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Phase I study with the DNA sequence-specific agent adozelesin

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Adozelesin, a synthetic analog of the antitumor antibiotic CC-1065, is a novel cytotoxic agent which inhibits DNA synthesis by binding to the minor groove of the DNA helix. Preclinical studies have shown a broad spectrum of activity against a variety of murine and human tumor xenograft models. We conducted a phase I study of adozelesin to (i) determine a recommended dose for phase II testing using a 10 min i.v. infusion, (ii) characterize the toxic effects of the drug using this schedule and (iii) document any antitumor activity observed. Adozelesin was administered as an i.v. infusion every 6 weeks. CBC and biological parameters were performed weekly. The starting dose of 10 $\mu g/m^2$, corresponding to 1/30 the mouse equivalent lethal dose, was escalated, according to a modified Fibonacci scheme, until dose-limiting toxicity was encountered. Forty-seven adult patients with solid malignancies were entered in the study. Successive dose levels used were 10, 20, 33, 50, 70, 105, 120, 150 and 180 $\mu g/m^2$. The main toxic effect was myelosuppression, which was dose limiting. The maximally tolerated dose was defined as 180 $\mu g/m^2$. A minor response with a 4 month duration was reported in one previously treated patient with melanoma. We conclude that the recommended phase II dose of adozelesin given as a 10 min infusion is 150 $\mu g/m^2$, repeated every 4 weeks.

Key words: Adozelesin, alkylating agents, DNA minor-groove binders, phase I.

Introduction

Adozelesin (U-73, 975) is a potent synthetic cyclopropylpyrroloindole (CPI) analog of the cytotoxic DNA binding antibiotic CC-1065.[1–5] The parent compound, originally isolated from broths of *Streptomyces zelensis*,[6] was found to be a potent inhibitor of DNA synthesis that stabilized the native B-form DNA helix by binding in the A–T-rich regions of the minor groove.[7–9] CC-1065 did not bind to denatured or single-stranded DNA, RNA or proteins.[10] Although CC-1065 had shown promising efficacy initially as an anticancer drug,[10,11] irreversible liver and kidney toxicity and delayed deaths were observed in mice.[12] Research was then oriented to structuring less toxic analogs,[13–17] and adozelesin (Figure 1) was chosen as the clinical candidate due to its superior solubility, stability in aqueous formulations and extreme potency.[18] The drug was extremely cytotoxic against various animal and human tumor cell lines, including the B16 melanoma and the pancreas 02 tumors which are known to be resistant to almost all available drugs.[19–24] It was found to be more potent than doxorubicin, cisplatin, 5-fluorouracil and cyclophosphamide against human gynecologic cancer cell lines.[25] Some observations implied that human tumor cells might be even more sensitive to adozelesin than rodent cells.[19]

In animal trials, adozelesin did not cause serious toxicity and delayed deaths. The main dose-limiting toxicity (DLT) was myelosuppression (Earhart, personal communication). No obvious schedule dependence could be discerned and phase I testing was undertaken with a variety of schedules,[26] including a brief infusion given daily for 5 days every 3 weeks, a 24 h continuous infusion, a brief infusion every 4 weeks and a brief infusion every 6 weeks.

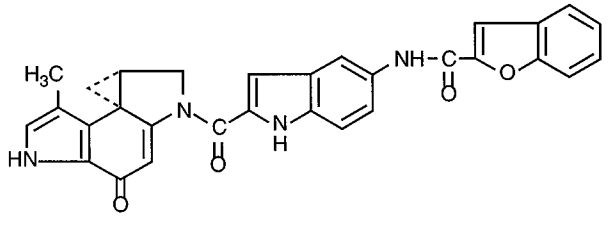

Figure 1. Structure of adozelesin.

