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ORIGINAL ARTICLE

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Plasma and cerebrospinal fluid pharmacokinetics of depsipeptide (FR901228) in nonhuman primates

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Abstract *Purpose*: Acetylation of histones by histone acetyl transferases (HATs) leads to transcriptional activation, while histone deacetylase (HDAC) activity leads to transcriptional repression. Abnormalities of histone acetylation are associated with the malignant phenotype. Depsipeptide (FR901228) inhibits HDAC and has shown anticancer activity in preclinical models. We studied the plasma and cerebrospinal fluid (CSF) pharmacokinetics of depsipeptide in a nonhuman primate model that is highly predictive of human CSF penetration. *Design*: Depsipeptide was administered intravenously at a dose of 10 mg/m^2 over 4 h to three different animals. Serial blood samples were obtained from all animals and serial CSF samples were obtained from two animals. Plasma and CSF concentrations of depsipeptide were measured using liquid chromatography/tandem mass spectrometry. Concentration-versus-time data were modeled using model-independent and model-dependent methods. *Results*: The peak plasma concentration (median ± SD) was 245 ± 50 n *M* and occurred within the first 2 h of the infusion. The terminal half-life was 205 ± 315 min, the AUC extrapolated to infinity was 50 ± 15 μ*M*·min, and the total body clearance was 350 ± 65 ml/min/m^2. In the two animals that had CSF sampling performed, the CSF peak concentration was 3.6 n *M* in one animal and 2.3 n *M* in the other, and the CSF half-lives were 250 and 325 min. The CSF penetration of depsipeptide (AUC_{CSF}:AUC_{plasma}) was 2% in each animal. Observed changes included anorexia, fatigue, elevation of creatine phosphokinase (CPK) enzyme levels (muscle fraction), and transient early leukopenia. All animals recovered without sequelae. *Conclusions*: Although the CSF exposure to depsipeptide after intravenous administration was only 2%, CSF concentrations approached the IC_{50} of depsipeptide in vitro for some tumors. Systemic administration of this agent may be useful for the treatment of leptomeningeal tumors.

Keywords Depsipeptide · FR901228 · CSF penetration · Pharmacokinetics

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Introduction

Histone proteins associate with DNA in the nucleosome. The binding of histones to DNA plays a role in DNA transcription and gene expression, and is partly regulated by the acetylation status of the histones. Acetylation of histones by histone acetyl transferases (HATs) leads to transcriptional activation, while histone deacetylase (HDAC) activity leads to transcriptional repression. Abnormalities of histone acetylation are associated with the malignant phenotype [1, 2, 3, 4, 5, 6].

Depsipeptide (FK228, formerly FR901228) was initially found to reverse the malignant phenotype of Ha-ras-transformed NIH 3T3 cells [7] and was subsequently identified as a potent histone deacetylase (HDAC) inhibitor [8]. Depsipeptide causes cell cycle arrest at the G_1/G_2M interphase, as well as internucle-

osomal chromatin breakdown [8]. The drug downregulates c-myc, and also downregulates cyclin D1 with upregulation of CDK inhibitor p21, resulting in inhibition of CDK activity, Rb dephosphorylation, and G_1 arrest [9]. There is also some evidence that depsipeptide may act, in part, through inhibition of angiogenesis [10]. Recent studies suggest that depsipeptide may function as a prodrug that undergoes intracellular reduction to the active compound [11].

In preclinical studies, depsipeptide has shown significant cytotoxic activity against multiple human tumor cell lines, murine tumors, and human tumor xenografts [12, 13, 14]. Preclinical studies in mice, rats, and dogs have demonstrated that infusions of 1 to 4 h produce the least toxicity and permit maximization of individual doses. Cardiac toxicity is seen with some dosing schedules, and local inflammation and necrosis may occur at catheter insertion sites. We studied the plasma and cerebrospinal fluid (CSF) pharmacokinetics of depsipeptide after systemic administration in a nonhuman primate model that is highly predictive of CSF drug disposition in humans [15].

