followed by 5 × 1.0 ml H_2O. Plasma samples were loaded onto the columns, washed with 3 × 1.0 ml H_2O and eluted with 4 × 1.0 ml methanol. The methanol fraction was centrifugated under vacuum until dry.

HPLC of both KW-2149 and M-16 was performed on a C_{18}-µ Bondapack reversed-phase column (150 × 6.0 mm inner diameter; YMC-Pack ODS-AQ/YMC Co., Kyoto, Japan) preceded by a Lichro-CART 4-4 guard column packed with Lichrospher 100/RP-8 (5 µm) media (Whatman, Clifton, NJ). KW-2149 and M-16 were eluted isocratically at ambient temperature with a solvent composition of 53% methanol and 47% water; the flow rate was 1 ml/min. For M-18, the same column configuration was used; however, the mobile phase was changed. A mixture of 0.05 M phosphate buffer (pH 7.0)/acetonitrile (70/30 v/v) was used for elution of M-18. KW-2149, M-16 and M-18 were detected at 375 nm using a Shimadzu SPD-6AV UV-VIS variable wavelength spectrophotometric detector (Shimadzu, Kyoto, Japan). Porfiromycin can be used as an internal standard; however, precision and reproducibility of the method allows quantitation of KW-2149, M-16 and M-18 by the method of an external standard. Plasma standard curves were obtained by plotting the resulting peak areas against the known concentrations added to blank plasma from an unrelated subject. Sensitivity of the HPLC assay was 5 ng/ml for KW-2149, 1 ng/ml for M-16 and 15 ng/ml for M-18. Recovery of KW-2149, M-16 and M-18 was 50%, the average recovery of KW-2149 and M-16 was 97 ± 5% and for M-18 was

98 ± 7%. The variation coefficient of variation of both assays was less than 7% (n = 6).

Pharmacokinetic calculations

The area under the plasma concentration–time curve (AUC; expressed in nG min/ml) for KW-2149, M-16 and M-18, was calculated using the trapezoidal method extrapolated to infinity. Total body clearance (TBC) was calculated using standard equations. Since the follow-up of plasma concentrations of either KW-2149 or metabolites could not exceed two or three apparent terminal half-lives, the precise determination of the half-life of the elimination phase was not possible.

Results

Patient characteristics

Thirty-seven patients received 97 courses of KW-2149 through 10 dose levels. The patient characteristics are shown in Table 1. One patient was initially considered to have an adenocarcinoma of unknown origin, but post-mortem examination revealed a primary non-small cell lung carcinoma (NSCLC).

Five patients were ineligible, and are therefore considered inevaluable for toxicity and response. A 50 year old man suffered from metastatic colorectal carcinoma. He received KW-2149 at the starting level of 5 mg/m^2 and developed grand mal seizures 10 days after drug administration. A computed tomography (CT) scan showed multiple brain metastases. A 42 year old woman suffering from metastatic NSCLC received one course at 25 mg/m^2. She had received prior treatment with cisplatin and was therefore considered ineligible. One patient started treatment in spite of discrete elevations of liver function tests; his two cycles of chemotherapy are left out of the current analysis, although these two courses were without toxicity other than mild nausea. Two more patients were ineligible due to the fact that they were entered with an abnormal LVEF. One patient was struck by cerebrovascular bleeding 10 days after treatment. This course was not evaluable due to lack of follow-up. Four more cycles were inevaluable for different reasons, mainly due to incomplete data.

Table 1. Patient characteristics

No. of patients (total/evaluable)	37 (33)[a]
Sex (male/female)	25/12
Median age, years (range)	57 (41–70)
ECOG performance status (range)	1 (0–2)
Prior therapy	
chemotherapy	20
radiotherapy	1
both	10
none	6
Tumor type	
colorectal	20
NSCLC	6
mesothelioma	3
pancreas	2
colorectal + pancreas	1
kidney	1
breast	1
ovary	1
unknown primary adenocarcinoma	2

[a]Including one patient who received two courses at 100 mg/m^2 and two courses at 75 mg/m^2.