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We report the results of a phase I clinical trial of adozelesin administered as a 10 min infusion every 6 weeks. Six weeks was chosen as the interval for treatment, because there was a concern that delayed hepatic toxicities (such as those seen with the parent compound CC-1065) might be seen 4 weeks after treatment with adozelesin. The main objectives of the trial were the determination of the maximally tolerated dose (MTD) and the pharmacokinetics of adozelesin. Further objectives were to determine the recommended dose and schedule for eventual phase II therapeutic trials, and to collect observations on the antitumor effect of adozelesin when such events occur. Our preliminary results were previously presented as abstracts.[27,28]

Patients and methods

Eligibility

Patients with a histologically confirmed solid tumor that was refractory to conventional therapy or for which no conventional therapy existed were eligible for this study. Measurable disease, while desirable, was not required. Other eligibility criteria included the following: (i) age 18 years or older; (ii) ECOG performance status 0–2 and anticipated life expectancy of at least 6 weeks; (iii) adequate organ function (white blood cell count $> 4000/\mu l$ and platelet count $> 100\,000/\mu l$); normal hepatic function (total bilirubin < 2.0 mg/dl, and aspartate aminotransferase and alanine aminotransferase normal or $< 2 \times$ upper limit of normal in case of liver involvement by tumor); normal renal function (creatinine < 2.0 mg/dl and normal urinanalysis); and serum calcium and electrolyte levels within normal limits (controlled diabetes permitted); (iv) no chemotherapy or radiotherapy within 4 weeks prior to entry onto protocol (6 weeks for mitomycin C or nitrosoureas); and (v) no active infectious process, severe malnutrition or intractable emesis. All premenopausal female patients with childbearing potential had to have a negative pregnancy test at entry and had to use adequate contraceptive methods during the study. All patients gave written informed consent in accordance with federal and institutional guidelines.

Treatment plan

Adozelesin was provided by the Upjohn Company (Kalamazoo, MI) as sterile glass ampules containing 1.2 ml of non-aqueous concentrate at a concentration of 1.0 mg/ml, comprised of a 2:1 mixture of polyethylene glycol 400:ethanol, with 10% Tween 80. Ampules were stored frozen (−20°C) and protected from direct sunlight, as well as fluorescent and UV light sources. The concentrate was diluted to 1/10 strength with 5% dextrose in water, and then the approximate dose was drawn into a plastic disposable syringe and injected over a 10 min period through the side-port of a freely flowing i.v. line containing 5% dextrose in water. At the first and the second level, three patients experienced a brief period of shortness of breath soon after receiving the drug, accompanied by facial flushing in one of them. After that, the schedule of administration was modified, using a Harvard pump, with an infusion rate not exceeding 1 ml/min. The starting dose of adozelesin was 10.0 $\mu g/m^2$ given as a single-dose per course [1/30 mouse equivalent lethal dose (MELD) on the single dose schedule] with courses repeated every 6 weeks, for up to 1 year of treatment for each patient. A course was defined as 1 treatment at the planned dose plus a 41-day observation period thereafter.

Doses were escalated according to a modified Fibonacci scheme in cohorts of at least three patients. Two patients had dose escalations (from 20 to 30 $\mu g/m^2$ and from 70 to 105 $\mu g/m^2$), because they both had stable disease and had tolerated the lower dose well, and there were patients ahead of them who had tolerated the higher dose without difficulty. At least three evaluable patients were entered at each dose level and observed for at least 4 weeks before entry of the next patient. If toxicity of grade 3 or greater was noted in any of the first three patients at a dose level, then three additional patients were enrolled at that dose level. The MTD was defined as the dose level at which three or more of the six patients experienced any grade 3 toxicity or two of six patients experienced any grade 4 toxicity. An additional four patients had to be treated at a dose one level lower than the MTD and at least three of 10 patients were to receive a minimum of three courses each at this dose level to ascertain whether or not there was cumulative toxicity. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria.

Pretreatment evaluation

Pretreatment evaluation included a medical history, physical examination, electrocardiogram, chest radiograph and radiologic examinations needed for

disease measurement. A pretreatment complete blood cell count was performed, with differential and platelet count as well as blood chemistry, including the analysis of urea nitrogen, serum creatinine, glucose, electrolytes, calcium, phosphate, bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, albumin, total protein, uric acid, creatinine and prothrombin time. Urinalysis was also performed.

Evaluation during study

Before and during administration, concomitant treatments and acute drug effects were recorded. Patients were followed with weekly CBC, platelet count and differential. Weekly serum BUN, creatinine, glucose, electrolytes, calcium, phosphate, bilirubin (total), AST (SGOT) and alkaline phosphatase analyses were performed. If significant hematological or biochemical toxicity (grade 2 or higher) was encountered in any patient, subsequent blood sampling was done twice weekly in that patient and in all other patients who were treated at that dose level or at higher doses. The type of sampling was based on the nature of the toxicity which was observed.

