Phase I Trial of the Histone Deacetylase Inhibitor, Depsipeptide (FR901228, NSC 630176), in Patients with Refractory Neoplasms


ABSTRACT

Purpose: The primary objectives of this trial were to define the maximum tolerated dose (MTD) and to characterize the toxicities and pharmacokinetics of depsipeptide (FR901228) given on a day-1 and day-5 schedule every 21 days. A secondary objective of the trial was to seek evidence of antineoplastic activity.

Patients and Methods: Patients with advanced or refractory neoplasms received depsipeptide by a 4-h i.v. infusion on days 1 and 5 of a 21-day cycle. On the basis of preclinical data suggesting that depsipeptide may have significant cardiac toxicity, patients were treated while receiving continuous cardiac monitoring and were followed with serial cardiac enzymatic determinations, electrocardiograms (ECGs), and nuclear ventriculograms (MUGA scans). The starting dose of the trial was 1 mg/m², and dose escalations proceeded through a total of eight dose levels to a maximum of 24.9 mg/m². The DLTs included grade-3 fatigue (3 patients), grade-3 nausea and vomiting (1 patient), grade-4 thrombocytopenia (2 patients), and grade-4 cardiac arrhythmia (1 patient, atrial fibrillation). The MTD was defined at the seventh dose level (17.8 mg/m²). Reversible ST/T changes and mild reversible dysrhythmias were observed on the post-treatment ECG. There were no clinically significant changes in left ventricular ejection fraction. One patient achieved a partial response. The plasma disposition of depsipeptide was well described by a first-order, two-compartment model. The mean volume of distribution, clearance, t₁/₂a, and t₁/₂b, at dose of 17.8 mg/m² was: 8.6 liters/m², 11.6 liters/h/m², 0.42 h, and 8.1 h, respectively. The mean maximum plasma concentration at the MTD was 472.6 ng/ml (range: 249–577.8 ng/ml). Biological assays showed that the serum levels achieved could cause the characteristic cell cycle effects of this agent when serum was added to PC3 cells in culture, as well as increased histone acetylation in patient-derived peripheral blood mononuclear cells.

Conclusion: The MTD of depsipeptide given on a day-1 and -5 schedule every 21 days is 17.8 mg/m². The DLTs are fatigue, nausea, vomiting, and transient thrombocytopenia and neutropenia. Whereas cardiac toxicity was anticipated based on preclinical data, there was no evidence of myocardial damage. However, reversible ECG changes with ST/T wave flattening were regularly observed. Biologically active serum concentrations were achieved, and 1 patient obtained a partial response. The recommended Phase II dose is 17.8 mg/m² administered on day 1 and 5 of a 21-day cycle.

INTRODUCTION

Depsipeptide (FR901228, NSC 630176) is a novel anticancer agent isolated from the fermentation broth of Chromobacterium violaceum (1). It has demonstrated potent cytotoxic activity against human tumor cell lines and in vivo efficacy against both human tumor xenografts and murine tumors (2, 3). Although it was identified as a Pgp substrate by COMPARE analysis of its NCI drug screen cytotoxicity profile, and confirmed as such in the laboratory, depsipeptide is highly potent against sensitive cell lines in the screen (4). Depsipeptide shows a lack of cross-resistance with the cytotoxic agents vincristine, 5-fluorouracil, mitomycin C, and cyclophosphamide. Although

Received 7/26/01; revised 11/27/01; accepted 12/13/01.

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initially identified by Fujisawa Pharmaceutical Co. in a screening program for agents able to reverse the malignant phenotype of Ha-ras-transformed NIH 3T3 cells, depsipeptide has been identified recently as a potent HDAC inhibitor (3, 5). Histone deacetylation is an important component of transcriptional control, and thus, inhibition of the deacetylase enzyme may be a mechanism of antineoplastic activity of depsipeptide (5, 6). Depsipeptide, however, is structurally distinct from other known HDAC inhibitors, such as the trichostatins and trapoxins, and may have other mechanisms of cytotoxic action (7).

Laboratory studies have demonstrated that depsipeptide, like other HDAC inhibitors, induces expression of a specific subset of genes linked to inhibition of cell growth and induction of differentiation (8, 9). Depsipeptide is able to cause both a p21-dependent G1 and a p21-independent G2 arrest, with the G2 arrest appearing more cytotoxic than the G1 arrest (10, 11). In human breast cancer cells, increased p21, phosphorylation of H11022, and apoptosis have been observed after depsipeptide treatment (12). In thyroid cancer cells, low concentrations of depsipeptide have been shown to increase expression of a functional Na+/K+ symporter in poorly differentiated thyroid carcinoma cells (13), thus offering a potential therapeutic strategy for resensitizing radioresistant thyroid cancer to radioiodine.

In preclinical studies, greater antitumor activity was observed with an intermittent schedule of depsipeptide administration than with daily administration because of greater host tolerance for depsipeptide and the ability to administer higher individual doses. In addition, it was observed that short infusions (>30 s to 4 min) and prolonged infusions (>24 h) caused the greatest toxicity and that infusions of 1–4 h produced the least toxicity and allowed for the highest individual doses. Two potentially serious toxicities were observed in the preclinical assessment of depsipeptide. Cardiac toxicity, including elevations in cardiac enzymes and necrosis, with chronic inflammation or neutrophilic infiltration of cardiac muscle on histopathological examination was observed in some dosing schedules. In addition, local inflammation and necrosis were noted at catheter insertion sites. This study was designed to determine the MTD and toxicity profile and characterize the pharmacokinetic profile of a 4-h infusion of depsipeptide given intermittently on a day-1 and day-5 schedule every 21 days. Precautions for potential cardiac and catheter site toxicities were included.

