Cardiotoxicity of Histone Deacetylase Inhibitor Depsipeptide in Patients with Metastatic Neuroendocrine Tumors

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Abstract

Purpose: This phase II study was undertaken to assess objective response and toxicity of histone deacetylase inhibitor depsipeptide in patients with neuroendocrine tumors.

Experimental Design: A total of 15 patients with metastatic neuroendocrine tumors received a 4-hour i.v. infusion of depsipeptide at 14 mg/m^2 on days 1, 8, and 15 every 28 days. Tumor response was assessed at 8-week intervals using Response Evaluation Criteria in Solid Tumors. Most patients were chemo-naïve (n = 12) but receiving long-acting octreotide for carcinoid syndrome (n = 11). All patients had Eastern Cooperative Oncology Group performance status of 0 to 1.

Results: The study was terminated prematurely due to an unexpected high number of serious cardiac adverse events so the objective response rate could not be determined. A total of 77 doses of depsipeptide with a median of four doses (range, 2-13) per patient were administered. The most common adverse events included nausea (86%), anorexia (73%), vomiting (66%), and fatigue (73%). A sudden death attributed to possible fatal ventricular arrhythmia occurred within 24 hours after the fifth dose of depsipeptide. Furthermore, asymptomatic grade 2 ventricular tachycardia (n = 2) and prolonged QTc (n = 3) probably related to depsipeptide were observed. Plasma depsipeptide levels measured in a subset of patients failed to reveal differences among patients with or without cardiac adverse events.

Conclusions: Depsipeptide was associated with a high number of potentially serious cardiac adverse events in patients with metastatic neuroendocrine tumor. As sudden death possibly associated with depsipeptide was observed in this trial, the risks for potentially life-threatening arrhythmia associated with this agent need to be comprehensively evaluated.

In recent years, the importance of epigenetic modification in the initiation and progression of human cancer has risen to the forefront (1). A novel class of drugs that inhibit the histone deacetylase (HDAC) enzymes are capable of targeting epigenetic silencing mechanisms, resulting in reversal of crucial steps in carcinogenesis and thus hold significant potential as anticancer therapy (2, 3). Depsipeptide is a novel HDAC inhibitor that has been shown to be a potent inducer of growth inhibition, apoptosis, and differentiation of multiple cancer cell lines *in vitro* and *in vivo* and is currently being tested in early-phase clinical trials (4). The pharmacokinetics and safety of depsipeptide in cancer patients have been studied in several National Cancer Institute (NCI)–sponsored phase I and II clinical trials (5–7), and >450 patients have received depsipeptide to date. Objective responses have been seen in patients with cutaneous and peripheral T-cell lymphoma, islet cell tumor, renal cell carcinoma, and leiomyosarcoma (6, 8).

Carcinoid and islet cell tumors are generally classified at the less aggressive end of the spectrum of neuroendocrine tumors. They are thought to derive from enterochromaffin cells that are distributed throughout the gastrointestinal and respiratory system. Plasma chromogranin A and 24-hour urine for 5-hydroxy indole acetic acid are known prognostic markers for survival in patients with carcinoid tumors (9). Although locoregional carcinoid and islet cell tumors are surgically manageable, metastatic disease is present in 50% of patients at the time of diagnosis and the overall 5-year survival for patients with distant metastasis is 20% (10, 11). Although somatostatin analogues, interferon-α, and hepatic artery chemoembolization provide palliation of the carcinoid syndrome symptoms associated with such tumors (12, 13), no systemic therapy has consistently been shown to elicit significant tumor responses or prolong survival.

To date, there have been no good preclinical models for neuroendocrine tumors aiding search to find new therapies into clinical arena. However, a minor response (40% tumor reduction) lasting several months had been seen in a patient with an islet cell tumor in an initial phase I trial (6). Based on

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Received 12/8/05; revised 2/12/06; accepted 2/28/06.

Grant support: National Cancer Institute grant CA76576.

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doi:10.1158/1078-0432.CCR-05-2689

this and its unique antitumor mechanisms, we conducted a phase II clinical trial in patients with metastatic carcinoid/islet cell tumors to test our hypothesis that depsipeptide has antitumor effects in metastatic neuroendocrine tumors. Based on toxicity and pharmacokinetic data available from initial phase I clinical trials in solid tumor and hematologic malignancies, the dose of 14 mg/m^2 administered as 4-hour i.v. infusion on days 1, 8, and 15 every 28 days was considered optimal for our trial. We report the results of the first prospective phase II study of depsipeptide in patients with metastatic neuroendocrine tumors.