Materials and methods

Drugs

Depsipeptide was supplied by the Division of Cancer Treatment, NCI (Bethesda, Md.) in vials of 10 mg lyophilized powder. Each 10-mg vial was reconstituted with 2 ml special diluent (containing a 4:1 mixture of propylene glycol and ethanol, also supplied by NCI) and then administered after dilution in 0.9% sodium chloride to a concentration of 0.04 mg/ml. All bags and tubing were wrapped in foil to protect the drug from light exposure during infusion.

Monkeys

This protocol was approved by the Institutional Animal Care and Use Committee. Three adult male rhesus monkeys (*Macaca mulatta*) weighing 12.3 to 14.5 kg were used in these experiments. The animals were fed Open Formula Extruded Non-Human Primate Diet twice daily and group-housed in accordance with Guide for the Care and Use of Laboratory Animals [16]. Drug was administered through a surgically implanted central venous catheter. Blood samples were drawn through a catheter placed in the contralateral femoral or saphenous vein. Ventricular CSF samples were obtained from a chronically indwelling fourth ventricular catheter attached to a subcutaneously implanted Ommaya reservoir [15]. The reservoir was pumped four times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF.

Experiments

Three animals received depsipeptide intravenously at a dose of 10 mg/m^2 administered over 4 h. Blood samples and ventricular CSF was collected immediately prior to administration, and at target times of 1, 2, and 3 h during the infusion, at the end of the infusion, and at 15 and 30 min, and 1, 2, 4, 6, 8, 10, 24 and 48 h after infusion. Plasma was separated immediately by centrifugation at 12,000 *g* for 10 min and frozen. CSF was frozen immediately after collection. Hematology and blood chemistry tests were obtained on a weekly basis for a minimum of 3 weeks after the depsipeptide infusion according to institutional guidelines. Creatine phosphokinase (CPK) was subsequently fractionated after marked elevations of total CPK were observed. Animals were also observed for a minimum of 3 weeks after infusion for any evidence of clinical toxicity.

Sample analysis

Depsipeptide concentrations in plasma and CSF samples were analyzed using a previously published LC/MS/MS method with slight modifications with a limit of quantitation of 0.1 ng/ml [17, 18]. Briefly, each 0.2-ml plasma sample was spiked with 500 ng BMLP (N-t-Boc-Met-Leu-Phe) in 100 µl normal saline as an internal standard. Then 0.2 ml potassium phthalate buffer (pH 4.0, 50 m *M*) and 2 ml ethyl acetate was added and the tube was agitated for 10 min by a horizontal shaker at room temperature. The ethyl acetate layer was separated by centrifugation at 2500 *g* for 5 min and the organic phase was evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted with 80 µl mobile phase and 30 µl was injected onto the LC/MS/MS system. Monkey CSF samples were analyzed similarly, except that 0.2 ml blank human plasma was added to 0.2 ml CSF before extraction. Calibration curves were constructed separately for monkey plasma and CSF.

The LC/MS/MS system consisted of a Perkin-Elmer Sciex API 300 triple-quadrupole mass spectrometer (Thornhill, Ontario, Canada) coupled to a Shimadzu HPLC system (Shimadzu, Columbia, Md.). The HPLC separation was achieved on a 50×2 mm BetaBasic C8 5-µm column with isocratic elution consisting of 70% acetonitrile and 0.1% acetic acid (v/v) at a flow rate of 0.2 ml/min. The split ratio was 9:1 (waste:source) so that the eluate was introduced into the API source at 20 µl/min. The mass spectrometer was equipped with an electrospray ionization source. Multiple reaction monitoring was used to monitor the precursor/product ion pairs of depsipeptide (*m*/*z* 541.2/424) and the internal standard BMLP (*m*/*z* 510.0/217).

Pharmacokinetic analysis

Plasma concentration-time data were modeled in ADAPT II using the maximum likelihood method [19]. Two- and three-compartment models were fitted to the data and the best fit was determined using Akaike's Information Criterion [20]. Clearance was determined from the equation $Cl = K_{10} \cdot V_c$ and half-lives for each phase were determined from the equation $t_{1/2} = \ln 2/\text{lambda}$ where lambda is the disposition rate constant for the phase [21]. For plasma and CSF, the areas under the concentration-time curve (AUC) were determined by the linear trapezoidal method and extrapolated to infinity using the terminal rate constant [21].