Dose escalation scheme

A total 86 evaluable courses were administered to 32 patients. This trial was initiated at a dose of 5 mg/m^2 as a single intravenous bolus injection once every 3 weeks. Further dose escalation was performed following a modified Fibonacci series (Table 2). One patient received two courses at the 100 mg/m^2 dose level and two further courses at the 75 mg/m^2 level. These are counted separately within both dose level steps.

Toxicity

Treatment with KW-2149 was well tolerated. No acute hypersensitivity reactions were observed. No patients suffered temperature rise suggestive of drug fever.

Table 2. KW-2149 dose escalation scheme

Dose (mg/m^2)	Number of patients	Number of courses
5	2	6
10	3	11
17	3	7
25	3	5
35	2	5
47	3	8
60	2	6
75	7[a]	16
90	4	15
100	3	9
	32	86

[a]Including one patient who received two courses at 100 mg/m^2 and two courses at 75 mg/m^2.

The first patient treated at the dose level of 5 mg/m^2 complained of aching pains along the vein through which the drug was injected. These symptoms were observed during the actual infusion and subsided immediately afterwards. No external signs of phlebitis or thrombosis were observed. This patient had been pretreated with 5-fluorouracil and had suffered several episodes of superficial phlebitis during those therapies. This was the only patient who received the drug as a 5 min infusion. From the second patient on, all patients received the drug over a 10 min period. These symptoms were not observed in any of the other patients. From the 75 mg/m^2 level, discrete alopecia was observed in several patients, starting after the second course and continuing as long as treatment was ongoing.

Gastrointestinal toxicity

Nausea and vomiting occurred from dose level 35 mg/m^2 onwards. Out of the five courses given at that level, grade II nausea was observed on four occasions. From the 47 mg/m^2 dose step, grade III nausea/vomiting occurred in nine of 54 courses. In one patient at the 100 mg/m^2 dose level who suffered from grade III vomiting during the first two courses, pretreatment with corticosteroids and metoclopramide effectively prevented nausea and vomiting during subsequent courses. On the whole nausea and vomiting were mild, occurring in general within 1 h after drug administration and in some patients lasting for up to 24–36 h. Although in several patients changes in liver tests were observed, no straightforward evidence for drug induced liver toxicity could be substantiated. In two patients a grade III change in liver tests was observed. In the first patient, treated at the 100 mg/m^2 dose level, an elevated AST level was observed on day 5 after drug administration. After 1 week the transaminase levels normalized. The other patient with grade III liver toxicity, limited to an increase in the γGT values, was also treated at the 100 mg/m^2 level. Here the time course after drug administration was similar to the first case.

Pulmonary toxicity

Pulmonary toxicity was observed in six patients (Tables 3 and 4). This toxicity was clinically significant in four patients. At the 60 mg/m^2 dose level, one patient with an extensive pretreated ovarian carcinoma developed moderate changes in pulmonary function with a discrete decrease in ventilatory measurements, but substantial changes in diffusion capacity. Both chest X-ray and chest CT scan showed bilateral pleural effusion. A bronchoscopy failed to show any evidence of tumor involvement but revealed substantial interstitial fibrosis in transbronchial biopsies. This toxicity became evident only 2 months after ending her four courses with KW-2149. In the 75 mg/m^2 group, two patients had a decline in lung function, without or with only minor symptoms. At that stage we were not completely convinced that drug toxicity was causing these changes. Further dose escalation to 90 mg/m^2 failed to show lung toxicity even in patients receiving up to six courses. This prompted us to escalate further to 100 mg/m^2. With the dose of 100 mg/m^2, however, all three patients suffered from grade III pulmonary toxicity. In this group all patients started complaining of exertional dyspnea and of a dry, non-productive cough. On auscultation no clear changes were observed except a decrease in basal alveolar breathing due to effusions. Measurements of carbon monoxide diffusion capacity showed substantial changes. Further measurements of ventilation mechanics showed changes which could be due to the development of pleural effusions. In five out of six patients showing this pulmonary toxicity, bilateral pleural effusions developed simultaneously with the dyspnea and in three of these patients pleural fluid was further examined. It was a clear exudate with no malignant cells nor increased inflammatory cells. In five patients pulmonary histology was obtained. This was done either by transbronchial biopsy or on autopsy. In four of these a variable degree of interstitial fibrosis with widening of the interalveolar septa was observed. Treatment with corticosteroids resulted in alleviating both the troublesome cough and to some degree the dyspnea.