Patients and Methods

Patient selection. For the original protocol (November 2003 version), eligibility criteria included histologically confirmed well-differentiated or moderately differentiated neuroendocrine carcinoma; metastatic and/or locally advanced measurable disease; no IFN-α, systemic chemotherapy, or radiation therapy within past 4 weeks; no hepatic artery chemoembolization within past 12 weeks; age ≥18 years; Eastern Cooperative Oncology Group performance status of 0 to 1; total bilirubin <1.5 mg/dL; transaminases <2.5 × upper limit of normal; absolute neutrophil count ≥1,500/μL; platelet count ≥100,000/μL, and serum creatinine <1.5 mg/dL. The standard dose of long-acting octreotide was permitted for the control of carcinoid syndrome symptoms if the dose was stable for 3 months before study entry. Exclusion criteria were as follows: pregnancy, breastfeeding, uncontrolled intercurrent illness including dysrhythmias and angina, history of myocardial infarction within 1 year of study entry, history of serious ventricular arrhythmia, QTc >500 ms, or left ventricular ejection fraction <40% by resting multiple-gated acquisition scan.

As of June 2004, the following eligibility criteria were added in the amended protocol in response to sudden death that occurred on our study: patient must not be eligible for standard therapy, no more than one prior systemic chemotherapy regimen, no left ventricular hypertrophy per Cornell electrocardiogram voltage criteria [S in V3 + R in aVL > 24 mm (men); S in V3 + R in aVL > 20 mm (women)], and patients must not be receiving hydrochlorothiazide to decrease the possibility of developing hypokalemia.

Study design. Depsipeptide was provided by the sponsor (Cancer Therapy Evaluation Program, NCI) of the study and was administered as a 4-hour i.v. infusion at a dose of 14 mg/m^2 on days 1, 8, and 15 repeated every 28 days. Continuous telemetry monitoring was required for 24 hours after completion of treatment with the first dose of depsipeptide for the patients treated before June 9, 2004, whereas such monitoring was required with every dose given after this date as per the amended protocol. Potassium and magnesium replacements were given before depsipeptide infusion if serum levels were <4.0 or <0.85 mmol/L, respectively. Oral ondansetron (16 mg) and metoclopramide (20 mg) were given as prophylactic antiemetics before each dose of depsipeptide. Antiemetic regimen was based on the prior experience in the early-phase clinical trials of depsipeptide. Therapy was given for a total of 24 weeks unless a patient met one of the following criteria: progressive disease, unacceptable adverse event, off-study drug for >4 weeks, or patient withdrawal from the study.

History, physical examination, complete blood count, serum chemistry, troponin I, and electrocardiogram were done within 7 days before initiation, before every dose, and within 2 to 4 weeks after the last dose of depsipeptide. Electrocardiograms were also done at 4 and 24 hours after starting depsipeptide infusion with the first two doses. Computed tomography scan, octreoscan, and serum tumor markers were done before and after therapy and every 8 weeks, whereas multiple-gated acquisition scan was done before and after therapy and every 12 weeks. Objective response was assessed according to Response Evaluation Criteria in Solid Tumors, and adverse events were assessed according to revised NCI Common Terminology Criteria for Adverse Events version 3.0. Manual measurements for QT intervals were made from each electrocardiogram by a cardiologist and corrected for heart rate using Bazett formula to obtain QTc. Troponin I was measured by acridinium ester–based chemiluminescent immunoassay using ADVIA Centaur system (Bayer Healthcare, Tarrytown, NY).

Dose modifications. Patients who developed grade 3 or 4 drug-related nonhematologic, noncardiac adverse events (except fatigue, nausea, asymptomatic hypocalcemia) were treated at a reduced dose of 10 mg/m^2/dose once their toxicity had resolved to grade 2 or less. For cardiac adverse events, the team cardiologist was consulted and depsipeptide was held till following adverse events resolved and the therapy was restarted at 10 mg/m^2/dose: sinus tachycardia (pulse >140/min after recumbency), new occurrence of atrial dysrhythmia, prolongation of QTc (≥500 ms or increase by ≥50 ms), T-wave inversion of >4 mm, or ST depression of ≥2 mm. In event of any of the following cardiac events, patients were taken off the study: troponin I > upper limit of normal, left ventricular ejection fraction <40% or decrease by ≥25% from the baseline ejection fraction, and ventricular arrhythmia (ventricular tachycardia or fibrillation ≥3 consecutive beats).