Patients were formally evaluated for antitumor response after every 6 weeks of therapy. Those who tolerated treatment were permitted to continue on therapy as long as there was no evidence of tumor progession.

At the beginning of the trial, three patients receiving adozelesin had respiratory problems. A pulmonary toxicity of the drug was suspected and the protocol was amended with monitoring of pulmonary tests. These tests included corrected carbon monoxide diffusing capacity (DL_{CO}), spirometry and arterial blood gas measurements, which were all obtained at baseline and repeated prior to each treatment. Eligibility criteria were also modified to include patients with DL_{CO} no less than 60% of predicted normal (adjusted for hemoglobin).

Pharmacokinetic and bioanalytical methods

Pretreatment plasma and urine samples for adozelesin determinations were collected in the initial course. Heparinized whole blood specimens (5 ml) were collected before, and at −5, 0, 5, 10, 15, 20, 30, 40, 50 and 60 min after the end of the 10 min adozelesin infusion. Patient identification, date, time due and time actually collected were recorded for each sample. Plasma was immediately separated from formed elements by centrifugation for 5–10 min and was frozen for subsequent analysis. During and for 12 h following the initial adozelesin infusion for each patient, all urine was collected as 4 h cumulative collections. Each 4 h urine volume was recorded, and an aliquot (15 ml) was frozen for subsequent adozelesin and creatinine determinations.

Concentrations of adozelesin in human plasma were determined using a validated, HPLC method. Briefly, 1 ml plasma was supplemented with internal standard (U-78188) and plasma protein precipitated with 1 ml acetonitrile. The acetonitrile–plasma extracts were diluted with 1 ml water and loaded on a phenyl solid-phase extraction column, washed with water:acetonitrile (80:20 v/v) and eluted with water: acetonitrile (40:60 v/v). The solid phase extracts were transferred to conical polypropylene autosampler vials for HPLC analysis. The prepared samples were chromatographed on a reverse phase column (Zorbax® RX-ODS 250 × 4 mm [i.d.]; Dupont) using an autoinjector for sample injection and a flow rate of 1.0 ml/min. The mobile phase was acetonitrile: 50 mM ammonium acetate buffer (50:50 v/v), at pH 7.0. The mobile phase was switched to acetonitrile: water (90:10 v/v) after the internal standard had eluted and the column was washed for 6 min to elute highly retained interferences, and then returned to the 50:50 isocratic mobile phase to equilibrate the column for the next injection. The UV absorbance of the column effluent was monitored at 305 nm. The peak height measurements of adozelesin and the internal standard were used for calculation of standard curve statistics and concentration of adozelesin in unknown samples. Quantification of adozelesin was achieved using peak height ratios with concentrations of adozelesin back-calculated from the through-the-origin slope obtained by unweighted linear regression analysis. The method is linear for adozelesin concentrations from 1.0 to 100 ng/ml. The method has a lower limit of quantification of 1 or 2 ng/ml plasma dependent on the individual assay run. The analytical method used for human urine samples was essentially identical to the validated method used for the quantitative determination of adozelesin in human plasma. The lower limit of quantification for this method was 5 ng/ml in urine.

Results

Forty-seven patients were enrolled who received a total of 101 courses of treatment of adozelesin.

Patient characteristics are listed in Table 1. All of the patients were assessable for toxicity. One patient (dose level 150 $\mu g/m^2$) died during the post-treatment follow-up period, with hepatic failure due to massive metastases. Another patient (dose level 120 $\mu g/m^2$) died due to *Pseudomonas* pneumonia, 5 weeks after receiving the fourth course of treatment. Both patients had normal blood counts at this time. There were 29 males and 18 females with a median age of 60 years (range 23–79) (Table 1). Patients had a performance status of 0–2 (median 1). Thirteen patients (28%) had colorectal cancer; the remainder had prostate, melanoma, breast, sarcoma, lung, renal, gastric, pancreatic, hepatocarcinoma and ovarian cancers.