Hematological toxicity

In general, hematological toxicity of KW-2149 was mild (Table 5). In this study the MTD for hematological toxicity remains undetermined. From the dose step of 60 mg/m^2 on, moderate leucopenia, i.e. grade I and II, and mild thrombocytopenia, grade III, occurred. In the only patient who received four courses of 60 mg/m^2, the kinetics of blood count recovery are suggestive of cumulative myelosuppression. There was no evident correlation between prior pretreatment and hematologic toxicity (Table 5). Even in the highest dose escala-

Table 3. Patients with lung toxicity

Dose level (mg/m^2)	Total dose (mg)	Tumor type[a]	Clinical symptoms[b]	Rx[c]	%DCO[d]	Pleural fluid	Therapy	Tumor response[e]
60	**240**	ovary	no	no	35.3	yes	none	PD
75	**225**	colon	no	yes	31.0	no	steroid	MR
75	**300**	NSCLC	yes	yes	32.5	yes	steroid	**PR**
100	**400**	NSCLC	yes	yes	46.0	yes	steroid	**PR**
100	**300**	NSCLC	yes	yes	22.0	yes	steroid	**PR**
100	**350**	PTU	yes	yes	36.8	yes	steroid	**PR**

[a]Histological type. [b]Presence or absence of pulmonary symptomatology. [c]Presence or absence of changes on chest X-ray. [d]Maximal change in total lung diffusion capacity expressed as a percentage. [e]Best tumor response observed.

Table 4. Overview of lung toxicity in phase I KW2149 i.v. bolus

Dose level (mg/m^2)	*N*	Cumulative dose (mg/m^2)	Lung toxicity (LT)[a]
47	15	188	no
	16	94	no
	17	94	no
60	18	**240**	**yes** (LT at 240)
	19	120	no
75	20	150	no
	21	75	no
	22	75	no
	23	150	no
	24	**225**	**yes** (LT at 225)
	25	75	no
	26	**300**	**yes** (LT at 150)
90	27	180	no
	28	180	no
	29	**540**	**no**
	30	**450**	**no**
100	31	**400**	**yes** (LT at 200)
	32	**300**	**yes** (LT at 200)
	33	**350**	**yes** (LT at 200)

[a]LT = dose at which lung toxicity could be detected.

Table 5. Hematological toxicity of KW-2149

	Dose level of KW-2149 (mg/m^2)			
	60	75	90	100
Total no. of patients	2	7	4	3
no prior therapy	0	3	2	3
moderate pretreatment	0	3	2	0
extensive pretreatment	2	1	0	0
Total no. of courses	6	15[a]	15	9
No. of courses with:				
leucopenia III	0	3	0	0
leucopenia IV	0	1	0	1
thrombopenia III	1	1	0	0
thrombopenia IV	0	0	0	1
No. of courses grade III/IV	1/6	3/15	0/15	2/9

[a]Including two courses of a patient who received her two first courses at the 100 mg/m^2 dose level.

tion step only one out of three patients had grade III or IV thrombocytopenia or leucopenia. No episodes of fever or infection were observed. No bleeding episodes were recorded nor did any of our patients need platelet transfusions. The patients with grade I and II leucopenia and thrombocytopenia all had been pretreated.

Cardiac toxicity

Although in three patients a decrease of 15% below the baseline ejection fraction was observed, no clear evidence for drug related cardiac toxicity was considered. Two of these patients were extensively pretreated with anthracyclines. No symptoms or signs of cardiovascular toxicity were observed. The changes in ejection fraction were not accompanied by changes on the ECG.

Antitumor activity

Antitumor activity was observed in four patients, all of whom also suffered pulmonary toxicity. Antitumor activity was observed in three patients with NSCLC and in one patient with a tumor of primary unknown origin (PTU). Antitumor activity was observed in one patient in the 75 mg/m^2 step. This 57 year old woman was initially considered to have a PTU with a solitary lung and multiple bone and liver metastases. Pathology of a surgical bone biopsy showed the presence of a mucus secreting, well-differentiated adenocarcinoma. After treatment with KW-2149, the levels of the tumor markers, CEA and CA 125, decreased. The lesion in the lung was clearly reduced in size after two courses and she continued to receive a total of four courses, after which treatment was interrupted because of lung toxicity. She died of progressive disease.

