A phase I and pharmacokinetic study of the selective, non-peptidic inhibitor of matrix metalloproteinase BAY 12-9566 in combination with etoposide and carboplatin

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Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that degrade the extracellular matrix during the processes of invasion, metastasis and angiogenesis. BAY 12-9566 (BAY) is a selective, non-peptidic biphenyl inhibitor of MMPs, with nanomolar inhibitory activity against MMP-2, -3 and -9, and anti-invasive, anti-metastatic and anti-angiogenic activity in a variety of tumor models. This phase I study of oral BAY was conducted to evaluate the safety and pharmacokinetics of BAY when administered in combination with etoposide (VP-16) or in combination with VP-16 and carboplatin (CBDCA) in subjects with advanced cancer. The first cohort of patients ($n=8$) received a cycle of VP-16, 60 mg/m^2, followed 1 week later by a fixed daily oral dose of BAY, 800 mg b.i.d., to which three potential possible doses of VP-16 (low dose: 60 mg/m^2; mid dose: 90 mg/m^2; high dose: 120 mg/m^2) were added every 3 weeks as tolerated. The second cohort ($n=5$) received VP-16 (120 mg/m^2) and CBDCA (AUC=5) followed 1 week later by a fixed daily oral dose of BAY (800 mg) b.i.d., to which VP-16 (120 mg/m^2) and CBDCA (AUC=5) were added. Dose-limiting toxicity (DLT) was defined as toxicity grade 3 or above. Maximum tolerated dose was declared if two or more patients experienced DLT. A performance status of 0–2 and acceptable organ function were required for eligibility. Plasma concentrations of BAY and VP-16 were measured to investigate pharmacokinetic interactions. Eight eligible patients with a variety of tumor types (median age 64 years, range 44–76) were enrolled in the first cohort, six of who whom completed all three levels of VP-16. Progressive disease occurred in five of the eight patients; three patients continued on study with treatment. Drug level and pharmacokinetics analysis of BAY and VP-16 were also determined. The combination of BAY and VP-16 was tolerable in the first cohort, permitting enrollment of the second cohort. In the second cohort ($n=5$), the combination of BAY, VP-16 and CDBCA was intolerable at the doses used due to excessive hematologic toxicity in the first five patients enrolled. Pharmacokinetics and toxicity analysis was performed for this group of patients. Only Level 1 of treatment was completed for Cohort II. At this point the study was halted due to toxicity and the results of an interim analysis that failed to demonstrate sufficient clinical activity of this compound in other clinical trials. We conclude that the combination of BAY and VP-16 was well tolerated. However, the combination of BAY, VP-16 and CDBCA produces significant hematologic toxicity. Findings from this study may help to direct further studies with other inhibitors of MMPs. *Anti-Cancer Drugs* 16:997–1002 © 2005 Lippincott Williams & Wilkins.

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Introduction

Matrix metalloproteinases (MMPs) are a family of at least 20 secreted and membrane-bound zinc endopeptidases [1]. These enzymes are capable of degrading all of the components of the extracellular matrix. MMPs are believed to be essential for the metastatic process in that they contribute to tumor invasion and they play a part in angiogenesis. Preclinical data suggests an association between various members of the MMPs and metastatic potential. In particular, MMP-2 and -9 have been implicated in tumor invasion in human cancers [1].

BAY 12-9566 (BAY) is a non-peptidic biphenyl inhibitor of MMPs, with nanomolar inhibitory activity against MMP-2, -3 and -9, and anti-invasive, anti-metastatic and anti-angiogenic activity in a variety of tumor models. Preclinical studies of BAY, using cultured tumor cells and human tumor xenografts, demonstrated a wide spectrum of anti-metastatic activity including activity against the Lewis lung carcinoma. While no effect of BAY was seen on primary tumor growth, the total number of metastases was inhibited by 86% compared with untreated control animals [2]. In the B16.F10 murine melanoma model, BAY given p.o. b.i.d. for 14 days inhibited s.c. tumor