PATIENTS AND METHODS

The study was approved by the NCI Institutional Review Board and the Institutional Review Board of Ohio State University. All patients were required to give written informed consent before study participation.

Eligibility Criteria. Patients with histologically confirmed evidence of malignancy for whom no known standard therapy was available that was either curative or definitely capable of extending life expectancy were eligible. Other eligibility criteria included age ≥18 years, ECOG performance status ≤2, and estimated life expectancy >12 weeks. Previous chemotherapy or biological therapy had to be discontinued for at least 4 weeks. Previous radiotherapy had to be completed 3 weeks before study entry and could not have encompassed >25% of the bone marrow. Patients were required to have the following laboratory values, obtained within 14 days of study participation: neutrophils ≥1,000/μL, platelets ≥100,000/μL, creatinine ≤1.5 × upper normal limit, bilirubin ≤1.5 × upper normal limit, aspartate aminotransferase/alanine aminotransferase (AST/ALT) ≤3 × upper normal limit, prothrombin time/ partial thromboplastin time ≤1.1 × upper normal limit. Patients were not eligible if they had an uncontrolled infection, central nervous system metastasis, or recent major surgery (within 21 days). Pregnant or lactating patients were not allowed on study.

Toxicity and Response Evaluation. Pretreatment evaluation included a complete history and physical examination, PT/PTT, electrolytes, renal function and liver function tests, CPK, LDH, urinalysis, MUGA scan, chest X-ray, 12-lead ECG, β-human chorionic gonadotropin (if appropriate), and HIV serology. All patients were evaluated with computed tomography scans of the chest, abdomen, and pelvis. A 24-h Holter monitor was obtained on all patients before treatment as a baseline study. Patients in the first cycle were observed with continuous cardiac monitoring during the 5-day interval starting with the first infusion on day 1 until 24 h after the second infusion on day 5. During these 5 days, patients were evaluated with daily 12-lead ECGs, CPK, LDH, and troponin-I determinations, as well as routine blood counts and serum chemistries. Patients were also evaluated by echocardiography on day 6 of each cycle. Urinalysis, cardiac enzymes, blood counts, and chemistries were performed weekly between cycles, as well as before each cycle. An ECG was performed before each cycle, and a MUGA scan was performed after every second cycle. Tumor measurements were obtained after every other cycle, and responses were scored according to WHO criteria. All toxicities were graded according to the NCI Common Toxicity Criteria. DLT was defined as any grade-4 hematological toxicity and any grade-3 or -4 nonhematological toxicity, excluding alopecia. The MTD was defined as the dose below which ≥2 of 3 or ≥2 of 6 patients experienced DLT. After determination of the MTD, 3 additional patients were treated at this dose to further define toxicity.

Dose Escalation. The starting dose was 1 mg/m², which represented one-third of the toxic low dose in dogs from the initial preclinical studies. At least 3 new patients were to be recruited for each dose level. Three additional patients (for a total of 6) were treated at the same dose level if 1 of the first 3 exhibited DLT. Patients who experienced grade-3 nonhematological toxicity or grade-4 hematological toxicity could continue to receive depsipeptide but at the next lowest dose level and only after all toxicity had resolved to at most grade 1. The drug was discontinued if patients experienced grade-4 nonhematological toxicity. Dose escalation followed a modified Fibonacci scheme (Table 1). The dose escalation scheme was accelerated after the 1st patient was treated at the fourth dose level. This protocol amendment was based on data from a concurrent Phase I study of depsipeptide given on a weekly schedule at Georgetown University. The accelerated fourth dose level (6.5 mg/m²) was designated 4B.

The day-5 dose was delayed to day 7 if blood counts, renal function, or liver function studies were abnormal and not within 10% of baseline values. If repeat values on day 7 were not within the 10% margin, the drug was held until the 1st day of the following cycle. The protocol was amended after the fifth dose...
level to allow treatment on day 5 if the AGC was ≥1,000/mm³ and the platelet count ≥75,000/mm³.

The protocol was also amended to allow intrapatient dose escalation when the sixth dose level was reached. Patients with responding or stable disease could receive the next higher dose level after 3 patients had received that dose level without DLT and had been monitored for at least 3 weeks after treatment.

Three patients had their doses escalated to the next highest dose level after having stable disease with multiple cycles of treatment on study.

**Drug Administration.** Depsipeptide was administered on day 1 and day 5 of a 21-day cycle as a 4-h i.v. infusion through a central venous catheter that had healed for at least 7 days after placement. Depsipeptide was reconstituted with 2 ml of diluent (containing a 4:1 mixture of propylene glycol and ethanol) from 10 mg of vials of lyophilized powder provided by the Division of Cancer Treatment, Diagnosis, and Centers/NCI and then administered after dilution in 0.9% sodium chloride to a concentration of 0.04 mg/ml.