On autopsy a primary lung carcinoma was diagnosed. Microscopy of the lung showed patchy areas with extensive fibrosis. At the 100 mg/m^2 dose level all three patients demonstrated antitumor activity. The first patient was a woman with metastatic NSCLC. She received four courses of KW-2149 and had a definitive reduction of her primary lung tumor after two courses. She continued therapy to receive a fourth cycle after which a PR was observed. Pulmonary toxicity prohibited further therapy. The second patient was a 59 year old man with a metastatic adenocarcinoma of the lung with hilar and mediastinal lymph nodes, and rapidly progressive bone metastases. His serum CEA level at the start of therapy was 518 ng/ml. He received three courses of KW-2149 each at the scheduled 3 weekly interval. This was accompanied with a major improvement of his pains and a rapid and sustained fall in the CEA level. He had a PR after his second cycle. Treatment was interrupted because of pulmonary toxicity. On corticosteroid treatment his dyspnea ameliorated together with the measured DCO but failed to return to baseline values. His disease progressed 2 months after his last course. The third patient in this group was a 57 year old female with a large liver mass (Figure 2). Histology of a liver biopsy showed an adenocarcinoma and further investigations failed to reveal other sites of disease. Of all tumor markers analyzed only the CA 19.9 level was elevated up to 300 U/ml. She had a major PR after four cycles of KW-2149 but also suffered from lung toxicity which improved after steroid therapy. She suffered from grade IV leucopenia and thrombocytopenia every second cycle. After the fourth cycle she developed brain metastases.