Tumor marker studies. Depending on the tumor type of specific patient, relevant serum tumor markers (such as pancreastatin, gastrin, pancreatic polypeptide, glucagon, substance-P, neurotensin, calcitonin, somatostatin, vasoactive intestinal peptide, gastrin releasing peptide, and adrenocorticorticotropic hormone) were obtained at baseline, every 8 weeks while on treatment and on the posttreatment visit. Pancreastatin was chosen instead of chromogranin-A as pancreastatin assay is routinely done in the local clinical laboratory at our center. Pancreastatin is a split product of chromogranin A and it also has good prognostic value (14, 15).

Pharmacokinetic studies. Peripheral blood was collected predose, immediately after completion (4-hour time point) and at 20 hours after completion of depsipeptide with intent to perform pharmacodynamic studies. However, pharmacokinetic studies were done in these procured samples in selected patients to see if cardiotoxicity correlated with plasma depsipeptide levels. A highly sensitive and specific atmospheric pressure ionization liquid chromatographic-tandem mass spectrometric method was used for measuring plasma concentrations of depsipeptide. The sensitivity of quantitation was 0.5 ng/mL. This method has been previously described in detail (16) and has been used successfully in phase 1 studies done by our group (7).

In a single patient who had sudden death, heart tissue was harvested at the time of autopsy after obtaining the consent from patient's next of kin. Heart tissue was then cut into small pieces, weighed, and added in 20 mL lysis buffer. The sample was then homogenized using an electronical Ultra-Turrax homogenizer (Tekmar, Co., Cincinnati, Ohio) at the highest speed for four strokes of 15 seconds on ice. Two aliquots of 0.5 mL of such homogenate was analyzed for depsipeptide concentration.

Statistical considerations. The minimax two-stage design of Simon (17) was chosen resulting in a trial with a decision to proceed to the second stage based on efficacy seen in the first 16 patients. Depsipeptide was to be considered ineffective if the true response probability was <10% (p_0). The regimen was to be worthy of further study if the target response rate was 30% or greater (p_1). These figures resulted in a two-stage design of 16 and 25 patients, with an α of 0.10 and β of 0.10.

The primary end point was to assess the objective response (partial remission or complete remission) of depsipeptide in metastatic neuroendocrine tumors. Stable disease was not considered an objective response to therapy given the relatively slow-growing nature of these cancers and due to a single-arm study design.

Results

Patients. After obtaining informed consents, 15 patients with metastatic neuroendocrine tumor were enrolled on the

NCI-approved and the Institutional Review Board–approved phase II study at The Ohio State University between April and September 2004 (n = 11 on original protocol, n = 4 on amended protocol). The full accrual to the first stage of the trial (n = 16) was not reached given premature termination of the trial due to an unexpected high number of potentially serious cardiac adverse events noted in our study. Patient characteristics are outlined in Table 1.

Treatment administered. Patients received a median of four doses (range, 2-13) with a median cumulative total dose of 48 mg/m^2 (range, 28-140). Out of total of 77 doses given in the study, 34 doses in nine patients were given at the reduced dose (10 mg/m^2/dose) per protocol guidelines due to adverse events [recurrent grade 2 nausea/vomiting (n = 4), abnormal liver function tests (n = 2), thrombocytopenia (n = 2), and prolonged QTc (n = 1)]. The reasons for early discontinuation of the drug before 24 weeks of planned therapy are included in Table 2. Of note, five patients withdrew their participation in the study due to new risks in the amended consent whereas four patients chose to continue the study and signed the amended consent before restarting the depsipeptide therapy on the amended protocol.

Table 1. Patient characteristics

Characteristic	No. (%)
Total patients	15
Age (y)	
Median	64
Range	37-69
Sex	
Female	5 (33)
Male	10 (67)
ECOG performance status	
0	6 (40)
1	9 (60)
Grade of neuroendocrine carcinoma	
Well differentiated	11 (73)
Moderately differentiated	4 (27)
Primary sites	
Small bowel	6 (40)
Colorectal	2 (13)
Lung	2 (13)
Pancreas	1 (7)
Unknown	4 (27)
Metastatic sites	
Liver alone	2 (13)
Liver and lymph nodes or bones	5 (33)
Liver, lymph nodes, and bones	7 (47)
Carcinoid syndrome/concurrent octreotide therapy	
Present	11 (73)
Absent	4 (27)
Prior treatment	
Debulking or palliative surgery	7 (47)
Hepatic artery chemoembolization	7 (47)
Systemic chemotherapy	3 (20)
Radiation	2 (13)
Immunotherapy	1 (7)
Baseline elevation of serum tumor markers	
Pancreastatin	14 (93)
Calcitonin	2 (13)
Gastrin	2 (13)
Glucagon	1 (7)
Neurotensin	1 (7)
Pancreatic polypeptide	1 (7)

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

Objective response. Due to premature termination of the study, the objective response rate of depsipeptide in metastatic neuroendocrine cancer could not be determined in this study. No patients achieved a partial remission or a complete remission. Three patients (20%) had progressive disease noted at week 6 (n = 1) and week 8 (n = 2). No significant reduction in serum tumor marker levels or octreotide uptake in octreoscan was observed during treatment in five patients who received at least two cycles of therapy.