**Pharmacokinetics.** Blood samples were drawn in heparinized tubes before the treatment and at 1, 2, 3, 4, 4.25, 4.5, 5, 7, 10, 16, 24, and 48 h after initiation of the depsipeptide infusion. Samples were collected before infusion, as well as 4 and 7 h after the start of the infusion on day 5. Samples collected on days 1 and 5 during the first and the second cycles were centrifuged, and the plasma was stored at −80°C until analysis. Samples were analyzed using a sensitive electrospray liquid chromatography/tandem mass spectrometry assay as described by Li and Chan (14). The data were analyzed using a two-compartment open model using the computer program ADAPT II. Pharmacokinetic parameters, volume of distribution (V), clearance (CL), distribution half-life (T₁/₂α), distribution half-life (T₁/₂β), and elimination half-life (T₁/₂ε), were obtained by using weighted least square analysis within the same program. The highest observed concentrations were used for Cmax. In addition, the data from the first cycle were used for model-independent analysis. For non-compartmental analysis, the area under the concentration versus time curve (AUC) was calculated by using the trapezoidal rule extrapolated to infinity. The following equations were used to calculate elimination half-life (T₁/₂ε), systemic clearance (CLtot), and apparent volume of distribution at steady state (Vdss): T₁/₂ε = 0.693/ke, CLtot = dose/AUC, Vdss = CLtot × mean residence time. The difference between cycles 1 (C1) and 2 (C2) was evaluated by the ratio of C2:C1 from patients who received the same dose for both cycles.

**Biological Assays.**

**Cell Cycle Assay.** An ex vivo biological assay was performed using serum samples from patients on the study. PC3 prostate cancer cells were grown in RPMI medium supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO₂ incubator. Exponentially growing cells at 50–70% confluence were incubated overnight with serum from patients obtained at various pharmacokinetic time points and diluted 1:1 with normal medium. The cells were then analyzed by standard methods: trypsinized, washed with PBS, and resuspended in a propidium iodide staining solution (PBS with 0.1% Triton X-100, 1 mg/ml RNase A, and 50 μg/ml propidium iodide; Ref. 11). The suspension was then passed through a nylon mesh filter and analyzed using a Becton Dickinson FACSort.

**Histone Acetylation Assay.** Cytospins were prepared from patient mononuclear cells isolated from whole blood by Ficoll-Hypaque gradient. The slides were fixed in 95% ethanol/5% acetic acid for 1 min at room temperature. Depsipeptide-treated (10 ng/ml overnight) PC3 cells were included as a positive control. After fixation, slides were washed twice with PBS for 15 min, blocked in 8% BSA in PBS for 1 h at room temperature, and washed 15 min in PBS before incubating overnight at 4°C with 5 μg/ml anti-α acetylated Histone H3 (Upstate Biotechnology, Lake Placid, NY) in 2% BSA in PBS. Subsequently, cells were washed twice with PBS for 5 min at room temperature and then stained with horse antirabbit FITC-conjugated secondary antibody (Vector Laboratories, Burlingame, CA). After staining with secondary antibody, slides were washed three times with PBS for 15 min and then counterstained with 4’,6-diamidino-2-phenylindole-containing anti-fade compound (Vector Laboratories).

**RESULTS**

Thirty-seven patients received a total of 88 cycles of depsipeptide at eight different dose levels. Patient characteristics are outlined in Table 2. The planned dose escalation scheme was...
modified in the course of the study. The following dose levels were evaluated: 1, 1.7, 2.5, 3.5, 6.5, 9.1, 12.7, 17.8, and 24.9 mg/m² on days 1 and 5 (Table 1). Only 1 patient was treated at a dose of 3.5 mg/m², because the dose escalation scheme was modified at dose-level 4 (see “Patients and Methods”). Patients (36) were assessable for toxicity during the first cycle of therapy. One patient was not assessable, because he required surgical intervention for hemoptysis after the day-1 treatment. This patient was not retreated until he recovered from surgery. At that point, his dose level had been identified as safe, and patients had been enrolled beyond 9.1 mg/m². He was, therefore, considered invaluable for toxicity. Two additional patients did not receive the day-5 dose during the first cycle of treatment. One patient at dose-level 2 (1.7 mg/m²) developed an increase in CPK with absence of MB fraction, associated with a fever, and the second, entered at 24.9 mg/m², developed erythema around the central venous access device site. Three patients had the day-5 dose delayed in the first cycle of treatment. One was delayed because of a decreased neutrophil count (at 17.8 mg/m²), a second because of a decreased platelet count (at 9.1 mg/m²), and the third because of a central venous catheter clot (at 17.8 mg/m²). All 3 received the day-5 dose without complications.

Two patients were removed from the study for potential toxicity. The 1st occurred at the first dose level because of a persistent elevation of CPK above normal without other findings. The MB fraction remained normal. The rise in CPK was evident on the 12th day of the second cycle (573 units/dl; normal: 52–386 units/dl) of treatment and returned to normal by the 5th day of cycle 3 (327 units/dl). The CPK rose again by the 12th day of cycle 3 (576 units/dl), and the patient was taken off study. The CPK was followed to day-70 post-treatment and fluctuated outside of the normal range (444–627 units/dl). There were no associated ECG, LDH, or troponin-I abnormalities or evidence of myopathy. No other patients experienced CPK elevations that could be related to depsipeptide treatment. The 2nd patient, enrolled at the 17.8 mg/m² dose level, was removed from the study after the development of splenic infarcts.