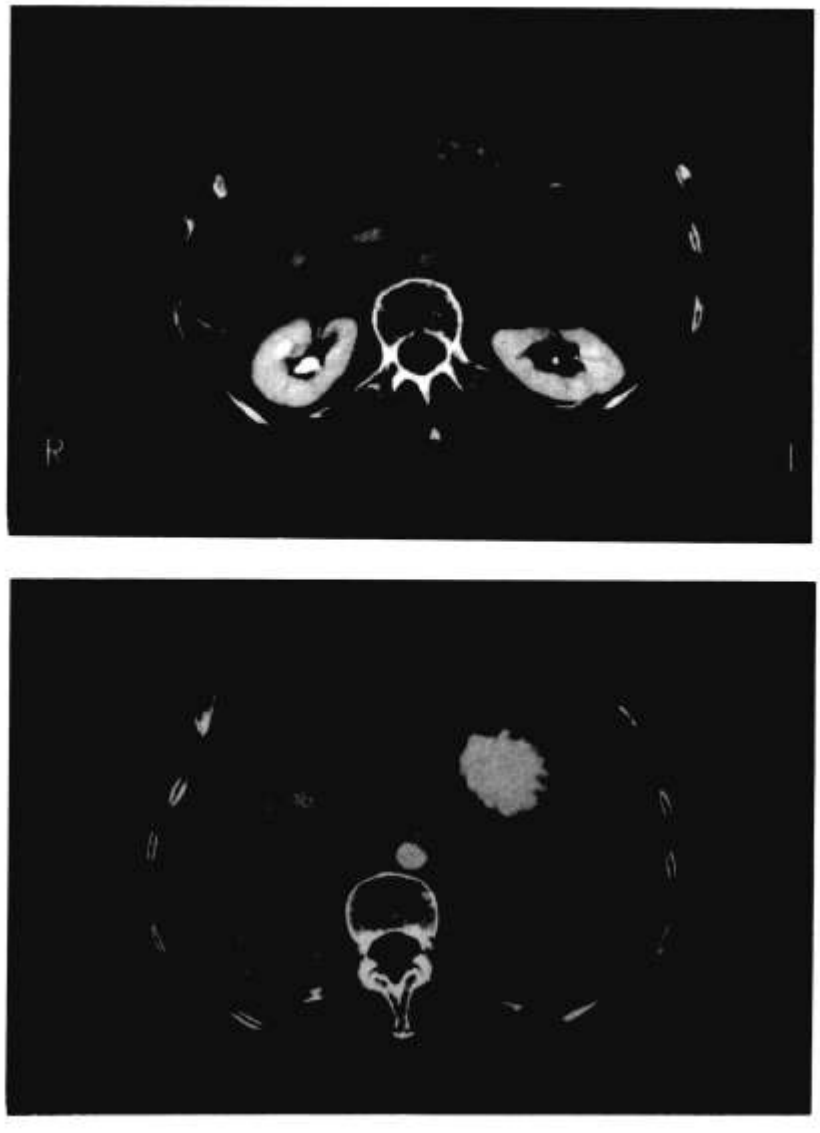

Figure 2. Comparative CT scans of the liver in a patient treated with KW-2149 at 100 mg/m^2. Prior to therapy (A) and after three (B) courses.

Table 6. Mean pharmacokinetic parameters of KW-2149, M-16 and M-18

Dose	N	KW-2149			M-16			M-18		
		C_{max} (ng/ml)	AUC (ng min/ml)	TBC (ml/min/m^2)	C_{max} (ng/ml)	AUC (ng min/ml)	$T\ C_{max}$ (min)	N	C_{max} (ng/ml)	$T\ C_{max}$ (min)
10	2	1261 ± 28	13202 ± 651	759 ± 37	100 ± 10	3130 ± 1458	16 ± 1	–	–	–
17	3	1517 ± 268	14810 ± 4226	1316 ± 296	166 ± 63	4330 ± 1886	18 ± 3	–	–	–
25	4	2941 ± 1273	31024 ± 12984	1308 ± 435	276 ± 42	9016 ± 710	19 ± 1	–	–	–
35	3	3371 ± 743	31175 ± 9131	1320 ± 349	470 ± 95	10034 ± 2204	15 ± 2	–	–	–
47	3	4292 ± 636	38796 ± 2828	1306 ± 93	459 ± 133	17368 ± 7406	17 ± 3	–	–	–
60	3	5475 ± 1315	55757 ± 9491	1138 ± 185	449 ± 11	18593 ± 173	18 ± 1	–	–	–
75	6	5704 ± 734	46831 ± 5598	1694 ± 158	633 ± 102	24157 ± 2749	14 ± 1	–	–	–
90	4	5602 ± 1195	44795 ± 10882	2353 ± 716	447 ± 41	16235 ± 1728	15 ± 1	2	200 ± 26	11
100	3	8561 ± 923	78937 ± 10644	1321 ± 202	585 ± 65	20353 ± 1403	16 ± 1.7	3	213 ± 15	11

Data are shown as the mean ± SE. C_{max}, the maximum conentration measured. AUC, area under the curve. TBC, total body clearance. $T\ C_{max}$, time measured from the start of the bolus infusion until the highest M-16 concentration was measured.