Adverse events. The worst grade experienced by an individual patient is used to report the adverse events. Grades 1 to 3 drug-related (possible, probable, and definite attribution to the drug) adverse events reported in 15 patients are outlined in Table 3. In addition, grade 5 sudden death (n = 1) and grade 4 lymphopenia (n = 1) possibly related to depsipeptide were observed. Although the uncommon events of sudden death (n = 1), grade 2 ventricular tachycardia (n = 2; Fig. 1), and grade 2 prolonged QTc (n = 3) were the most serious, the common adverse events observed were gastrointestinal (nausea, 86%; anorexia, 73%; and vomiting, 66%) and constitutional (fatigue, 73%). Of note, we did not observe troponin I elevations in the any of our patients on the study.

Patient no. 006 was a 48-year-old White male with metastatic carcinoid tumor with carcinoid syndrome and a history of hypertension who experienced sudden death 20 hours after completion of the fifth dose of depsipeptide that was administered as an outpatient. While driving with the family, the patient suddenly collapsed and was unresponsive. Resuscitation measures were unsuccessful. Autopsy done at the Ohio State University did not reveal evidence of myocardial infarction, pulmonary embolism, or stroke, and failed to identify any immediate cause of sudden death. Cardiomegaly (640 g) with biventricular hypertrophy (left greater than right) was noted. Endocardial valves were flexible and free of fibroses or calcifications during the autopsy exam. In the absence of identifiable organic cause of death in autopsy, it was presumed that sudden death was related to fatal ventricular arrhythmia that might be related to one or more of the following factors: left ventricular hypertrophy, chronic relative hypokalemia, and depsipeptide. This patient had history of hypertension with evidence of left ventricular hypertrophy on electrocardiogram, had normal pretreatment resting multiple-gated acquisition scan, and had chronic relative hypokalemia (serum potassium within 3.0-3.9 mmol/L range). Chemotherapy used during hepatic artery chemoembolization procedure done 2 years before study entry included 30 mg doxorubicin and 30 mg mitomycin. Concomitant medications included stable doses of long-acting octreotide, losartan/hydrochlorothiazide, metoprolol XL, metoclopramide, and ondansetron. The patient did not have any arrhythmia during inpatient telemetry monitoring up to 24 hours after completing the first dose of depsipeptide. The patient did not have any dose reduction or delays during subsequent doses of depsipeptide and required 140 mEq (i.v.

Table 2. Treatment administered (n = 15 patients)

Patient no.	Treatment on original protocol						Amendment after sudden death on the study: Treatment on amended protocol								
	Course I			Course II			Course I			Course II			Course III		
	D1	D8	D15	D1	D8	D15	D1	D8	D15	D1	D8	D15	D1	D8	D15
001	×	×	×	×	×	×	Patient withdrawal due to new risks in the consent—off study								
002	×	×	▲	Study on hold			Patient withdrawal due to new risks in the consent—off study								
003	×	▲	▲	▲	Study on hold		▲	▲	▲	▲	▲	▲	▲	▲	▲ D/C
004	×	×	×	Study on hold			×	×	▲	▲	▲	▲	▲	▲	▲ D/C
005	×	×	×	×	PD – off study		PD – off study								
006	×	×	×	×	×	Sudden death	Sudden death – off study								
007	×	×	×	Study on hold			Patient withdrawal due to new risks in the consent—off study								
008	×	▲	▲	Study on hold			Patient withdrawal due to new risks in the consent—off study								
009	×	×	Study on hold				Patient withdrawal due to new risks in the consent—off study								
010	×	Study on hold					×	×	▲	▲	▲	▲	PD—off study		
011	×	Study on hold					×	▲	▲	D/C					
012	Not accrued at this point						×	×	×	×	×	×	PD—off study		
013	Not accrued at this point						×	▲	▲	Ventricular tachycardia—off study					
014	Not accrued at this point						×	▲	▲	▲	Ventricular tachycardia—off study				
015	Not accrued at this point						×	▲	D/C						

NOTE: ×, 14 mg/m^2/dose of depsipeptide; ▲, 10 mg/m^2/dose of depsipeptide.
Abbreviations: PD, progressive disease; D/C, discontinued due to study termination.