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growth by 50% and the total number of metastases by 58% [2]. In nude mice implanted with the human colon cancer cell line HCT 116 and treated with BAY for 42 days, tumor growth was decreased by 40% and tumor invasion by 60% [3,4]. Other studies of BAY in animal models have shown inhibition of pulmonary metastasis, and anti-invasive and anti-angiogenic properties [5,6]. In phase I studies, BAY has shown mild gastrointestinal toxicity as well as nasal congestion, headache and drowsiness [7–11]. A recently published National Cancer Institute of Canada Clinical Trials Group phase I study of BAY in combination with 5-fluorouracil/leucovorin demonstrated no clinical or pharmacological interaction. Neutropenic fevers and diarrhea were the dose-limiting toxicities (DLTs) [12].

We performed a phase I study of BAY combined with etoposide (VP-16) and carboplatin (CBDCA) as a potential combination of an anti-metastatic agent with chemotherapy for advanced solid malignancies. We assessed the safety, tolerability and pharmacokinetics of BAY and VP-16 alone as well as the combination of BAY, VP-16 and CBDCA.

Materials and methods

Patient selection

Patients with solid tumors refractory to standard chemotherapy or for whom no effective therapy existed were eligible for entry into this trial. All patients were >18 years of age, with an ECOG performance score of 0–2, life expectancy of ≥ 12 weeks and confirmed pathologic diagnosis of cancer. Laboratory inclusion criteria included: ANC $\geq 1500/mm^3$, platelets $\geq 100\,000/mm^3$, bilirubin $< 1.5\times$ upper limit of normal (ULN), ALT and AST $< 4\times$ ULN, and creatinine $< 1.5\times$ ULN. Women of child-bearing age also required a negative pregnancy test. The Mayo Clinic Institutional Review Board granted approval and all patients signed consent before entering the study. Exclusion criteria included: major surgery within 14 days, large field radiation therapy, chemotherapy or immunotherapy within 4 weeks, prior radiation to $\geq 25\%$ of the bone marrow, prior MMP inhibitor, uncontrolled central nervous system disease and New York Heart Association class 3/4 heart disease.

Study design and toxicity criteria

The study was designed as a 'cohorts of three' phase I clinical trial with dose escalation and two separate patient cohorts [13]. DLT was defined as toxicity above grade 3 that was probably or possibly related to one of the study medications. Maximum tolerated dose (MTD) was declared if more than two patients experienced DLT. National Cancer Institute Common Toxicity Criteria version 3.0 was used to determine and grade toxicities.

Treatment

BAY was supplied by Bayer (West Haven, Connecticut, USA). For Cohort I, eight patients received VP-16 60 mg/m^2 i.v. on day 1. One-week later BAY 800 mg was started twice daily by mouth. A second dose of VP-16 60 mg/m^2 was given at week 3. VP-16 was escalated to 90 and 120 mg/m^2 at weeks 6 and 9, respectively, if the previous dose was without DLT as tolerated in the same patient cohort. Toxicity evaluation and pharmacokinetic sampling were performed for all patients in this cohort. For Cohort II, five patients received VP-16 120 mg/m^2 followed by CBDCA AUC = 5 (using the Calvert Formula) on day 1. One-week later BAY 800 mg by mouth twice a day was started. A second cycle of VP-16 120 mg/m^2 followed by CBDCA AUC = 5 was given at week 3. If Cycle 2 was to be tolerated CBDCA was escalated to AUC = 6 with the third cycle (week 6) in the same patients. Toxicity assessment and pharmacokinetics were also performed for this cohort of patients.

Specimen collection

Blood samples (5 ml) were drawn via venopuncture or indwelling i.v. cannula into heparin-containing tubes, and immediately cooled in a slurry of ice and water. Plasma was separated by low-speed centrifugation in a refrigerated centrifuge maintained at 4°C. Following centrifugation, plasma was transferred to a plastic tube, capped and immediately frozen at −70°C. Blood samples for etoposide measurements were drawn on the first day of each cycle before drug administration, at the end of the 60-min infusion and at the following times after the end of the 1-h infusion: 10, 20, 40, 60, 120 and 240 min, and 8, 12 and 24 h. On Cycles 2 and 3, blood specimens were drawn before the infusion, at the end of the infusion, and 2 and 8 h after the infusion. Blood samples for BAY measurements were drawn the day before beginning etoposide treatment on Cycle 2 (2 weeks after beginning BAY, 3 weeks after the first course of etoposide), and on the first day of Cycles 2 and 3. Specimens were collected before drugs were given on that day, and 2, 4, 8 and 12 h after the BAY dose was given.