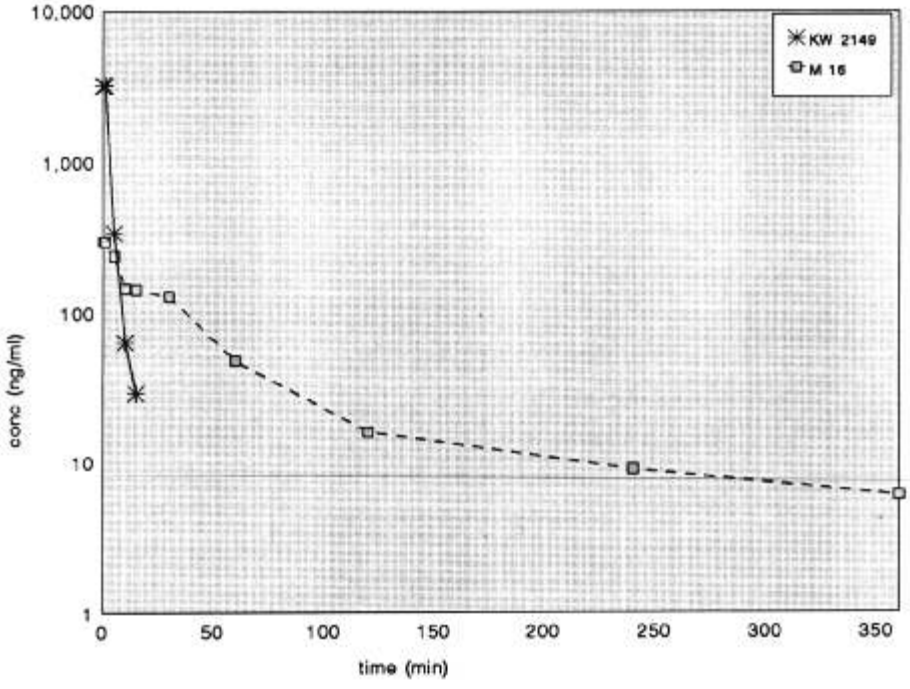

Figure 3. A representative time–concentration graph of KW-2149 and M-16 in a patient treated at 90 mg/m^2 demonstrating the rapid decay of KW-2149 and the detection of M-16.

Pharmacokinetics

Drug monitoring was initiated from the second level onwards, i.e. 10 mg/m^2. Complete plasma concentration–time curves were obtained for the first cycle of KW-2149 in all patients, in 20 patients for the second cycle, in three patients for three cycles and in one patient for all four cycles. Mean pharmacokinetic data for both the parent compound and M-16 are listed in Table 6. The plasma concentration–time curve of KW-2149 is best characterized by a rapid disappearance of the parent drug with a calculated half-life between 1.5 and 5 min (Figure 3).

In only 10 of the 55 cycles, KW-2149 could be quantified in plasma up to 60 min after the end of the administration. Both peak KW-2149 concentrations (r = 0.957, p = 0.0001) and AUC of KW-2149 (r = 0.895, p = 0.0011) increased linearly with dose. The relationship between TBC and dose was less constant (r = 0.633, p = 0.067). The plasma concentration–time curve of M-16 was characterized by its immediate presence in plasma at the end of the infusion with a maximum concentration 5 min after the end of the infusion. Both C_{max} (r = 0.827, p = 0.006) and AUC (r = 0.861, p = 0.0029) of M-16 increased linearly with the dose of KW-2149. In most patients M-16 could be measured up to 240 min after the end of the infusion with an apparent $t_{1/2}$ ranging from 10 to 115 min. M-18 could be determined in one patient treated at 90 mg/m^2 and in all three patients treated at the 100 mg/m^2 step. In these four patients M-18 behaved similarly to M-16, being detectable immediately at the end of the infusion but remaining detectable in plasma only for 30 min. No correlation was observed between hematological toxicity or lung toxicity and any of the pharmacokinetic data.

Discussion

KW-2149 is one of many MMC analogs synthesized with substituents at the 7-*N* position. This position is of particular importance since it controls the reduction potential of the quinone moiety of the molecule. These analogs were selected from extensive structure–activity studies because of supposed increased activity. The search for MMC analogs with both an increased activity and an improved toxicity profile led to the synthesis and development of KW-2149.[11] This agent, with an incrased molecular weight compared to MMC (598.7 versus 350) and superior water solubility, forms on weight basis 20-fold more effectively DNA–DNA and DNA–protein cross-links. The antitumor activity in preclinical testing proved to be at least equal or superior to MMC. Toxicity testing data were reassuring, with both LD_{10} and LD_{50} values in mice on a weight basis 4–5 times higher than those of MMC.

Of particular importance was the superior hematological tolerance of KW-2149 with regard to thrombocytopenia in a murine model.[16]

In the present phase I study the initial dose was selected as 1/10th of the LD_{10} in mice being 5 mg/m^2. As shown in Table 2, multiple escalations were necessary before significant toxicity occurred. We ended up administering 100 mg/m^2, a 20-fold increase compared with our initial dose. This is an impressive dose compared with MMC, even considering the difference in molecular weight. The hematological tolerance was excellent with only moderate leucopenia and thrombocytopenia occuring in the lower doses in patients with extensive pretreatment, of whom many had been pretreated with escalated-dose chemotherapy and growth factor support. In the non-pretreated patients hematological toxicity was mild. The blood counts of one patient in the 100 mg/m^2 group, without prior chemotherapy exposure and without evidence of bone marrow involvement, showed a tendency for cumulative toxicity with nadirs occurring after every other course. These results corroborate the preclinical data on bone marrow toxicity.