**Hematological Toxicity.** The number of patients experiencing neutropenia or thrombocytopenia during the first cycle at each dose level is outlined in Table 3. Overall, significant hematological toxicity did not occur at dose levels <9.1 mg/m² on any cycle of treatment. Two patients with grade-4 thrombocytopenia were among the cycle-1 toxicities at 24.9 mg/m² that were defined as dose limiting. Episodes of neutropenia and thrombocytopenia had a distinct time course unlike that of chemotherapeutic agents in current clinical use. As in the examples shown in Fig. 1, there was a rapid drop in both platelet count and neutrophil count, with a rapid recovery. Data from cycles 1 (○), 2 (●), and 3 (■) are shown, with no evidence of cumulative toxicity.

We also evaluated multiple treatment cycles at each dose level. At 9.1 mg/m², out of a total of eight cycles of treatment given to 3 patients, 1 patient had the day-5 dose delayed for 1 day because of thrombocytopenia (platelet count = 110,000/mm³). A 2nd patient had the day-5 dose delayed for 1 day because of neutropenia (AGC = 740/mm³, C3) and had the day-5 dose omitted in two cycles because of both neutropenia and thrombocytopenia [AGC = 934/mm³ (C2) and 785/mm³ (C4); platelet counts = 85,000/mm³ (C2) and 61,000/mm³ (C4)]. This patient was responding to treatment and, therefore, had escalation of the day-1 dose after safety was confirmed in other cohorts. This patient continued to have the day-5 dose omitted in the next four cycles, one given at 12.7 mg/m² and three at 17.8 mg/m². Excluding this patient, but including all other patients who received at least one cycle of depsipeptide at 17.8 mg/m², there were 26 treatment cycles in 13 patients at 17.8 mg/m². Among these patients, none had day-5 dose delays or omissions for thrombocytopenia, and only 2 patients had the day-5 dose omitted for neutropenia. There were a total of 13 episodes of grade-3 and 3 episodes of grade-4 neutropenia and 9 episodes of grade-3 and 2 episodes of grade-4 thrombocytopenia in the 26 cycles received by this same group of 13 patients. Whereas too few patients received multiple cycles of treatment at dose levels >9.1 mg/m² to make conclusive judgements, the transient hematological toxicity of depsipeptide does not seem cumulative.

**Nonhematological Toxicity.** Table 4 summarizes the major nonhematological toxicities associated with the first cycle of depsipeptide for each dose level. Drug-related grade-3 or -4 nonhematological toxicities were defined as dose limiting and were noted in the first cycle in patients receiving 24.9 mg/m². The most common toxicity was severe fatigue, observed in 3 patients in the first cycle at 24.9 mg/m². One patient had grade-3 nausea and vomiting followed by an episode of atrial fibrillation requiring treatment (grade 4) on day 7. One patient transiently developed grade-3 hypocalcemia on day 2, and 2 patients developed grade-3 hypophosphatemia on days 5 and 10, respectively. Together with the two episodes of grade-4 thrombocytopenia, these nonhematological toxicities occurring in the first cycle of therapy with depsipeptide defined 24.9 mg/m² as a dose that exceeded the MTD. Only one grade-3 nonhematological toxicity was observed in the first cycle at a dose <24.9 mg/m², and that was transient grade-3 hypocalcemia occurring on day 6 in a patient treated at 17.8 mg/m². This was not considered related to depsipeptide until it was also observed in a patient receiving 24.9 mg/m².

Nonhematological toxicities occurring in multiple cycles of therapy were similar to those observed after the first cycle. Six patients experienced grade-3 fatigue after either the first or subsequent cycles of depsipeptide, 1 patient at a dose of 17.8 mg/m² and 5 patients at a dose of 24.9 mg/m². Grade-2 fatigue was observed in 6 of 26 cycles administered to 13 patients at...
17.8 mg/m². Two patients refused additional therapy because of fatigue. Initially in the study, patients were not given routine nausea and vomiting prophylaxis. At doses of ≥3.5 mg/m², patients began to experience grade-2 nausea and vomiting. These symptoms were often delayed, and patients were subsequently given prophylaxis with ondansetron, prochlorperazine, and occasionally metoclopramide. Grade-2 nausea and/or vomiting was noted in 12 of 26 cycles administered in 13 patients at 17.8 mg/m²; no grade-3 episodes were noted at this dose level. Hypocalcemia occurred in 15 (grade 2) and 1 (grade 3) of 26 cycles administered in 13 patients at 17.8 mg/m² and in 8 (grade 2) and 1 (grade 3) of 14 cycles administered in 8 patients at 24.9 mg/m², but the episodes were not associated with symptoms or significant clinical findings. Alopecia was not observed. Despite concerns regarding catheter-site toxicities generated by the preclinical data, only 1 patient experienced a catheter-site complication that was not thought attributable to depsipeptide administration.