plus oral) of potassium and 2 g magnesium supplements before the fifth dose of depsipeptide as serum potassium and magnesium levels were 3.3 mmol/L and 1.8 mg/dL, respectively. The postsupplement potassium and magnesium levels were 3.6 mmol/L and 2.6 mg/dL, respectively. The QTc intervals ranged from 440 ms (baseline) to 468 ms during the first four doses. Electrocardiogram was unremarkable (QTc of 414 ms) just before the fifth dose of depsipeptide, whereas the electrocardiogram was not done as per original protocol after completion of the fifth dose. Subsequent to this serious adverse event, the protocol was amended to require close inpatient telemetry monitoring for 24 hours with every dose of depsipeptide and to exclude patients with known risk factors of ventricular arrhythmia (see details under Patients and Methods).

Patients no. 013 and 014 were enrolled on the amended protocol and experienced grade 2 asymptomatic ventricular tachycardia. Of note, both of these patients did not have prolonged QTc or arrhythmia during inpatient telemetry monitoring up to 24 hours after completing of the first two or three doses of depsipeptide. Systemic chemotherapy agents used in both of these patients included only etoposide and cisplatin or carboplatin. Patient no. 013 is a 68-year-old White male with metastatic moderately differentiated neuroendocrine carcinoma and a history of hypertension who developed two episodes of asymptomatic ventricular tachycardia (4- and 12-beat run) within 24 hours of the third dose of depsipeptide (Fig. 1A). At that time, the patient did not have any evidence of myocardial infarction and his dipyridamole stress myocardial perfusion test done within 48 hours of the event showed normal left ventricular motion and left ventricular ejection fraction was 55%. He had normal baseline resting multiple-gated acquisition, electrocardiogram, and serum potassium (4.4-4.9 mmol/L) and magnesium (2.3-2.8 mmol/L) levels before and within 24 hours of depsipeptide infusion. The patient required dose delay and reduction due to thrombocytopenia per protocol with his second and third doses of depsipeptide.

Patient no. 014 is a 50-year-old White female with metastatic islet cell tumor with carcinoid syndrome and a history of hypertension who developed grade 2 asymptomatic ventricular tachycardia (8-beat run) ~3 hours after initiation of the fourth dose of depsipeptide (Fig. 1B). This patient had normal baseline stress multiple-gated acquisition, electrocardiogram, and her serum potassium (4.3-4.4 mmol/L) and magnesium (2.1 mmol/L) levels were normal before and within 24 hours of depsipeptide. Patient 014 required dose reduction due to nausea per protocol starting with her second dose of depsipeptide.

Furthermore, patients no. 001, 002, and 004 who had baseline QTc of 464, 435, and 438 ms, respectively, developed asymptomatic grade 2 prolonged QTc (492, 499, and 495 ms, respectively) within 24 hours after completion of depsipeptide that resolved spontaneously by the subsequent doses. Of note, none of these patients developed ventricular tachycardia. Finally, reversible diffuse ST-T changes were noted in 7 of 15 patients. An example of such changes noted in patient 004 is shown in Fig. 2.

Pharmacokinetic studies. As pharmacokinetics of depsipeptide are well studied in phase I trials, we did not intend to perform detailed pharmacokinetics in our patients. However, given the unexpected high number of potentially serious cardiac adverse events, we examined if any variation in their pharmacokinetics in patients with metastatic carcinoid/islet cell

tumors compared with the phase I patient population could explain high number of cardiac events. We therefore chose four patients who had cardiac adverse events (patient no. 002, 006, 013, and 014) and three control patients (patient no. 005, 007, and 008). Plasma depsipeptide levels were measured during the first two doses of depsipeptide administration at the predose, immediately after completion (4-hour time point) and at 20 hours after completion of depsipeptide. In addition, we also did pharmacokinetics in the samples obtained during the third, fourth, and fifth doses of depsipeptide in patient no. 006 who had sudden death. As predicted from pharmacokinetics noted in the phase I trials, plasma depsipeptide levels at predose were undetectable. At the 20-hour time points, depsipeptide levels were <10 ng/mL in all seven patients. Mean plasma depsipeptide concentration at 4 hours in patients who had cardiac adverse events was 723 ng/mL (SD ± 479) versus 744 ng/mL (SD ± 335) in control patients. Furthermore, there was no significant intrapatient increase in 4-hour plasma depsipeptide in the dose that was associated with cardiac event compared with the first dose of depsipeptide. There was no detectable depsipeptide in heart tissue that was harvested during autopsy for patient no. 006 who had sudden death.

