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Phase I trial of Adozelesin using the treatment schedule of daily × 5 every 3 weeks

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Summary

CC-1065 is a unique alkylating agent that preferentially binds in the minor groove of double-stranded DNA at adenine-thymine-rich sites. Although it has broad antitumor activity in preclinical models its development was discontinued because of deaths observed during preclinical toxicology studies. Adozelesin is a potent synthetic analog that was chosen for clinical development because it had a similar preclinical antitumor spectrum, but did not produce deaths similar to CC-1065 at therapeutic doses. Phase I evaluations using a variety of Adozelesin treatment schedules have been conducted. This report describes our experience using a multiple dose treatment schedule. Endpoints including antitumor response, maximum tolerated dose, dose limiting toxicity as well as other toxicities and the recommended Phase II starting dose were determined. Adozelesin was given as a 10 minute IV infusion for 5 consecutive days every 21 days or upon recovery from toxicity. The dose range evaluated was 6–30 mcg/m^2/day. All patients had refractory solid tumors and had received prior cytotoxic treatment. Thirty-three patients (22 men: 11 women) were entered onto the study and 87 courses were initiated. Dose limiting toxicity was cumulative myelosuppression (leucopenia, thrombocytopenia). The maximum tolerated dose was 30 mcg/m^2/day. The only other significant toxicity was an anaphylactoid syndrome that occurred in 2 patients. A partial response was observed in a patient with refractory soft tissue sarcoma. The recommended Phase II starting dose of Adozelesin using a 10 minute IV infusion for 5 consecutive days is 25 mcg/m^2/day to be repeated every 4–6 weeks to allow recovery from myelotoxicity, based on our experience. Additional Phase I and II studies with Adozelesin are recommended.

Introduction

Anticancer drugs that covalently bind to DNA and consequently interfere with DNA replication (alkylating agents and platinum analogs) constitute a diverse group of agents with regards to their origin, structure and clinical usefulness [1]. The novel antibiotic, CC-1065 (NSC 298223) was isolated from broths of *Streptomyces zelensis*, and was found to have potent antitumor activity [2]. After determining its structure [3], CC-1065 was shown to stabilize the native DNA helix by binding in the minor groove [4, 5]. The minor groove binding was found to occur in two steps. The initial step, which was reversible; was followed by covalent bonding to the N^3 position of adenine [6]. Further, this minor groove binding to adenine was shown to preferentially occur in areas of DNA that contain adenine-thymine-rich sequences located on the 5' side. Thus, CC-1065 showed specificity for its preferred DNA alkylating site. CC-1065 demonstrated cytotoxicity at multiple stages of the cell cycle, but cell

kill was greatest for mitotic cells [7]. Development of CC-1065 as a potential clinical candidate was discontinued when it produced deaths in mice occurring 30–60 days following i.v. treatment at therapeutic doses [8]. Evaluation of synthetic analogs of CC-1065 allowed identification of the structural portion of the molecule responsible for the delayed deaths [9], and potent compounds with improved therapeutic ratios over CC-1065 [10]. Thus, analogs of CC-1065 were identified that demonstrated a broader antitumor spectrum with a decrease in toxic effects and no delayed death from which Adozelesin (NSC D615284, U-73915) was chosen as the first for clinical development.

Adozelesin demonstrated antitumor activity against murine leukemia models (P388, L1210) and solid tumor models (B_{16} melanoma, Lewis lung carcinoma, M5076 sarcoma, colon 38 carcinoma, pancreatic 02 carcinoma) [10]. Cytotoxicity was also demonstrated against human tumor xenografts (CX-1, LX-1, Caki-1, ovarian 2780 carcinoma) [10]. Toxicity studies in mice and rabbits indicated lesions at the injection site as well as toxic effects involving lymphoid tissue, bone marrow, liver, gastrointestinal tract and reproductive organs [10]. This is a report of our Phase I trial in adults with solid tumors using the treatment schedule of a 10 minute infusion for 5 consecutive days every 3 weeks.

Materials and methods

Drug

Adozelesin concentrate was supplied by the Upjohn Company (Kalamazoo, MI) in sterile glass ampoules that contained 1.2 ml of drug (1.0 mg/ml) dissolved in PET vehicle (60% polyethylene glycol 400, 30% ethanol, 10% Tween 80). These ampoules were stored at –20°C and protected from light until time of dilution for patient administration.