**Fig. 1** Hematological effects of depsipeptide. Counts from the first three cycles in 2 patients (A and B) were plotted after treatment. Results from cycle 1 are shown in the ○, cycle 2 with the ●, and cycle 3 with the □. These patients received 17.8 mg/m², except in A, where the patient received the first cycle of depsipeptide one dose above the MTD. The legend indicates whether the day-5 dose was administered on day 5 or day 6 and the dose received (mg/m²).

**Table 4** Nonhematological toxicity in cycle 1

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>17.8 mg/m² (n = 9)</th>
<th>24.9 mg/m² (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients with any</td>
<td>Patients with grades 3 and 4 toxicity</td>
</tr>
<tr>
<td></td>
<td>No. %</td>
<td>No. %</td>
</tr>
<tr>
<td>Anorexia</td>
<td>4 44</td>
<td>0 0</td>
</tr>
<tr>
<td>Cardiac EKG changes</td>
<td>7 78</td>
<td>0 0</td>
</tr>
<tr>
<td>Dysrhythmia</td>
<td>1 11</td>
<td>0 0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6 67</td>
<td>0 0</td>
</tr>
<tr>
<td>Fever</td>
<td>7 78</td>
<td>0 0</td>
</tr>
<tr>
<td>Nausea</td>
<td>7 78</td>
<td>0 0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>6 67</td>
<td>0 0</td>
</tr>
<tr>
<td>Taste change</td>
<td>6 67</td>
<td>0 0</td>
</tr>
<tr>
<td>Headache</td>
<td>4 44</td>
<td>0 0</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>7 78</td>
<td>1 11</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
<td>1 11</td>
<td>0 0</td>
</tr>
</tbody>
</table>

*ECG, electrocardiogram.
Cardiac Monitoring and Toxicity. A major concern during the protocol, based on the preclinical studies, was to identify any occurrence of cardiac toxicity. Because of this, patients were monitored for cardiac toxicity as described in the “Patients and Methods” section above. Beginning with the fourth dose level (3.5 mg/m²), ECG changes consisting of T-wave flattening in the anterolateral or inferior leads were observed (grade 1; Fig. 2). At a dose of 24.9 mg/m², 2 patients developed deep T-wave inversions with ST-segment depression (grade 2; Fig. 2), which returned to normal before the subsequent cycle. The ECG changes were noted to some degree within 24 h and were most marked on day 6, the day after the second dose of the cycle. Because ECG changes were not obtained between days 6 and 21, it is not known how long the ST/T-wave changes persisted. Three patients experienced asymptomatic arrhythmias after depsipeptide, detected during the inpatient cardiac monitoring period. Episodes included asymptomatic atrial bigeminy (on a background of preexisting sinus bradycardia) in 1 patient, a 3-s sinus pause while a 2nd patient was sleeping, and an asymptomatic five beat run of ventricular tachycardia in a 3rd patient. None of these was considered a DLT or definitely related to depsipeptide. One patient, described above, experienced an episode of atrial fibrillation that was symptomatic and dose limiting. The patient was subsequently retreated at a lower dose (17.8 mg/m²) without recurrence of the atrial fibrillation. Cardiac ejection fraction was measured in all patients before treatment and subsequently as described in “Patients and Methods.” The mean pretreatment ejection fraction was 56.4 ± 9.1%, and the mean of all post-treatment ejection fraction measurements was 53.1 ± 5.7%, values not found to be significantly different, P = 0.097. No patient developed any degree of clinical heart failure or a measured ejection fraction outside of the normal range. Among patients receiving doses of ≥17.8 mg/m², the mean ejection fraction pre and postdepsipeptide was 57.3 ± 7.4% and 56.6 ± 5.4%, respectively. Finally, no Troponin-I elevations were observed.

Pharmacokinetics. Table 5 provides an overview of the pharmacokinetic parameters of depsipeptide at the various dose levels in this trial. The plasma disposition of depsipeptide was well described by a first-order, two-compartment open pharmacokinetic model. Fig. 3 shows concentration versus time curves generated using this model and data from 2 different patients treated at 1 (Fig. 3A) and 17.8 mg/m² (Fig. 3B). Using the model, the mean volume of distribution, clearance, distribution half-life, and elimination half-life with a dose of 17.8 mg/m² was 8.6 liters/m², 11.6 liters/h/m², 0.42 h, and 8.1 h, respectively, with two compartmental analysis. In addition, the maximum concentration (Cmax) and AUC of depsipeptide increased as the dose increased (Fig. 4, A and B). The ratio of Cmax and AUC for C2 versus C1 showed no apparent differences. Both two-compartmental and noncompartmental methods of analysis provided similar pharmacokinetic parameters (Table 5, A and B). As such, either method can be used for pharmacokinetic analysis in future studies with depsipeptide.

