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Determination of the antitumor agent depsipeptide in plasma by liquid chromatography on serial octadecyl stationary phases

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Abstract

A high-performance liquid chromatographic assay has been developed and validated for the determination of the antitumor agent depsipeptide (FR-901228) in plasma samples from patients with advanced cancer. After the plasma proteins were precipitated with acetonitrile, the supernatant was extracted with ethylacetate. Depsipeptide was chromatographed on two serial octadecylsilica stationary phases using a mobile phase consisting of acetonitrile–potassium phosphate buffer (0.03 *M*, pH 3) (27:73, v/v), at a flow-rate of 2.0 ml/min and at ambient temperature. The method was linear over a 50 to 2000 ng/ml range and the intra- and inter-day coefficients of variations were less than 8%. The method was applied to the determination of the plasma concentration–time profile for 14 patients with advanced cancer receiving from 1 to 7.5 mg/m^2 of depsipeptide per day as a continuous 4-h infusion.

Keywords: Depsipeptide

1. Introduction

Depsipeptide (FR-901228) is a unique bicyclic peptide containing a non-cystine disulfide bridge isolated from *Chromobacterium violaceum* Strain WB968 (Fig. 1A) [1–3]. This compound has been found to have inhibitory activity against oncogene function in bacteria. For example, depsipeptide decreased the mRNA expression of the C-myc oncogene, yet had no effect on Ha-ras mRNA expression. The compound preferentially inhibited RNA synthesis and caused cell cycle arrest at G_0/G_1 phases without producing DNA single strand breaks or cross-links.

The development of novel peptide analogues as therapeutic agents has recently been of increasing interest in drug research. As many peptide analogues are often administered at a low dosage, a highly sensitive analytical method is required to support pharmacokinetic studies. Derivatization of the peptide analyte with a fluorescent dye is usually carried out to improve its detectability when using high-performance liquid chromatography (HPLC) [4–8]. Recently, different methods have been developed requiring no derivatization. Oxytoxin, a cyclic nonapeptide, has been quantified by isocratic HPLC with coulometric detection [9]. Cyclosporin A

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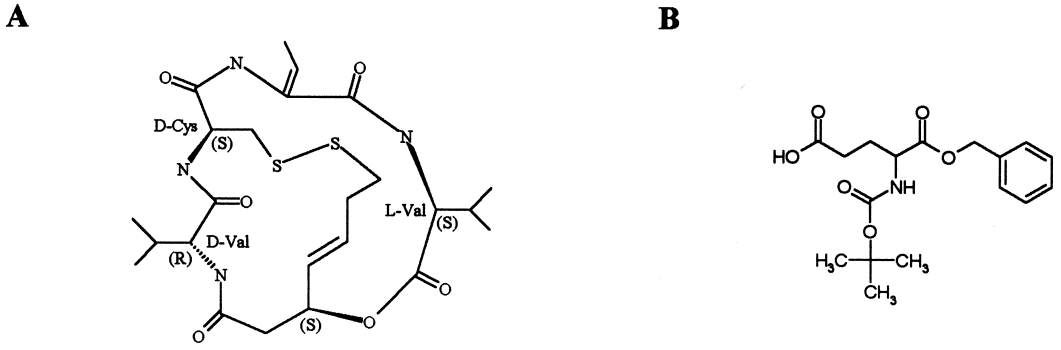

Fig. 1. Structures of the compounds used in this study: (A) depsipeptide; (B) internal standard.

[10,11], melanotan-II, a melanotropin fragment analog, [12] and also a cyclic lipopeptide of the echinocandin class [13], have been assayed by HPLC using UV detection.

Regarding this new trend in peptide analysis, a HPLC method has been developed and validated for the determination of depsipeptide in plasma. Depsipeptide does not have a strong chromophore amenable for UV detection at relatively long wavelengths (Fig. 1A). Although depsipeptide has a relatively large molecular size, a high lipophilicity and a low aqueous solubility, it was difficult to resolve this compound from other interfering substances present in biological fluids. To overcome this problem, two octadecylsilica stationary phases were placed in series to separate depsipeptide from the endogenous plasma interferences. This manuscript reports the successful application of this reversed-phase HPLC method, coupled to UV detection, to the quantitative determination of depsipeptide in plasma. The resulting assay has been used to analyze plasma samples from pediatric patients receiving a continuous 4-h infusion of depsipeptide as part of a phase I study.