Table 1. Patient characteristics

Characteristic	No. of patients ($n = 47$)
Male/Female	29/18
Median years of age (range)	60 (23–79)
Performance status	
0	15
1	27
2	5
Prior therapy	
chemotherapy	16
radiotherapy	7
both	18
none	6
Diagnosis	
colorectal	13
prostate	8
melanoma	7
breast	5
sarcoma	4
non-small cell lung cancer	3
renal	2
gastric	2
pancreas	1
hepatocarcinoma	1
ovarian	1

Hematological toxicity

Myelotoxicity was the dose-limiting toxicity (Tables 2 and 3) and was more severe in patients who had undergone prior cytotoxic treatment. Twenty-six patients (56%) had neutropenia and 12 of them were grade 3 or 4. Thrombocytopenia was observed in 27 patients (57%) and 11 of them were grade 3 or 4. Anemia was documented in 38 patients (81%) and was grade 3 or 4 in six cases (13%). Anemia was thought to be worsened by adozelesin treatment in 13 patients. Thirty-two patients received two or more courses of treatment. Of these patients, 13 moved on to the next cycle without dose modification, while dose was increased in two patients and reduced in 11 patients because of hematological toxicity (two patients at dose level 70 $\mu g/m^2$, one patient at 120 $\mu g/m^2$, seven patients at 150 $\mu g/m^2$ and one patient at dose level 180 $\mu g/m^2$). Treatment was delayed for the same reason in four patients (all were from level 7 at a dose of 150 $\mu g/m^2$). However, one of these four patients had to receive concomitant radiotherapy to the lumbar spine, which might have contributed to his prolonged thrombocytopenia and leukopenia.

Tables 2 and 3 summarize the hematologic toxicities observed with adozelesin. Neutropenia was dose dependent. Grade 3 neutropenia was first observed at 120 $\mu g/m^2$ (40% of patients). At 150 and 180 $\mu g/m^2$, respectively, 50 and 67% of patients had greater than grade 2 neutropenia. The granulocyte nadir occurred on day 15 (range 2–50) and the median duration was 7 days (2–37). For doses above 120 $\mu g/m^2$, thrombocytopenia was reported in 40% of patients. The nadir occurred on day 20 (range 6–42) and median duration was 7 days (range 2–27). Twenty-two of the 42 patients (47%) were heavily pretreated. A heavy pretreatment was defined as chemotherapy which was very likely to produce lasting marrow injury (more than three different

Table 2. Number of patients with hematological toxicity at each dose level

	Grade	Level (total no. of patients in that level)								Sub-total	Overall (47)	
		1 (4)	2 (7)	3 (3)	4 (3)	5 (5)	6 (10)	7 (12)	8 (3)		Total	(%)
Anemia	1–2	1	5	1	2	4	6	10	3	32	38	(81)
	3–4	0	0	0	0	1	3	2	0	6		
Leukopenia	1–2	0	1	0	1	2	5	7	1	17	29	(62)
	3–4	0	0	0	0	1	4	5	2	12		
Neutropenia	1–2	1	1	1	0	2	2	6	1	14	26	(56)
	3–4	0	0	0	0	0	4	6	2	12		
Thrombocytopenia	1–2	0	1	0	0	2	5	6	2	16	27	(57)
	3–4	0	0	0	0	1	4	5	1	11		

Table 3. Hematological toxicity of adozelesin

Dose ($\mu g/m^2$)	Level	No. of patients	No. of cycles	Course 1		All courses	
				Neutrophils	Platelets	Neutrophils	Platelets
10	1	4	7	6.0 (3.3–7.3)	406 (139–570)	5.4 (1.5–7.3)	227 (139–570)
20	2	7	17	5.3 (1.8–8.2)	338 (165–738)	5.3 (1.7–9.3)	311 (143–738)
33	3	3	6	3.2 (1.7–3.7)	254 (150–306)	3.4 (1.7–4.6)	249 (150–306)
50	4	3	4	2.7 (2.3–5.6)	232 (221–256)	2.9 (2.3–5.6)	232 (221–268)
70	5	5	13	2.1 (1.1–8.3)	157 (48–352)	2.0 (1.1–8.3)	140 (26–352)
120	6	10	20	1.2 (0.3–3.8)	106 (7–239)	1.5 (0.2–3.8)	92 (7–239)
150	7	12	30	1.0 (0.1–2.8)	95 (7–293)	1.3 (0.1–2.8)	95 (7–293)
180	8	3	4	0.4 (0.04–1.6)	88 (4–105)	0.5 (0.04–1.6)	54 (4–105)