Biological Assays. To confirm that the level of depsipeptide in the serum was sufficient to achieve the biological effects observed in vitro, two different assays were evaluated. First, serum was obtained from patients before treatment with depsipeptide and then at subsequent time points. This serum was added in a 1:1 dilution with normal media to a monolayer of proliferating PC3 cells. As a control, 10 ng/ml depsipeptide were added to PC3 cells alone without any patient serum. Fig. 5 shows the results obtained with serum derived from a patient at the indicated time points after treatment with 17.8 mg/m² depsipeptide. In the top right panel, there is a cell cycle arrest after the in vitro addition of 10 ng/ml depsipeptide. Similarly, the addition of patient serum obtained between 4 and 5 h after initiation of the depsipeptide infusion results in a cell cycle arrest. However, with serum obtained at 7 h, the degree of cell cycle arrest is minimal. This correlates well with the elimination half-life of 8.1 h obtained from pharmacokinetic analysis. Comparable results were obtained for all patients enrolled at the MTD. As a second assay performed to assess depsipeptide effects, histone acetylation was evaluated by immunofluorescence. Normal circulating mononuclear cells were obtained from patients pre and post-treatment. As shown in Fig. 6, there is an increase in histone acetylation in PC3 cells after in vitro treatment with depsipeptide. In the second and third rows, representative patient mononuclear cells are shown before and after treatment with depsipeptide (4 and 24 h in the second and third rows, respectively). Increased histone acetylation is observed. These results can be compared with the increase in histone acetylation observed in normal mononuclear cells to which depsipeptide has been added in vitro (bottom row).

Responses. As this was a Phase I trial, the response was a secondary end point. One partial response was observed at a dose of 9.1 mg/m² in a patient with renal cell carcinoma. Eight patients had stable disease at the initial evaluation, 24 patients had progressive disease, and 4 patients were not evaluable for response. The patient with a partial response had a >50%
reduction in size of supravacular, mediastinal, and retroperitoneal adenopathy. This response was observed after two cycles and persisted for an additional six cycles.

**DISCUSSION**

This Phase I study of depsipeptide was conducted to determine the MTD and define the toxicity of this novel agent when given as a 4-h infusion on a day-1 and day-5 schedule of a 21-day cycle. DLTs were fatigue, nausea, vomiting, and thrombocytopenia. One patient developed atrial fibrillation during an episode of severe nausea and vomiting. The MTD was defined as 17.8 mg/m² given over 4 h. Biological assays confirmed the ability of serum from patients treated with depsipeptide to induce cell cycle arrest in PC3 cells and confirmed an increase in histone acetylation in circulating mononuclear cells.

It has been demonstrated that FR901228 is a HDAC inhibitor (5). Histone acetylation provides an enzymatic mechanism to regulate transcription by affecting the interaction between DNA and histones. HDAC inhibitors have been shown to induce expression of genes linked to growth inhibition and cellular differentiation (15, 16). HDAC inhibitors synergize with retinoic acid to stimulate leukemia cell differentiation (17–19). We observed induction of the multidrug-resistance gene MDR1/Fipg and epidermal growth factor with features of a differentiated

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**Table 5  Pharmacokinetic parameters of depsipeptide (FR901228) of two-compartment open model (A) and noncompartmental analysis (B)**

<table>
<thead>
<tr>
<th>A. Dose (c) (mg/m²)</th>
<th>CL (b) (liters/h/m²)</th>
<th>(T_{1/2a}) (h)</th>
<th>(T_{1/2b}) (h)</th>
<th>(V_s) (liters/m²)</th>
<th>(C_{max}) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1.0\ (n = 3, c = 5))</td>
<td>8.8 ± 4.5</td>
<td>0.24 ± 0.14</td>
<td>8.3 ± 13.4</td>
<td>3.7 ± 3.6</td>
<td>35.6 ± 22.2</td>
</tr>
<tr>
<td>(1.7\ (n = 3, c = 5))</td>
<td>12.0 ± 7.5</td>
<td>0.61 ± 0.38</td>
<td>49.5 ± 58.6</td>
<td>9.2 ± 3.4</td>
<td>40.8 ± 20.9</td>
</tr>
<tr>
<td>(2.5\ (n = 3, c = 6))</td>
<td>21.6 ± 17.6</td>
<td>0.48 ± 0.68</td>
<td>6.1 ± 31.2</td>
<td>9.3 ± 10.1</td>
<td>49.5 ± 31.7</td>
</tr>
<tr>
<td>(3.5\ (n = 1, c = 2))</td>
<td>10.6 ± NA</td>
<td>0.63 ± NA</td>
<td>9.5 ± NA</td>
<td>10.5 ± NA</td>
<td>129.9 ± NA</td>
</tr>
<tr>
<td>(6.5\ (n = 3, c = 5))</td>
<td>10.5 ± 2.2</td>
<td>0.30 ± 0.11</td>
<td>6.6 ± 2.2</td>
<td>5.0 ± 1.6</td>
<td>211.8 ± 77.1</td>
</tr>
<tr>
<td>(9.1\ (n = 4, c = 7))</td>
<td>21.7 ± 11.4</td>
<td>1.20 ± 2.3</td>
<td>19.0 ± 24.5</td>
<td>10.5 ± 13.5</td>
<td>162.6 ± 53.8</td>
</tr>
<tr>
<td>(12.7\ (n = 3, c = 5))</td>
<td>38.2 ± 26.4</td>
<td>0.26 ± 0.20</td>
<td>12.7 ± 9.1</td>
<td>20.7 ± 20.2</td>
<td>224.7 ± 241.0</td>
</tr>
<tr>
<td>(17.8\ (n = 11, c = 19))</td>
<td>11.6 ± 5.8</td>
<td>0.42 ± 0.25</td>
<td>8.1 ± 6.0</td>
<td>8.6 ± 6.3</td>
<td>553.8 ± 299.5</td>
</tr>
<tr>
<td>(24.9\ (n = 8, c = 12))</td>
<td>19.2 ± 10.6</td>
<td>0.34 ± 0.18</td>
<td>4.3 ± 2.3</td>
<td>8.6 ± 3.3</td>
<td>478.2 ± 316.6</td>
</tr>
</tbody>
</table>