Results

Pharmacokinetics

The pharmacokinetics of depsipeptide after i.v. administration over 4 h in the nonhuman primate are best described by a biexponential model. Pharmacokinetic parameters are listed in Table 1 and a graph of depsipeptide concentration as a function of time is shown in Fig. 1. The peak plasma concentration (median ± SD) was 245 ± 50 n *M* and occurred within the first 2 h of the infusion. The terminal half-life was 205 ± 315 min, the AUC extrapolated to infinity was 50 ± 15 µ*M*·min, and the clearance was 350 ± 65 ml/min/m^2. In the two

Table 1 Depsipeptide pharmacokinetic parameters in plasma after a 4 hour infusion of 10 mg/m^2 in the nonhuman primate

Animal	k_{10} (min^{-1})	K_{12} (min^{-1})	K_{21} (min^{-1})	$T_{1/2\alpha}$ (min)	$T_{1/2\beta}$ (min)	AUC (μM·min)[a]	Clearance (ml/min/m^2)
1	0.15520	0.01161	0.004031	4.147	185.100	48.8	350
2	0.26270	0.01602	0.003715	3.872	205.300	42.7	410
3	0.05365	0.01788	0.001252	9.649	741.600	67.9	275
Median	0.1552	0.01602	0.00372	4.2	205	48.8	350
SD	0.1044	0.00322	0.00152	3.3	316	13.2	65

[a]Model-independent parameter; others are from ADAPT 2 compartment model

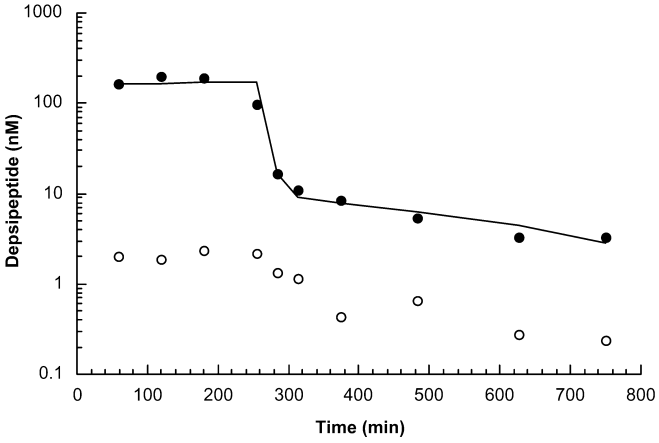

Fig. 1 Representative plasma concentration-time curve after i.v. administration of depsipeptide at 10 mg/m^2 over 4 h to a nonhuman primate (animal 2) (● measured plasma depsipeptide concentration, ○ measured CSF depsipeptide concentration, — plasma depsipeptide concentration predicted by the two-compartment model)

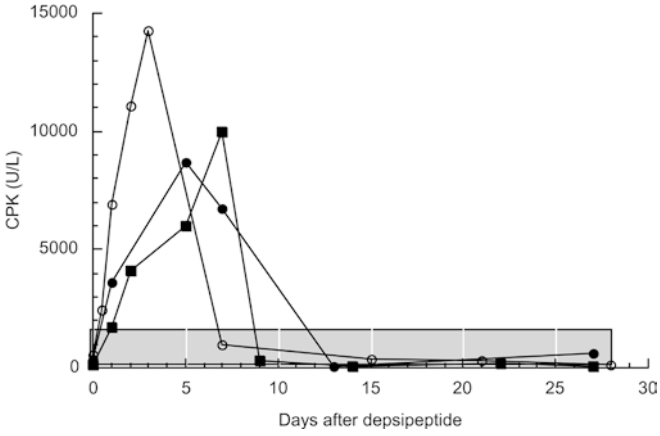

Fig. 2 Serum CPK levels in each animal ($n=3$) in the 15 days following drug administration. *Shaded area* represents the normal range (● animal 1, ○ animal 2, ■ animal 3)