regimens, e.g. nitrosoureas, mitomycin C or carboplatinum) and/or a prior radiotherapy to 30% or more of the bone marrow. Severe neutropenia in course 1 was more prevalent among the heavily pretreated cases (5 versus 2), but the difference was not statistically significant ($p = 0.414$). Meanwhile, severe thrombocytopenia (six patients) was observed solely among heavily pretreated patients and the difference was statistically significant ($p = 0.022$) (Table 4).

Non-hematological toxicities

Adozelesin was generally well tolerated by the patients, with most of the non-hematologic toxicities being mild to moderate (grade 1 or 2). Non-hematological toxicities are detailed in Table 5.

Hyperglycemia was observed in 37 patients (79%) and three of them were severe (grade 3–4). Hyperglycemia and hypocalcemia, which was evident in 18 (38%) of our patients, were observed in preclinical animal studies. Sixteen of the 18 patients with hypocalcemia also had hyperglycemia, but this association was not statistically significant ($p = 0.95$). Lower levels of post-treatment serum calcium were correlated with lower levels of serum albumin, which possibly contributed to the hypocalcemia.

Liver function tests were frequently elevated (28 cases with elevated alkaline phosphatase, 25 cases with elevated transaminases and seven cases with hyperbilirubinemia). However, adozelesin was thought to be the cause of the abnormal function tests in five of the 24 patients with elevated transaminase values (20%).

An increase of serum creatinine (grade greater

Table 4. Thrombocytopenia and dose modification according to the dose level and pretreatment of patients

Patient no.	Adozelesin dose ($\mu g/m^2$)	Pretreatment	Platelet nadir (cycle 1)	Platelet nadir (no. of cycles)	Dose reduction
7-01	150	+++	122	35 (2)	yes
7-02	150	+	204	106 (2)	yes
7-03	150	++	156	131 (2)	yes
7-04	150	+++	102	45 (3)	yes
7-05	150	0	79	58 (2)	yes
7-06	150	+	293	206 (3)	yes
7-07	150	+	95	–	–
7-08	150	++	139	19 (3)	no
7-09	150	+	7	–	–
7-10	150	+++	19	–	–
7-11	150	0	78	67 (3)	yes
7-12	150	+	52	52 (1)	yes
8-01	180	0	105	54 (2)	yes
8-02	180	0	88	–	–
8-03	180	+++	4	–	–

+++: heavy pretreatment: more than three different regimens of chemotherapy or chemotherapy which is very likely to produce lasting marrow injury (mitomycin C, nitrosoureas or carboplatinum) and/or prior radiotherapy to 30% or more of the bone marrow.
++: two to three different regimens of chemotherapy.
+: one regimen of chemotherapy.

Table 5. Number of patients with non-hematological toxicity at each dose level

	Grade	Level (total no. of patients on that level)								Sub-total	Overall (47)	
		1 (4)	2 (7)	3 (3)	4 (3)	5 (5)	6 (10)	7 (12)	8 (3)		Total	(%)
Elevated alkaline	1–2	1	4	3	2	1	6	5	0	22	28	(60)
phosphate	3–4	0	0	0	1	1	1	3	0	6		
Elevated transaminase	1–2	1	3	1	1	1	6	6	1	20	25	(53)
	3–4	0	0	1	0	1	1	2	0	5		
Elevated bilirubin	1–2	0	0	1	0	1	0	1	0	3	7	(15)
	3–4	0	0	0	0	0	1	3	0	4		
Elevated creatinine	1–2	0	4	0	0	1	2	4	0	11	12	(26)
	3–4	0	0	0	1	0	0	0	0	1		
Hyperglycemia	1–2	2	3	3	2	4	7	11	2	34	37	(79)
	3–4	0	0	0	1	0	2	0	0	3		
Nausea/vomiting	1–2	1	2	2	0	0	3	7	0	15	18	(38)
	3–4	0	1	0	0	0	0	2	0	3		
Anorexia	1–2	2	1	1	0	0	0	4	0	8	9	(19)
	3–4	0	0	0	0	0	1	0	0	1		
Infection	1–2	1	1	0	0	1	3	1	0	7	9	(19)
	3–4	0	1	0	0	0	1	0	0	2		

than 0) during treatment was noted in 12 patients (26%). However, only one patient, who had prostate cancer with obstructive uropathy, had a grade 3 elevation of serum creatinine, which was thought to be unrelated to adozelesin treatment.