**B. Dose \(c\) (mg/m²) | \(n\) | AUC\(0-1\) (µg/ml/h) | AUC\(0-∞\) (µg/ml/h) | \(T_{1/2a}\) (h) | \(V_{ss}\) (liters/m²) | CL\(0-∞\) (liters/h/m²) |
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>(1.0)</td>
<td>3</td>
<td>0.13 ± 0.09</td>
<td>0.14 ± 0.10</td>
<td>11.2 ± 14.9</td>
<td>48.8 ± 65.5</td>
<td>10.2 ± 6.9</td>
</tr>
<tr>
<td>(1.7)</td>
<td>3</td>
<td>0.15 ± 0.07</td>
<td>0.16 ± 0.07</td>
<td>14.4 ± 9.8</td>
<td>38.5 ± 27.0</td>
<td>21.5 ± 9.7</td>
</tr>
<tr>
<td>(2.5)</td>
<td>3</td>
<td>0.12 ± 0.11</td>
<td>0.12 ± 0.11</td>
<td>2.3 ± 2.9</td>
<td>38.5 ± 27.0</td>
<td>35.4 ± 26.5</td>
</tr>
<tr>
<td>(3.5)</td>
<td>1</td>
<td>0.23 ± NA</td>
<td>0.24 ± NA</td>
<td>11.8 ± NA</td>
<td>37.9 ± NA</td>
<td>14.7 ± NA</td>
</tr>
<tr>
<td>(6.5)</td>
<td>3</td>
<td>0.52 ± 0.06</td>
<td>0.53 ± 0.06</td>
<td>8.1 ± 3.7</td>
<td>18.8 ± 3.7</td>
<td>12.5 ± 1.4</td>
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<tr>
<td>(9.1)</td>
<td>4</td>
<td>0.75 ± 0.50</td>
<td>0.79 ± 0.49</td>
<td>20.6 ± 16.3</td>
<td>126.8 ± 155.0</td>
<td>14.5 ± 7.4</td>
</tr>
<tr>
<td>(12.7)</td>
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<td>0.35 ± NA</td>
<td>0.35 ± NA</td>
<td>8.5 ± NA</td>
<td>55.3 ± NA</td>
<td>34.1 ± NA</td>
</tr>
<tr>
<td>(17.8)</td>
<td>9</td>
<td>2.21 ± 1.35</td>
<td>2.27 ± 1.34</td>
<td>11.7 ± 8.7</td>
<td>37.1 ± 43.8</td>
<td>10.5 ± 6.4</td>
</tr>
<tr>
<td>(24.9)</td>
<td>8</td>
<td>1.94 ± 1.50</td>
<td>1.95 ± 1.52</td>
<td>7.5 ± 5.5</td>
<td>20.4 ± 8.3</td>
<td>18.5 ± 10.1</td>
</tr>
</tbody>
</table>

\(a\) Values, mean ± SD from cycles 1 and 2. 
\(b\) CL, systemic clearance; \(T_{1/2a}\), distribution half-life; \(T_{1/2b}\), elimination half-life; \(V_s\), volume of distribution of central compartment; \(C_{max}\), observed maximum concentration; \(n\), number of patients per dose group (patients who had a dose reduction with the second cycle; the data is reflected for both cycles); \(c\), number of pharmacokinetic profiles per dose group; \(V_{ss}\), volume of distribution at steady state; AUC\(_{0-∞}\), AUC time curve (0 to the last measurable concentration time point); AUC\(_{0-1}\), AUC time curve (0 to infinity). 
\(c\) One patient from the dose group was excluded from the toxicity evaluation; Values, mean ± SD from cycle 1. 
\(d\) Pharmacokinetic profile was not available from 2 patients in the dose group.

**Fig. 3** Depsipeptide plasma concentration time curves from 2 representative patients receiving different dosing regimens. A, 1 mg/m²; B, 17.5 mg/m²; ○, observed concentrations; ——, fitted for two-compartment model.
phenotype in human colon cancer and neuroblastoma cell lines after the addition of the HDAC inhibitor sodium butyrate (20–22). Likewise, depsipeptide induces multidrug-resistance gene 1 expression in human colon cancer cell lines (data not shown). Unlike sodium butyrate, which has also been studied in clinical trials, depsipeptide is active in the nm range, and the induced Pgp is functional and able to transport rhodamine.

Although both neutropenia and thrombocytopenia occurred after administration of depsipeptide, the drop in cell counts was rapid and transient, suggesting a mechanism other than toxicity to early myeloid precursors as occurs with myelosuppressive cytotoxic agents in current clinical use. Bone marrow aspirates and biopsies were not performed as a part of this study; therefore, the level of maturation at which the myelosuppressive action of depsipeptide occurs remains unknown. Although patients had delays in the administration of the day-5 dose because of either thrombocytopenia, neutropenia, or both, no complications referable to those toxicities were observed. Taking into account that the thrombocytopenia and neutropenia were maximal at 10 and 5 days, respectively, similar to the life span in blood, these results suggest that depsipeptide may affect mature cells.