Table 3. Depsipeptide-related adverse events reported in all 15 patients

Adverse event	Grade 1	Grade 2	Grade 3
		n (%)	
Gastrointestinal			
Anorexia	3 (20)	8 (53)	—
Nausea	5 (33)	6 (40)	2 (13)
Vomiting	4 (27)	5 (33)	1 (7)
Dyspepsia/bloating	2 (13)	2 (13)	—
Dysgeusia	3 (20)	3 (20)	—
Diarrhea	2 (13)	2 (13)	1 (7)
Dry mouth	2 (13)	—	—
Constitutional			
Fatigue	4 (27)	6 (40)	1 (7)
Weight loss	3 (20)	1 (7)	—
Fever	2 (13)	—	—
Rigors	1 (7)	1 (7)	—
Cardiopulmonary			
Palpitations	—	1 (7)	—
Ventricular tachycardia	—	2 (13)	—
QTc prolongation	3 (20)	3 (20)	—
ST depression/T-inversion	—	5 (33)	—
T-wave flattening	2 (13)	—	—
Hypotension	1 (7)	—	—
Dyspnea	1 (7)	1 (7)	—
Dizziness	2 (13)	—	—
Hematologic			
Anemia	3 (20)	2 (13)	2 (13)
Thrombocytopenia	7 (46)	2 (13)	—
Neutropenia	—	—	1 (7)
Lymphopenia	5 (33)	4 (27)	—
Hepatic			
Elevated AST/ALT	4 (27)	1 (7)	—
Hyperbilirubinemia	3 (20)	1 (7)	—
Miscellaneous			
Hypocalcemia	4 (27)	1 (7)	—
Blurry vision	2 (13)	—	—
Orbital cellulitis	—	—	1 (7)

NOTE: Grade 4 to 5 adverse events: one grade 4 lymphopenia and one grade 5 sudden death.
Abbreviations: AST, aspartate aminotransferase; ALT, aspartate aminotransferase.

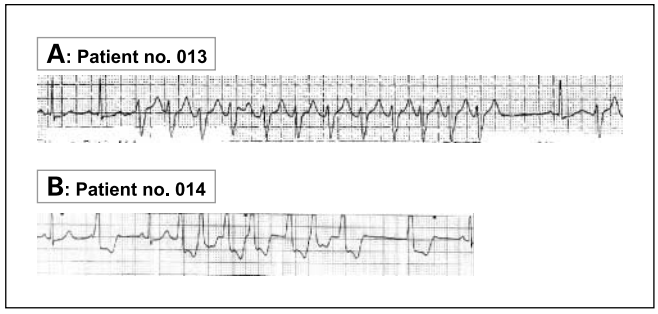

Fig. 1. Ventricular tachycardia possibly related to depsipeptide. *A*, electrocardiogram rhythm strip showing 12-beat run of ventricular tachycardia in patient no. 013 at 20 hours after initiation of the third dose of depsipeptide. *B*, electrocardiogram rhythm strip showing 8-beat run of ventricular tachycardia in patient no. 014 at 3 hours after initiation of the fourth dose of depsipeptide.

Discussion

This NCI-sponsored phase II clinical trial of single-agent depsipeptide in patients with metastatic neuroendocrine tumors was terminated prematurely due to a concern that there was an unexpected high number of potentially serious cardiac adverse events. In this limited setting, our study failed to show any objective tumor response in patients with metastatic neuroendocrine tumors. Consequently, we decided that the potential risk in this patient population who had no obvious objective response merited early discontinuation of the trial.

To determine whether these toxicities were attributed to the unique characteristics of our patient population, we considered several possibilities. Although it is feasible that peptide release in patients with carcinoid/islet cell cancer could result in cardiac arrhythmia as a part of carcinoid crisis, the sudden onset of cardiac events noted in our patients along with lack of associated symptoms (flushing, diarrhea, severe fluctuations in blood pressure) of carcinoid crisis makes it an unlikely explanation. We raised the question whether patients with neuroendocrine tumors generally have a high incidence of asymptomatic cardiac arrhythmia. However, such patients are routinely monitored at our center with continuous telemetry for 3 to 4 days during their inpatient hospitalization for hepatic artery chemoembolization procedure (five to six procedures per month) and we have not observed any new onset of arrhythmia in this patient population outside the setting of full-blown carcinoid crisis. We considered if specific concomitant medications (such as octreotide, ondansetron, and metoclopramide) commonly used in our patient population could have resulted in drug interactions with depsipeptide. Although octreotide can prolong QTc, we did not observe prolonged QTc at the pretreatment evaluation in the 11 patients receiving octreotide therapy. Additionally, two patients (no. 002 and 013) who