Pharmacokinetics

Etoposide plasma concentrations were determined by a modification of the reverse-phase high-performance liquid chromatography (RP-HPLC) procedure of Sinkule and Evans [14]. BAY plasma concentrations were determined using the method described by Heath *et al.* [11].

Data analysis

Statistical analysis was carried out under the auspices of the Mayo Clinic Cancer Center (MCCC) Statistics Unit Phase I program. A standard set of algorithms has been constructed to process phase I studies. The program includes textual and graphical summaries of toxicity and other clinical data along with laboratory variables. Descriptive statistics form the basis of analysis with

simple correlation coefficients used to relate the clinical and laboratory data.

Pharmacokinetic parameters were calculated by non-compartmental analysis using the program WINNONLIN (version 1.5). Etoposide AUC_{0-t} values were determined by trapezoidal approximation from the start of treatment to the last detectable plasma concentration (C_{last}). Residual area after C_{last} was calculated by $AUC_r = C_{last}/k_{el}$, where k_{el} is the terminal elimination rate constant calculated by linear least-squares regression of the last three or four time points in the plasma concentration–time profiles. The elimination half-life was calculated by $t_{1/2} = 0.693/k_{el}$. BAY $AUC_{0-12\,h}$ (steady-state AUC) values were determined by trapezoidal approximation of plasma concentration–time data collected during the 12-h drug administration interval.

Results

A total of thirteen patients were enrolled into this study, eight patients in Cohort I and five patients in Cohort II. Of the eight patients in Cohort I, six completed all three levels of VP-16. For the five patients in Cohort II, only Level 1 was completed due to significant hematologic toxicity. Patient characteristics are shown in Table 1. For Cohort I, median age was 64 years (range 44–76). For Cohort II, median age was 57 years (range 42–66). The majority (53%) of patients had lung cancers, 23% had colon cancers, and the rest had melanoma, thymoma and an unknown primary tumor. Two had received no prior chemotherapy and four had prior radiotherapy. The study population had an excellent performance status. All patients were available for toxicity analysis and for pharmacokinetic studies.

Table 1 Characteristics of patients enrolled into the phase I study of BAY, VP-16 and CBDCA

	Cohort I: BAY + VP-16 ($n=8$)	Cohort II: BAY + VP-16 + CBDCA ($n=5$)
Median age (years)	64 (44–76)	57 (42–66)
Gender		
male	5	3
female	3	2
Tumor type		
lung non-small cell	3	4
colon	2	1
melanoma	1	
thymoma	1	
unknown	1	
Prior chemotherapy		
yes	7	4
no	1	1
Prior radiation		
yes	2	2
no	6	3
Performance status (ECOG)		
0	3	2
1	4	2
2	1	1

Pharmacokinetic results

Etoposide pharmacokinetics were characterized in seven patients enrolled in Cohort I to investigate potential drug–drug interactions (Table 2). Five of those seven patients received 2 cycles of treatment and three patients continued to receive 3 cycles of treatment. Two of the first seven had disease progression and the other two had significant toxicity with the regimen. Plasma profiles for one patient treated with 3 cycles of etoposide and BAY are illustrated in Fig. 1. Following 60 mg/m^2 infusion, the mean peak plasma etoposide concentration was 11.7 ± 2.8 μg/ml. The disappearance of etoposide was biphasic with a mean terminal elimination half-life of 293 min. The mean clearance and steady-state volume of distribution values were 17.9 l/h/m^2 and 7411 ml/m^2. The etoposide clearance was modestly reduced (below 15%, not statistically significant) when administered with BAY during Cycle 2 (Figs 1 and 2). The etoposide peak plasma concentration was higher in Cycle 3 compared to Cycles 1 or 2 when a greater etoposide dose (90 mg/m^2) was administered, but the etoposide clearance was not substantially altered compared to administration alone.