The dose-limiting toxicity was the pulmonary toxicity occurring in six patients. The pattern of toxicity was one of interstitial lung disease with shortness of breath, a dry cough and interstitial accentuation on chest X-ray. In several patients this was accompanied by exudative pleural effusions. Treatment with steroids early on improved symptoms and reversed lung function parameters to some extent. The pathological data showed increased interalveolar widening with an increased deposition of collagen and elastin. In the pathological specimens available, often obtained only after institution of steroid therapy, no overt increased interstitial inflammation could be observed. Some dose–effect relationship was observed for lung toxicity in these patients (Tables 3 and 4). This was exemplified in all three patients in the highest dose group developing significant pulmonary toxicity. An increased risk for the development of pulmonary toxicity also seems to be dependent on total cumulative dose given.

Neither in mice, rats or dogs had this type of toxicity been observed. It is reasonable to speculate on the increased reducibility of the quinone part in KW-2149 compared with MMC and hence to a greater ease and increased efficiency of oxygen radical formation as a possible explanation for this type of toxicity. This drug definitively produces lung toxicity of the interstitial type, in the dose and schedule used. The toxicity seems to be dose related; either dependent on dose per individual course and/or on cumulative dose (Tables 3 and 4).

The knowledge of MMC-induced interstitial pneumonitis as a dose-dependent side effect observed in patients with a cumulative dose in excess of 30 mg/m^2 is very similar to the toxicity observed with KW-2149.[19-22] Antitumor activity was observed in four patients. All three patients at the highest dose step had a partial response. The antitumor activity in three patients with NSCLC seems to corroborate the preclinical data.[18] The duration of the responses is difficult if not impossible to interpret as in all patients therapy was interrupted prematurely because of lung toxicity. Whether the occurrence of lung toxicity and the observed responses in lung cancer both point to a common mechanism is a matter of current research.

The pharmacokinetic analysis showed rapid clearance of the parent compound and rapid appearance of both M-16 and M-18. In general the data do not fit a compartment model. Most KW-2149 plasma concentration–time curves demonstrate a monophasic decline with a short half-life of 1.5–5 min. At doses of 90 mg/m^2, a biphasic decline was frequently observed with prolonged elimination half-lives up to 35 min. This dose-dependency is only partially related to the increase of AUC with increasing dose. Nevertheless, the TBC was found to be relatively stable. We have a substantial number of concentration–time data on second, third and fourth courses in the same patients (data not shown). We always observed a similar pattern as the one described in the first cycle. The relatively short half-life is comparable with the data on murine pharmacology with [^{3}H]KW-2149 showing the half-life of the unchanged drug to be 9.7 min compared with 18 min for MMC.[23]

The immediate detectability of M-16, the methylsulfide form, and of M-18, the symmetrical disulfide dimer, is most likely due to plasma and/or red blood cell metabolism, with a possible role for enzymes such as S-methyltransferase and disulfide reductase. Pharmacokinetic data of KW-2149 obtained in this trial showed a linear and proportional relation between dose administered and AUC. This linear correlation with the dose of the parent compound is also present for peak plasma concentration and AUC of M-16. One can postulate that KW-2149 is a prodrug for M-16 and especially for M-18, which are both active *in vitro* but with different activities in cell lines with different resistance mechanisms.[14] In mice a similar disposition and metabolism was observed with production of M-16 and M-18 in the absence of lung toxicity.[23] Pharmacokinetic analysis, including detailed analysis of production of M-16 and M-18, was not helpful in predicting lung toxicity. This phase I study has demonstrated that

the major dose-limiting toxicity of KW-2149 and/or its metabolites is lung toxicity. The hematological profile of KW-2149 is clearly superior to the one of MMC compared on a molar base. The antitumor efficacy is interesting and warrants further evaluation with different scheduling and further study of the mechanisms of toxicity. Attempts to apply a pharmacokinetically guided dose escalation scheme in phase I trials is of limited benefit with drugs causing non-dose-dependent and/or subacute and/or unexpected toxicities.[24] In the first patient at 60 mg/m^2, lung toxicity became evident only weeks after ending the fourth cycle. The cumulative dose dependency of the lung toxicity, underscores the importance of treating patients in phase I trials for a substantial number of courses.

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