Patient eligibility and evaluation

All patients had confirmed solid tumors that were refractory to treatment. Performance status of grade 1 or better by Eastern Cooperative Group criteria [11] was required. Patients must not have received chemotherapy or radiotherapy within 4 weeks (6 weeks for nitrosoureas or mitomycin-c) of planned treatment with Adozelesin nor have received any previous treatment with bleomycin. Further eligibility requirements included adequate bone marrow function (peripheral white blood cell count [WBC] $\geq 4.0 \times 10^9$ cells/ml and platelets $\geq 100 \times 10^9$ cells/ml), renal function (serum creatinine ≤ 2.0 mg/dl and normal urinalysis), liver function (total bilirubin ≤ 2.0 mg/dl and normal transaminases); normal metabolic state (calcium, phosphorus and electrolytes within normal range); pulmonary function studies (spirometry, arterial blood gases on room air) with carbon monoxide diffusion capacity (DL_{CO}) no less than 60% of the predicted normal (corrected for hemoglobin); and a central venous access device for Adozelesin administration. A baseline physical examination, chest roentgenogram as well as other radiographic studies to document the extent of disease were required within one week of entering the study. Pregnant patients, those with an active infectious process or severe malnutrition were excluded. Written informed consent was obtained according to Federal and institutional guidelines.

Weekly physical examination and blood or serum studies to evaluate possible antitumor or toxic effects (particularly for liver, kidney, bone marrow) were performed. When hematological (WBC, platelets) toxic effects occurred, peripheral blood counts were performed twice weekly. Chest roentgenogram and pulmonary function studies were performed prior to beginning each treatment course to evaluate for pulmonary toxic effects. Repeats of previously positive radiological studies were performed every 6 weeks or sooner when indicated. Toxicity was evaluated using the National Cancer Institute Common Toxicity Criteria.

Treatment

Adozelesin concentrate was diluted (1:10) with PET vehicle that had been stored at room temperature. The appropriate patient dose was then placed in a 1.0 ml syringe and microbore IV tubing. Drug dilutions were made within two hours of drug ad-

ministration. The drug was administered over 10 minutes using a microsyringe pump into the side port (closest to the patient) of a 5% dextrose in water IV which was also fitted with microbore tubing and infusing at a rate that was ≤ 1 ml/min.

The starting dose was 6 mcg/m^2/day (1/10 the dose that produced lethality to 10% of mice following IV treatment with Adozelesin for 5 consecutive days). Dose escalations were based on a modified Fibonacci scheme until grade 2 or more toxicity was encountered. Thereafter, escalations were in 25% increments provided the grade 2 or more toxicity was limited to reversible myelosuppression. A minimum of 3 patients who had not received prior Adozelesin treatment were treated at each dose level until grade 2 or more toxicity was encountered. Then, a minimum of 3 additional Adozelesin naive patients were treated. Dose limiting toxicity (DLT) was defined as any grade 3 or greater toxicity that occurred in ≥ 3/6 patients treated with the same dose. A total of 10 patients were treated at the top 2 dose levels which included the maximum tolerated dose (MTD), *i.e.* the dose that produced ≥ grade 3 toxicity in ≥ 3/6 patients.

Patients were allowed to be retreated at 3 week intervals provided a) no toxicity occurred or had resolved to pretreatment levels, and b) there was no obvious evidence of disease progression at the time of retreatment. Patients whose maximum toxicity was grade 2 or 3 were retreated with 75% of their previous dose, while those who developed grade 4 toxicity were treated with 50% of their previous dose. Dose reductions were based solely on maximum toxicity grade.

Anti-emetics were not routinely used prior to treatment and no patients received hematologic growth factors.

Results

Dosage levels and patient characteristics

The starting dose (6 mcg/m^2) and 4 escalations (12, 20, 25, 30 mcg/m^2/day) were studied. Sixty-three full courses of treatment consisting of drug administration for 5 consecutive days along with 3 weeks of follow up were evaluated at these levels. Three courses were aborted, 2 because of acute toxicity during drug infusion and one because of protocol violation. Details of patient characteristics are shown in Table 1. Patients were entered between August, 1991 and August, 1993. The median number of prior chemotherapy regimens was 2. Some patients (17/33), as shown in Table 2, received 2 or more courses at the same dose level.