BAY steady-state pharmacokinetics were characterized in seven patients enrolled in Cohort I to investigate potential drug–drug interactions (Table 2). Six of those seven patients received 2 cycles of treatment and four patients continued to receive 3 cycles of treatment. The mean steady-state clearance of BAY when given alone was 452 ± 83 ml/h. The value was unaffected by coadministration of 60 or 90 mg/m^2 etoposide (Figs 2A and 2B).

Toxicity

The combination of BAY and VP-16 was tolerable in the first cohort studied, but an excess of hematological toxicity was seen when using combined BAY, VP-16 and CDBCA (Table 3). In Cohort I, six of eight patients were able to complete all three dose levels. The other two patients had excessive toxicity. However, excessive grade 4 hematological toxicity was seen in Cohort II at the initial dose level. In this cohort, grade 4 neutropenia was seen in four of the five patients, grade 4 anemia or thrombocytopenia was present in one patient each. On the other hand, gastrointestinal toxicity manifested mostly as diarrhea was seen as grade 2 toxicity in four patients (Table 4). Other less common gastrointestinal side-effects were anorexia, constipation and vomiting. Grade 2 alopecia was seen in three of the cases in both cohorts. Grade 1 fatigue occurred in only two patients (Table 3).

Disease response

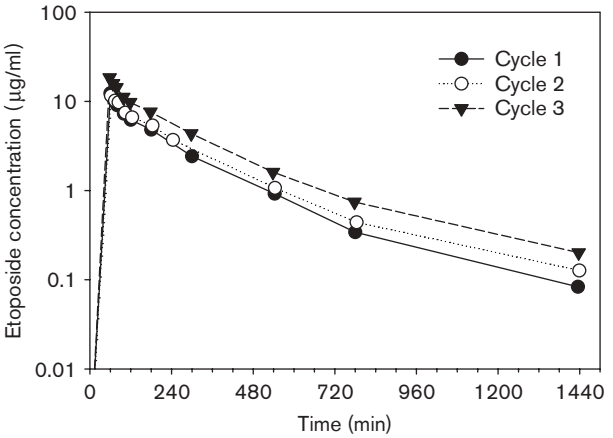
Three patients in Cohort I had stable or improving disease with the BAY and VP-16 combination. Tumor types included one patient each with thymoma, lung cancer and unknown primary cancer. All studies with BAY have been halted at our institution because

Table 2 Pharmacokinetic schedule for BAY, VP-16 and CBDCA

	Cycle 1	BAY initiation	BAY steady-state pharmacokinetics	Cycle 2	Cycle 3
Week	0	1	day 21	3	6
VP-16 pharmacokinetics[a]	×			×	×
CBDCA pharmacokinetics[b]	×			×	×
BAY pharmacokinetics[c]		negative control	×	×	×

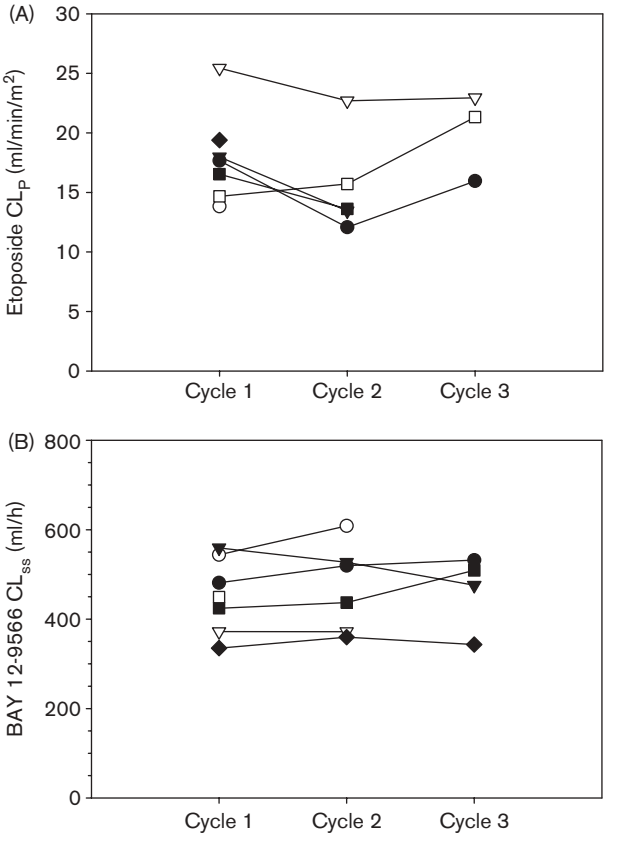
[a]Time 0, 10, 20 and 40 min, and 1, 2, 4, 8, 12 and 24 h.
[b]Time 0, 1 h 5 min, 1 h 20 min, 1 h 40 min, and 2, 3, 4, 8 and 24 h.
[c]Time 0, 2, 4, 8 and 12 h.