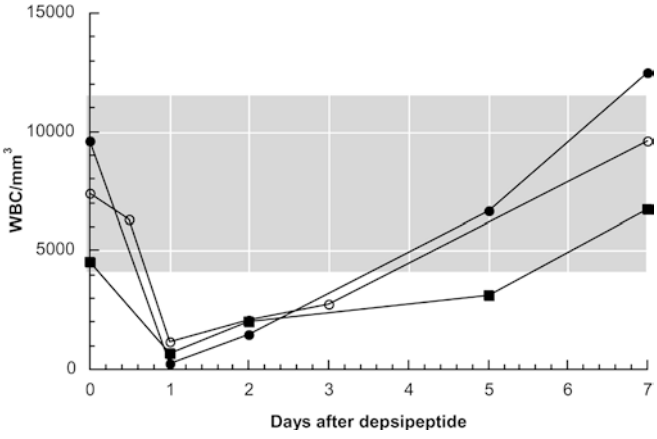

Fig. 3 Serum total white blood cell counts in each animal ($n=3$) in the 8 days following drug administration. *Shaded area* represents the normal range. (● animal 1, ○ animal 2, ■ animal 3)

animals that had CSF sampling performed, the CSF peak concentration was 3.6 n *M* in one animal and 2.3 n *M* in the other, and the CSF half-lives were 250 and 325 min. The CSF penetration of depsipeptide (AUC_{CSF}: AUC_{plasma}) was 2% in each animal.

Other findings

After the depsipeptide infusions the animals were noted to have a poor appetite and fatigue or listlessness. These symptoms resolved within 6 days of depsipeptide administration. In the first animal, a CPK of 6700 IU/l (upper limit of normal 1680 IU/l) was noted on routine laboratory evaluation 1 week after drug administration. The CPK resolved to baseline during week 2. A graph showing the total serum CPK level in all three animals at baseline and for 2 weeks following drug administration is shown in Fig. 2. Fractionation of the CPK demonstrated that the predominant fraction was the muscle fraction. Other laboratory abnormalities included mild elevations in serum lactate dehydrogenase (LDH) and an early decrease in the total white blood cell count. Figure 3 shows the total white blood cell count in the animals at baseline and for 1 week after drug administration. The leukopenia was atypical in that it occurred within 24 h after drug administration and had resolved by day 7. No animals received vehicle only, so data from controls are not available.

Discussion

In this study we showed that depsipeptide pharmacokinetics after i.v. administration over 4 h in the nonhuman

primate are best described by a biexponential model, with a terminal half-life of 205 ± 315 min and a clearance of 350 ± 65 ml/min/m^2. The half-life is considerably longer and the clearance lower in nonhuman primates than those observed in preclinical studies in rats [17]. Our results are in good agreement with recently published results from a phase I trial in adults, in which the terminal half-life was 8.1 h (486 min) and the clearance was 11.6 l/h/m^2 (approximately 200 ml/min/m^2) [22].

The changes observed in the nonhuman primates were fatigue and poor appetite accompanied by an elevation in the CPK and acute leukopenia. It is not clear whether changes observed during the study were caused by administration of depsipeptide since there was no vehicle control group set in the study. The dose used in our study, 10 mg/m^2, was less than the maximum tolerated dose of 17.8 mg/m^2 identified in adult phase I studies.

Depsipeptide penetrates poorly into the CSF, with an AUC_{CSF}:AUC_{plasma} of 2%. This may be explained by its high degree of protein binding, since for most drugs only the free fraction equilibrates across the blood-brain barrier [23]. The peak concentrations observed in the CSF were 2–3 n *M*. The IC_{50} of tumor cell lines in the National Cancer Institute screening panel are 2–10 n *M* [24, 25]. Since the recommended adult phase II dose (17.8 mg/m^2) is nearly twice the dose we administered to the nonhuman primates, the CSF concentrations achieved in human CSF may approach the IC_{50} of tumor cell lines despite the poor overall CSF penetration of this agent.

Phase II studies of depsipeptide in adults are underway, as is a phase I study in children with refractory cancer.

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