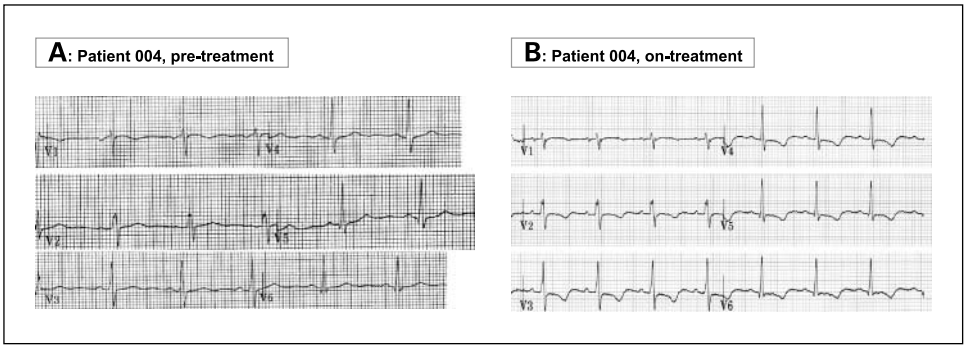

Fig. 2. Depsipeptide-induced diffuse ST-T wave changes on electrocardiogram. *A*, pretreatment baseline electrocardiogram (V1-V6 leads) in patient 004. *B*, electrocardiogram obtained at 20 hours after completion of fifth dose of depsipeptide in patient 004 showing diffuse ST depression/T inversion that were totally reversible by the sixth dose.

experienced potentially serious cardiac adverse events were not receiving concomitant octreotide. Concerning possible drug interactions with antiemetics, use of ondansetron and metoclopramide was not unique to our depsipeptide study and such a regimen has been used in other clinical trials with depsipeptide. Thus, we did not observe any consistent predisposing risk factor accounting for cardiotoxicity in our patients. However, the possible attribution of one of the risk factors (such as concomitant medications, drug interactions, ischemic heart disease, hypertensive cardiomyopathy, pharmacogenomic status, and electrolyte imbalance) cannot be fully excluded. Furthermore, carcinoid tumors can cause unique cardiac abnormalities including right-sided valvular heart disease, which is generated by the exposure of the tricuspid and pulmonary valves to the paraneoplastic effects of vasoactive substances released by the carcinoid tumors and thus subclinical carcinoid heart disease in our patient population might be a predisposing risk factor for serious cardiac adverse events. Of note, however, patient 006 did not have evidence of fibroses or calcification of endocardial valves on autopsy, suggesting that there may be other unknown risk factors that predispose to these toxicities. Finally, it is also possible that we observed high number of asymptomatic cardiac toxicity as our trial required continuous cardiac monitoring with every dose of depsipeptide (total of 53 doses of depsipeptide among 15 patients) on the amended protocol.

As of August 2005, there have been five (patients with solid tumor as well as hematologic malignancies) other reported incidents of sudden death occurring in ~450 patients enrolled on depsipeptide trials (Investigational New Drug reports from Cancer Therapy Evaluation Program, NCI). These reports, along with our phase II trial experience, raise a concern regarding how best to assess the potential for serious cardiac arrhythmias in preclinical and early clinical drug development studies. To date, extensive preclinical studies have been conducted in mice, rats, dogs, and beagle dogs that examined various dose schedules of depsipeptide. Cardiotoxicity was observed in dogs after i.v. administration of depsipeptide daily for 2 weeks or twice weekly for 4 weeks. Cardiotoxicity included elevation of serum cardiac enzymes, prolonged QTc changes, and histopathologic findings of chronic inflammatory changes in myocardium or epicardial and endocardial hemorrhage of right atrium or of left ventricle. Subsequent preclinical studies conducted by NCI in dogs and mice showed that intermittent (q4 days × 3) schedule was better tolerated than daily dosing. Furthermore, toxicity in general and cardiotoxicity specifically, seemed to be related to the rate of administration; 4-hour infusion was much better tolerated than a 30-second bolus or 10-minute injection. Based on this preclinical experience, an intermittent dosing schedule delivered as a 4-hour i.v. infusion was found to be optimum and was used for initial phase I clinical trials.