Fig. 1



Representative plasma profiles for one patient who received 3 cycles of treatment. Cycle 1: etoposide 60 mg/m^2; Cycle 2: etoposide 60 mg/m^2 + BAY 800 mg b.i.d.; Cycle 3: etoposide 90 mg/m^2 + BAY 800 mg b.i.d.

Fig. 2



(A) Graphs of etoposide plasma clearance. (B) BAY steady-state clearance for each cycle of treatment. Individual patient data is represented by symbols. Individual patient data for each cycle are linked by solid lines.

of a shortened time to cancer progression and survival time in another separate study of patients with small cell lung cancer.

Discussion

Current evidence shows that MMPs, a family of zinc-dependent endopeptidases, play a critical role in tumor invasion and metastasis. BAY is a non-peptidic biphenyl inhibitor of MMPs, with nanomolar inhibitory activity against MMP-2, -3 and -9, and anti-invasive, anti-metastatic and anti-angiogenic activity in a variety of tumor models [15]. Preclinical studies of BAY using cultured tumor cells and human tumor xenografts demonstrated a wide spectrum of anti-metastatic activity including activity against the Lewis lung carcinoma [2]. While no effect of BAY was seen on primary tumor growth, the total number of metastases was inhibited by 86% compared to untreated control animals [2]. Similar effects on tumor growth, and anti-invasive, anti-metastatic and anti-angiogenic activity has been documented in other animal studies [3–6].

We and others have shown that BAY alone at a dose of 800 mg orally twice daily is well absorbed and tolerated [7–11]. BAY has been studied in combination with chemotherapy agents such as 5-fluoruracil/leucovorin, doxorubicin, paclitaxel and carboplatin [12,16,17]. Thrombocytopenia was a significant side-effect in the BAY and 5-fluoruracil/leucovorin phase I trial [12]. Apparently, this side-effect was not seen in a parallel study performed by the same group with BAY and doxorubicin [16]. Furthermore, the combination of BAY and paclitaxel/carboplatin was safe with hematologic toxicity in the form of thrombocytopenia and mild gastrointestinal toxicity [17].