Other non-hematologic effects (grade greater than 2) included: nausea–vomiting (three patients), infections (two patients), malaise (one patient) and anorexia (one patient). These toxicities were mild and did not appear to be dose dependent. The infections (pneumonia and urinary infection) were not associated with neutropenia.

At the beginning of the trial, four of seven patients who were treated at the first and second dose levels of the study (10 and 20 $\mu g/m^2$) developed shortness of breath and changes on their chest X-rays. All of these patients had pre-existing disease in the lung. The first patient was a 60-year-old man with a long history of silicosis and adenocarcinoma of the lung. The second patient was a 62-year-old man with a history of chronic obstructive pulmonary disease with periodic exacerbations and adenocarcinoma of the lung. Both of these patients, who had also received thoracic radiotherapy, developed pulmonary infiltrates during treatment. The third patient had an adenocarcinoma of the rectum with metastatic spread in the liver, pelvis (bone) and the lung. The fourth patient had metastatic adenocarcinoma of the prostate and had received X-ray therapy to the breasts prior to receiving diethylstilbestrol. The accrual on the protocol was temporarily halted because of suspected pulmonary side effects from the adozelesin. These respiratory problems were not seen at the other site conducting this study. Because of this discrepancy and because the relationship between the drug and the pulmonary symptoms was uncertain, the investigators plus a panel of experts were assembled to review all of the patients' charts, clinical courses, X-rays and pathological data. The first and third patients had an autopsy, and the pathology revealed widespread metastatic disease of the lung. As a result of that review, it was concluded that the agent was probably not responsible for the pulmonary problems. Extensive additional animal testing in monkeys had also shown no pulmonary symptoms. The protocol was amended with a monitoring of pulmonary function tests. These earlier pulmonary problems were not seen in the subsequent patients treated. Extensive DL_{CO} and arterial blood gas monitoring showed no deteriorating pulmonary function.

One patient had chest pain for 3 min during infusion, but her electrocardiogram remained normal. Five patients had documented electrocardiogram changes after adozelesin treatment: nonspecific ST and T wave changes (two patients), temporary sinus tachycardia, arterial flutter and one premature ventricular beat. The relationship between these abnormalities and the infusion of adozelesin is uncertain, given the fact that patients in phase I studies frequently develop ECG abnormalities unrelated to drug administration.[29,30]

Other possible toxic effects observed (all less than grade 3) were (number of patients in parentheses):

diarrhea (3), constipation (3), weight loss (5), stomatitis (2), facial flush (3), headache (2), hyponatremia (2), hypokalemia (2), hyperkalemia (1), hyperchloremia (1), hypoglycemia (3), hypercalcemia (1) and proteinuria (1). Three patients had decreases of performance status during treatment.

Pharmacokinetic analysis

Plasma specimens were collected and adozelesin concentrations determined from 43 patients receiving doses ranging from 10 to 180 $\mu g/m^2$. The concentrations observed in patients receiving doses less than 150 $\mu g/m^2$ were generally below or only slightly above the limit of quantification of the assay. At the recommended phase II dose level (150 $\mu g/m^2$), maximum adozelesin concentrations ranged from 3.4 to 21.3 ng/ml. Concentrations decreased rapidly and were generally below the limit of quantitation (1 ng/ml) by 10–30 min after the end of infusion (see Figure 2). Therefore, a full characterization of the pharmacokinetics of adozelesin could not be carried out.

Urine specimens (pre-dose, 0–4, 4–8 and 8–12 h specimens) from five patients receiving the two highest dose levels (150 and 180 $\mu g/m^2$) were analyzed for adozelesin content. Detectable levels of adozelesin were not observed in any of the urine specimens assayed.