On the basis of preclinical data, both cardiac toxicity and catheter site complications were expected to be dose limiting. To maximize safety in the conduct of the trial, cardiac monitoring on this study was extensive, and central venous access catheters were allowed to heal for 7 days before dosing. There was no evidence of skin necrosis at the catheter site, and skin injury sites, such as needle sticks for pharmacokinetic sampling, healed normally without excessive bleeding or evidence of skin necrosis, a discrepancy between the preclinical data and the results of the trial.

Cardiac toxicities recognized in this trial consisted mainly of asymptomatic dysrhythmias and nonspecific ST/T changes on the 12-lead ECG. With continuous cardiac monitoring, three episodes of asymptomatic dysrhythmias and one episode of symptomatic atrial fibrillation requiring treatment were recorded. We cannot ascertain whether these episodes represent cardiac toxicity because of depsipeptide or dysrhythmias that would have occurred nevertheless but were detected because of the intensive monitoring. Serial measurements of cardiac output by MUGA scanning showed no clinically significant decline in cardiac output before and after treatment. The possibility that a greater degree of cardiac toxicity could become manifest with a more prolonged treatment cannot be excluded, and cardiac mon-
onitoring should be included in additional trials of this agent. Nausea and vomiting were associated with depsipeptide infusion. The nausea was delayed and, at the level which exceeded the MTD, was only partially responsive to ondansetron, prochlorperazine, and metoclopramide. It is possible that some of the electrocardiographic abnormalities observed were related to the use of these antiemetics, which have been reported to precipitate electrocardiographic changes and arrhythmias (23–27).

The major DLT of depsipeptide was profound fatigue. At 24.9 mg/m², all patients experienced some degree of fatigue, and one-third of patients experienced fatigue that was profound, functionally limiting, and lasted up to 1 week. The fatigue was not associated with anemia and was not clearly associated with other defined biochemical abnormalities. The 4 patients experiencing hypophosphatemia at 24.9 mg/m² may indicate that depsipeptide could induce cellular changes in ATP levels or availability. At the MTD, only 1 patient experienced dose-limiting, grade-3 fatigue in cycle 3, which recurred in cycle 4, although his dose was reduced to 12.7 mg/m². Six of the 9 patients enrolled at the MTD experienced some fatigue in the first cycle, and in the total 26 cycles administered to 13 patients at 17.8 mg/m², regardless of dose level of entry, there were 15 episodes of fatigue.

In summary, the toxicities attributable to depsipeptide administered in a day-1 and day-5 schedule are distinct from those associated with conventional chemotherapeutic agents. There was no alopecia observed, no mucositis, and a rapid recovery of blood counts that was dissimilar to the bone marrow suppression, resulting from an anthracycline or an alkylating agent. The chief DLT was fatigue; this was mild at the MTD but severe at doses exceeding the MTD. Interestingly, grade-3 toxicities for another HDAC inhibitor, tributyrin, consisted of nausea, vomiting, and myalgia, whereas fatigue occurred as a grade-1 toxicity (28). In a trial combining IL-2 with arginine butyrate, fatigue was the DLT (29). Although fatigue as a toxicity may also be nonspecific, it is tempting to speculate that it is related to HDAC inhibition.

HDAC inhibitors like depsipeptide have great potential as
anticancer agents, because the compounds induce expression of genes involved in cellular differentiation. This constellation of genes includes several that make attractive targets for combined anticaner therapy. We have shown in laboratory studies that the sodium-iodide symporter is induced in thyroid cancer cells (13). This would make depsipeptide potentially useful in combination with radioiodine. The HDAC inhibitor arginine butyrate has been shown in preliminary studies to induce expression of the IL-2 receptor (30). This receptor is the therapeutic target for an IL-2 toxin conjugate that has been approved for treatment of patients with cutaneous T-cell lymphoma (31). Indeed, preliminary results using depsipeptide in patients with cutaneous T-cell lymphoma suggests significant activity in that disease (32). Finally, there are in vitro studies suggesting that combination therapy with retinoids and nonretinoid differentiating agents may be more effective than retinoids alone (33, 34). If depsipeptide was to induce expression of the retinoic acid receptor, a basis for combined therapy with retinoids would be established. On the basis of its potency and cytotoxicity, depsipeptide appears to be a novel and potent HDAC inhibitor that may allow this class of drugs to enter clinical use.

ACKNOWLEDGMENTS

We thank the contributions of members of Fujisawa Pharmaceutical Co. for the supply of the compound and helpful review of the data and Ed Sausville and the Developmental Therapeutics Program for shepherding FR901228 through preclinical development. We also thank Wilma Medina-Pérez and Dr. Susan Mertins for cell culture work.

REFERENCES


Phase I Trial of the Histone Deacetylase Inhibitor, Depsipeptide (FR901228, NSC 630176), in Patients with Refractory Neoplasms

Victor Sandor, Susan Bakke, Robert W. Robey, et al.


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