Table 1. Adozelesin daily × 5 Phase I patient characteristics

Men: women	22 : 11
Performance status	0–18
	1–15
Median age in years	57
Range	37–79
Prior cytotoxic therapy	All
Histologic diagnoses:	
Adenocarcinoma	
Colon or rectal	11
Renal cell	3
Breast	2
Prostate	2
Unknown primary	2
Lung	1
Gastric	1
Soft tissue sarcoma	6
Malignant melanoma	2
Squamous cell of tonsil	1
Mesothelioma	1
Malignant thymoma	1

Table 2. Adozelesin daily × 5 Phase I patients treated with multiple courses

mcg/m^2/d dose level	Number of retreatment courses					
	1	2	3	4	5	6
6	1[+]	1	0	1[*]	0	0
12	3	0	0	0	0	1[*]
20	6	0	0	0	0	0
25	2	0	0	1	0	0
30	2	0	0	0	0	0

[+]Number of patients retreated at each dose level with 1–6 courses.
[*]This patient was retreated with 4 courses at the 6 mcg/m^2/d dose level and 6 courses at the 12 mcg/m^2/d dose level.

Toxicities

Myelosuppression

The dose limiting toxicity was myelosuppression (leucopenia, thrombocytopenia) which is summarized as maximum toxicity grade per patient dose

Table 3. Adozelesin daily × 5 Phase I maximum patient myelosuppression

mcg/m²/d dose level	Patient #	Common toxicity grade			
		1	2	3	4
6 and 12	6	0	0	0	0
20	7	1	1	1	0
25	10	1	2	5	0
30	10	2	2	3	2

#Total number of patients treated at that level.

Table 4. Adozelesin daily × 5 Phase I maximum leucopenia

mcg/m²/d dose level	Courses #	Common toxicity grade			
		1	2	3	4
6 and 12	23	0	0	0	0
20	13	2	2	1	0
25	17	2	3	2	0
30	13	2	3	3	1

#Total number of courses started at that level.

Table 5. Adozelesin daily × 5 Phase I maximum thrombocytopenia

mcg/m²/d dose level	Courses #	Common toxicity grade			
		1	2	3	4
6 and 12	23	0	0	0	0
20	13	0	1	0	0
25	17	0	2	4	0
30	13	1	1	3	2

#Total number of courses started at that level.

level in Table 3. Myelosuppression was not observed at the first and second dose levels but became apparent at level 3. At the 20 mcg/m²/day dose level, 3 patients had myelosuppression (1 each with grades 1–3). The grade 3 occurred as the result of a protocol violation when the patient was retreated prior to resolution of myelosuppression. The patient with grade 2 required a two week delay to recover to required blood counts, than had grade 1 myelosuppression when retreated at a reduced dose. The patient who had grade 1 required a 1 week delay to recover required blood counts then had grade 2 myelosuppression when retreated without dose reduction. Half of those treated at the highest 2 dose levels had grade 3 or 4 myelotoxicity which appeared to be dose related since more patients had grade 4 at the highest dose studied. At the highest dose level (30 mcg/m²/day), 6/10 patients were retreated. Five of the retreated patients required dose reductions (4 at 22.5, 1 at 15 mcg/m²/day) on their second or third treatment with Adozelesin, and 4 of these had treatment delays that ranged 2–5 weeks. Four patients at the highest dose level were not retreated because of progressive disease and all of these had some degree of myelotoxicity (leucopenia = 2 grade 1, one each grades 2 and 3; thrombocytopenia = one each with grade 0, 1, 3, 4).

A summary of the maximum leucopenia and thrombocytopenia for these patients based on the number of treatment courses is shown in Tables 4 and 5. Quantitatively, leucopenia occurred more often (21 of 66 courses) than thrombocytopenia (14 of 66 courses). However, more grade 3 and 4 thrombocytopenia occurred (9 of 14 toxic courses) compared to leucopenia (7 of 21 toxic courses).

Others

Other toxicities were rare. Two patients (one each at 12 and 20 mcg/m²/day) had an acute reaction (anaphylactoid) syndrome that occurred midway through the 10 minute infusion. This syndrome consisted of dyspnea, chest tightness or pain and extreme anxiety. One of these patients (20 mcg/m²/day) also had hypotension, mild nausea and vomiting and brief orthostatic syncope that occurred at the same time as the anaphylactoid syndrome. One patient's symptoms occurred on day 2 of course 2 (12 mcg/m²/day) and the other patient's symptoms occurred on day one of course one. All symptoms resolved spontaneously within 20 minutes of stopping the infusion. Neither of these patients were rechallenged with drug infusion.