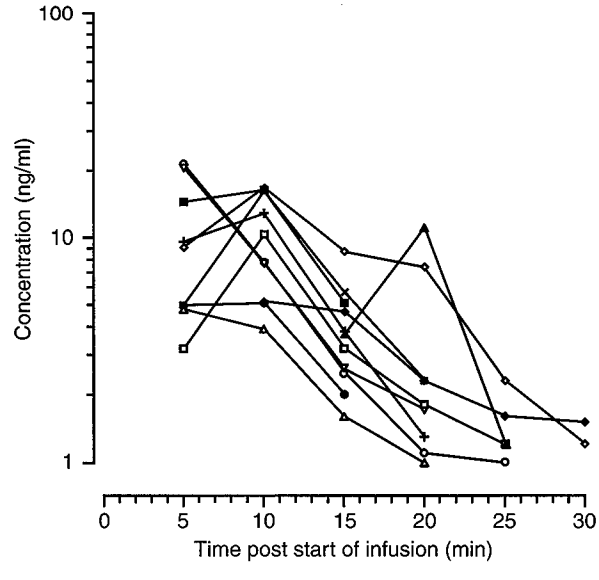

Figure 2. Semilogarithmic plot of adozelesin plasma concentrations in patients receiving 150 $\mu g/m^2$ as a 10 min i.v. infusion.

Tumor response

All of the patients were evaluable for toxicity and 36 of them had measurable lesions. Although tumor response was not the major goal of the study, patients with measurable disease were followed up for objective signs of response.

One patient with malignant melanoma had documented skrinkage of metastatic liver lesions (44%) during her first three courses of adozelesin. However, treatment had to be delayed and then terminated due to prolonged neutropenia and thrombocytopenia initially, and subsequent upper gastrointestinal bleeding (erosive gastritis probably due to naproxen sodium). This minor response had a 4 month duration.

The MTD was 180 $\mu g/m^2$, and neutropenia and, particularly, thrombocytopenia were DLTs. The recommended dose for phase II studies is 150 $\mu g/m^2$ administered every 4 weeks.

Discussion

Adozelesin is a novel cytotoxic agent of a new class of sequence-selective DNA alkylating agents which inhibit DNA synthesis by binding to the minor groove of the DNA helix.[10] Preclinical studies have shown a broad spectrum of activity against a variety of murine and human tumor xenograft models.[19–21]

In this trial, adozelesin was administered i.v. as a 10 min infusion repeated every 6 weeks. Forty-seven patients with advanced solid tumors received 101 courses of adozelesin at eight dose levels ranging from 10 to 180 $\mu g/m^2$. Significant myelosuppression was encountered at the highest dose level (180 $\mu g/m^2$). Myelosupression could be delayed and was occassionally prolonged, particularly thrombocytopenia. These patients were heavily pretreated and this characteristic appears to be a risk factor for the degree of myelosuppression and especially for thrombocytopenia. The non-hematologic toxicities of adozelesin in this study were mild. An original suspicion of pulmonary toxicity was not noted with additional experience with the drug. Moreover, no significant pulmonary toxicity was observed in the other phase I studies of adozelesin.[29,30] A minor response of 4 month duration was observed in a patient with melanoma.

Toxic effects reported to date on other phase I studies (such as a brief infusion given daily for 5 days every 3 weeks, a 24 h continuous infusion and a brief infusion every 4 weeks) have included thrombocytopenia and less severe neutropenia.[31,32]

The pattern of toxicity observed in this study is reminiscent of that seen with other groups of alkylating agents, especially nitrosoureas, which produce delayed myelosuppression that occurs 2–3 weeks after drug administration. Thrombocytopenia is more severe than neutropenia and is the main DLT. Perhaps, the use of thrombopoietin would allow the further development of this new class of agents.

The MTD of adozelesin was 180 $\mu g/m^2$ with neutropenia and, particularly, thrombocytopenia being the DLTs. The recommended dose for phase II studies is 150 $\mu g/m^2$ administered every 4 weeks. The toxicity profile, the clear *in vitro* antitumor activity of adozelesin and the observation in our study of a minor response in a patient with melanoma justify its continued evaluation. Phase II testing in patients with solid tumors is proceeding, with a brief infusion of 150 $\mu g/m^2$ given every 4 weeks.

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