Table 3 Etoposide pharmacokinetics for patients enrolled in Bayer study D97-024

Patient	Cycle 1 (60 mg/m^2 VP-16)					Cycle 2 (60 mg/m^2 VP-16 + 800 mg/m^2 b.i.d. BAY)					Cycle 3 (90 mg/m^2 VP-16 + 800 mg/m^2 b.i.d. BAY)				
	C_{max} (μg/ml)	$t_{1/2z}$ (min)	AUC (μg/ml·min)	Cl (ml/min/m^2)	V_{ss} (ml/m^2)	C_{max} (μg/ml)	$t_{1/2z}$ (min)	AUC (μg/ml·min)	Cl (ml/min/m^2)	V_{ss} (ml/m^2)	C_{max} (μg/ml)	$t_{1/2z}$ (min)	AUC (μg/ml·min)	Cl (ml/min/m^2)	V_{ss} (ml/m^2)
1	12.9	344	3398	17.7	8895	12.8	566	4972	12.1	7608	13.5	365	5640	16.0	9510
2	9.5	457	4338	13.8	9542										
3	8.8	371	3382	18.0	9762	10.4	356	4519	13.5	9032					
4	12.3	160	2359	25.4	5796	11.6	169	2644	22.7	5647	18.5	179	3922	22.9	5992
5	15.8	231	3705	16.5	5364	14.0	325	4500	13.6	6036					
6	14.0	253	4090	14.7	5567	12.7	241	3819	15.7	5722	16.4	209	4220	21.3	6293
7	8.5	232	3094	19.4	6952										
Mean	11.7	293	3481	17.9	7411	12.3	331	4091	15.5	6809	16.1	251	4594	20.1	7265
SD	2.8	102	656	3.8	1944	1.4	150	908	4.2	1476	2.5	100	918	3.7	1950
% CV	24.1	35	19	21.4	26	11.1	45	22	27.2	22	15.5	40	20	18.2	27

Table 4 Summary of the number of patients developing toxicities (grade) potentially related to BAY, VP-16 and CBDCA

Toxicity	Cohort I (BAY/VP-16)				Cohort II (BAY/CBDCA/VP-16)			
	1	2	3	4	1	2	3	4
Hematologic								
anemia			1				1	1
neutropenia	1	3	1			1		4
thrombocytopenia	4		1	1		1	1	1
Gastrointestinal								
anorexia	5	1			3			
constipation		1						
diarrhea	4				2			
nausea	2	1	1		4			
vomiting	21		1		1			
stomatitis	1							
Other								
alopecia	2	3			1	3		
fatigue	2							
headache	1							

We designed this phase I study to determine the toxicity, clinical response and pharmacokinetic profiles of BAY in combination with VP-16 and CBDCA in patients with advanced malignancies. The combination of BAY and VP-16 was tolerable with six of eight patients in Cohort I completing all three dose levels. For this group of patients, mild drug-related hematologic and gastrointestinal toxicities were seen. These toxicity profiles of BAY plus etoposide are similar to the ones reported in other phase I studies [7,8]. Three patients (38%) in Cohort I had stable or improving disease with treatment (one patient each with thymoma, lung or unknown cancer). Progressive disease occurred in the other five (62%) patients.

For the five patients enrolled into Cohort II, excessive grade 4 hematologic toxicity was seen at the initial dose level with no episodes of neutropenic fever. The grade 4 neutropenia seen in four (of five) patients treated with the combination of BAY, VP-16 and CBDCA is much higher than those hematologic toxicities reported with the combinations of BAY and 5-fluorouracil/leucovorin, BAY and doxorubicin, and BAY and carboplatin/paclitaxel, which is likely related to the regimen of VP-16 and CBDCA used in this study [12,16,17]. Accrual was halted with a planned dose reduction. All studies with BAY were halted because of a shortened time to cancer progression and survival time in another separate study of patients with small cell lung cancer. Thus, no dose reduction of the combination was possible.

Although complete etoposide pharmacokinetics were obtained for only a few patients treated with the combination of BAY, the present data suggest that it is unlikely that there is a substantial interaction between etoposide and BAY. In particular, BAY in combination with VP-16 has little effect on VP-16 clearance. Pharmacokinetics for BAY, VP-16 and CBDCA have not been evaluated due to the termination of the study.

The observed benefit and limited toxicity seen with the combination of BAY and VP-16 suggests that further studies with an inhibitor of MMPs in combination with VP-16 should be considered. Although BAY is not being developed further, the results of this study indicate that non-peptidic biphenyl inhibitors of MMPs are unlikely to alter the pharmacokinetics of VP-16. The fact that we could not administer the standard doses of VP-16 + CBDCA with BAY raises the possibility that BAY can alter CBDCA disposition. If BAY alters CBDCA protein binding or renal clearance, this could have

resulted in the greater than expected toxicity. In this study, we did not evaluate the effect of BAY on CBDCA disposition, but studies of agents with similar chemical structure should take this finding into consideration.

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