Subsequently, a few early phase trials of depsipeptide required extensive cardiac monitoring. Sandor et al. (5) reported phase I trial of depsipeptide given on days 1 and 5 as 4-hour i.v. infusion every 21 days with dose levels ranging from 1 mg/m^2 up to 24.9 mg/m^2 in 37 patients with refractory solid tumors. This clinical trial required the following cardiac monitoring during days 1 to 5 of the first cycle of therapy: continuous cardiac monitoring, and daily electrocardiograms and cardiac enzymes. In addition, patients were also evaluated by echocardiography on day 6 of each cycle. An electrocardiogram was done before each cycle, and a multiple-gated acquisition scan was done after every second cycle. Three patients had asymptomatic cardiac arrhythmia (one atrial bigeminy, one 3-second sinus pause, and one five-beat run of ventricular tachycardia). One of these patients was reported to have an episode of grade 4 atrial fibrillation and was retreated at lower dose level without recurrence. Furthermore, reversible T-wave flattening (grade 1) and T-wave inversions with ST depression (grade 2) have been reported in ~78% to 88%. Another phase I clinical trial of depsipeptide administered treatment as a 4-hour i.v. infusion on days 1, 8, and 15 with dose levels ranging from 1 to 17.7 mg/m^2 in total 33 patients with advanced cancer (6). Serial cardiac enzymes and electrocardiograms were done in all the patients on this trial, whereas continuous cardiac monitoring was required during first 24 hours after the first dose of depsipeptide only if there were laboratory abnormalities suggestive of cardiac toxicity. This trial reported minimal cardiotoxicity that included diffuse T-wave inversions in 10 patients. The relative low occurrence of asymptomatic cardiac arrhythmia observed in both of these studies compared with ours may be due to the fact that telemetry monitoring was limited to the first two doses of depsipeptide.

Recently, our group reported a clinical trial (n = 20) in patients with chronic lymphocytic leukemia and acute myeloid leukemia in whom depsipeptide was given as a 4-hour i.v. infusion at 13 mg/m^2/dose on days 1, 8, and 15 (7). Serial electrocardiograms, multiple-gated acquisition scans, troponin levels, and telemetry monitoring for 24 hours after each dose of depsipeptide were required. Minimal cardiac effects observed besides ST and T wave abnormalities were asymptomatic transient left bundle branch block and an isolated elevation of troponin. It is unclear why we observed high number of potentially serious cardiac adverse events in our neuroendocrine patients, whereas we saw minimal cardiotoxicity in the

above hematologic malignant patient population. Both of these studies were done by our group at the same center, required extensive cardiac monitoring, and used similar dose/schedule of depsipeptide.

Thus, preclinical studies of depsipeptide at specific dosing schedules revealed cardiomyopathy, myocardial ischemia, and prolonged QTc; however, they failed to predict cardiac arrhythmias associated with depsipeptide. Most of the early phase clinical trials, therefore, focused on serial cardiac enzymes, multiple-gated acquisition scans and electrocardiograms, and lacked extensive serial monitoring for cardiac arrhythmia. To date, we did not observe any association between prolonged QTc and ventricular tachycardia/sudden death. Thus, it is possible that prolonged QTc may not be a good predictor for ventricular arrhythmia associated with depsipeptide. Although some of the cardiac adverse events (prolonged QTc or ventricular tachycardia) that were observed in our trial were not associated with serious clinical consequences, such adverse events are of concern given the potential for associated life threatening event.

It is unclear if the cardiotoxicity of depsipeptide is mediated through HDAC inhibition. Three other classes of structurally diverse molecules inhibiting HDAC include short chain fatty acids (e.g., phenylbutyrate), benzamides (e.g., CI-994, MS-275), and hydroxamic acids (e.g., trichostatin A and suberoylanilide hydroxamic acid). As some of these agents are in the early phases of clinical trials, it is too early to comment if cardiotoxicity represents a class effect of HDAC inhibitors. If these promising agents are being given with much less intensive cardiac monitoring, we may not know if the absence of reports of cardiac abnormalities reflects the same degree of scrutiny involved with the depsipeptide trials. Recently, Rowinsky et al. reported their experience in cardiac monitoring in patients participated in three phase I trials with a cinnamic acid hydroxamate HDAC inhibitor, LAQ824. Prolonged QTc and unsustained torsade de pointes associated with LAQ824 were observed without clinical consequences (18).

In conclusion, the sudden death in a single patient on this trial is alarming. The actual experience on all trials with this agent should be carefully examined. Sudden death possibly associated with depsipeptide is not unique to our trial given the five additional cases reported among ~450 patients treated with depsipeptide on various phase I/II clinical trials. The experience in drug development with depsipeptide illustrates the limitations of preclinical animal models in defining risks of cardiac arrhythmia for patients entering early phase clinical trials. Finally, as HDAC inhibitors hold significant promise as an anticancer therapy, further studies should be done to identify the risk factors for potentially life-threatening cardiac adverse events associated with these agents.

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