One patient treated at 25 mcg/m²/day developed progressive dyspnea after her fifth course of treatment which required hospital admission 7 days after completion of drug administration. Evaluations showed hypoxia (arterial pO_2 of 42 mm Hg on room air), normal chest roentgenogram, 60% and 38% decrease in DL_{CO} and forced vital capacity, respectively. Computerized axial tomography (CAT scan) of the chest showed nodular and linear patchy opacifications predominately in both lung bases suggestive of either acute viral infection or

drug reaction. The hypoxia was quickly and easily corrected (arterial pO_2 of 104 mm Hg) with oxygen (4L) administered by nasal cannula and the dyspnea resolved. Additional studies including ventilation-perfusion scan and pulmonary arteriograms showed no evidence of pulmonary embolus. Within 3 days the patient was able to maintain adequate oxygenation on room air without dyspnea and was discharged home 4 days after admission. Dyspnea did not re-occur during the 2 months follow up after hospital discharge off Adozelesin treatment. Pulmonary function studies obtained at the last follow up 2 months after hospital discharge showed persistent abnormalities of the DL_{CO} and forced vital capacity. The chest roentgenogram remained normal while the chest CAT scan showed improvement. Since no exact cause for these pulmonary alterations was found, they were ruled possibly Adozelesin related. No patients demonstrated consistent asymptomatic changes in pulmonary function studies or chest roentgenogram.

Responses

One patient with soft tissue sarcoma had a partial response of 7 months duration. Three patients, one each with melanoma, soft tissue sarcoma, rectal adenocarcinoma had stable disease for 7, 3 and 2 months, respectively.

Discussion

Alkylating agents are arguably the largest group of cytotoxic agents used to treat cancer patients. Although their chemical structures are quite varied, most share the ability to form positively charged carbonium ions in aqueous solutions. The positively charged species then attacks electron rich sites, including nucleic acids in DNA, to form a covalent bond. Adozelesin is a new synthetic alkylating agent that binds in the minor groove of double stranded DNA in regions that are rich in adenine and thymine [12, 13].

The DLT of Adozelesin with this daily × 5, 10 minute infusion every 3 weeks schedule was myelosuppression manifested as both leucopenia and thrombocytopenia. At both the highest and immediately preceding dose levels of Adozelesin (30 and 25 mcg/m^2/day, respectively) half of the patients experienced ≥ grade 3 myelosuppression, and all recovered; while at 20 mcg/m^2/day only 1 of 7 patients experienced grade 3 toxicity. Additionally, at doses ≥ 20 mcg/m^2/day the myelosuppression resulted in subsequent dose reductions for some patients as well as treatment delays in a subset of those to allow for recovery of blood counts. The only other significant toxicity that was definitely treatment related was anaphylactoid reactions that occurred in 2 of the 33 patients. The reactions were not dose related, appeared to be idiosyncratic and did not require specific treatment other than discontinuing the infusion.

Our recommended Phase II starting dose of Adozelesin is 25 mcg/m^2/day. This results in a total dose of 125 mcg/m^2 per treatment course using this schedule and is similar to the recommended Phase II starting dose per treatment course by other schedules, *i.e.*, 100 mcg/m^2 given by 24 hour continuous infusion [14] and 150 mcg/m^2 given by 10 minute infusion [15]. The DLT for these other Adozelesin Phase I solid tumor trials was myelosuppression (leucopenia, thrombocytopenia). Cumulative and prolonged myelosuppression was observed in some patients following treatment in our study and the 24 hour infusion study, but was not reported with the single 10 minute infusion schedule. Thus, repeated or prolonged drug exposure schedules may predispose to cumulative myelosuppression. Anaphylactoid reactions were not observed in the 24 hour infusion schedule, but were observed in the other two trials. Thus, prolonged infusion appears somewhat protective from this phenomenon. Similar reactions have been reported for other drugs dissolved in Cremephor EL® (polyethoxylated castor oil) and dehydrated alcohol [16]; indicating that polyethylanoglycol (contained in PET) and polyethoxylated derivatives of castor oil (contained in Cremephor EL®) have a propensity for induction of anaphylactoid reactions. We did not observe other toxicities that were clearly drug related. However, non-myelosuppressive and non-anaphylactoid side effects reported from other trials included mild to moderate fatigue, nausea, vomiting and anorexia [14, 15]. The non-myelosuppressive toxicities did not appear to be dose related.

Further clinical evaluations of Adozelesin including Phase II studies in patients with solid tumor malignancies as well as Phase I and II studies in hematologic malignancies are warranted given its favorable toxicity profile and broad spectrum of preclinical antitumor activity.

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