2. Experimental

2.1. Chemicals

Depsipeptide {(E)-(1*S*,4*S*,10*S*,21*R*)-7-[(*Z*)-ethylidene] - 4,21 - diisopropyl - 2 - oxa - 12,13 - dithia - 5,8, 20, 23 - tetraazabicyclo - [8, 7, 6] - tricos - 16 - ene - 3,6,9,19,22-pentanone; Fig. 1A}, was provided by Fujisawa Pharmaceutical (Osaka, Japan). The internal standard (I.S.) (*N-tert.*-boc-D-glutamic acid α-benzyl ester; Fig. 2A) was purchased from Sigma (St. Louis, MO, USA). The source for HPLC grade acetonitrile and ethylacetate was EM Science (Gibbstown, NJ, USA). Sodium fluoride, *o*-phosphoric acid and potassium phosphate monobasic were purchased from Fisher Scientific (Jessup, MD, USA). Pooled control human plasma was supplied by the Blood Bank of the Georgetown University Medical Center (Washington, DC, USA).

2.2. Apparatus

HPLC was carried out using a Hewlett-Packard series 1100 system comprising a G1310A isocratic pump, a G1314A variable-wavelength UV detector and a G1313A auto-sampler (Hewlett-Packard, Wilmington, DE, USA). The solutes were detected at 210 nm. The data collection was carried out with a Hewlett-Packard Vectra 120 MHz Pentium computer equipped with the HP Chemstation software (rev A.04.02). Two serial Ultrabiosep octadecylsilica 10 μm stationary phases packed in 150×4.6 mm columns (Hypersil, Cheshire, UK) were used to carry out the chromatography.

2.3. Chromatographic procedures

The chromatographic separation of depsipeptide and the I.S. was achieved with a mobile phase consisting of acetonitrile–potassium phosphate buf-

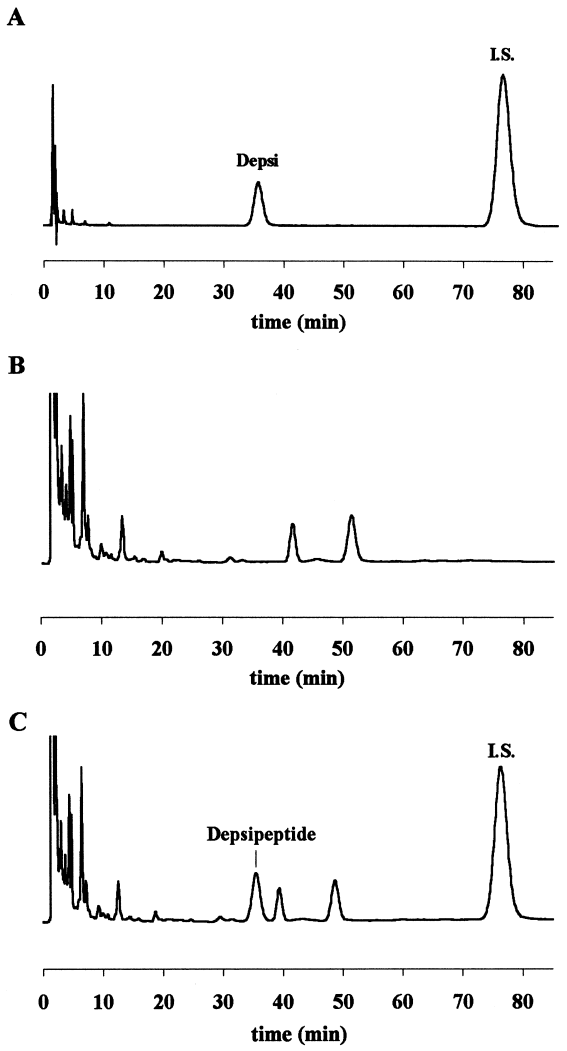

Fig. 2. Chromatograms of the analysis of (A) 500 ng/ml depsipeptide and 50 μg/ml internal standard in water; (B) blank plasma; (C) plasma spiked with 500 ng/ml depsipeptide and 50 μg/ml internal standard.

fer (0.03 *M*, pH 3) (27:73, v/v). A flow-rate of 2.0 ml/min and ambient temperature were used throughout the study.

2.4. Stock solutions

A stock solution of depsipeptide (1 mg/ml) is prepared in 80% methanol and diluted with water to 100 μg/ml. Stock plasma solutions containing 50, 80, 100, 200, 500, 800, 1000, 1600 and 2000 ng/ml of depsipeptide solution were prepared by serial dilution. A stock solution of I.S. (100 μg/ml) was prepared in 2% methanol. All the standards were prepared in bulk at the start of the validation, divided into appropriate aliquots and stored frozen at −20°C prior to analysis.

2.5. Sample preparation

Aliquots (1 ml) of the appropriate depsipeptide spiked stock plasma solutions were placed in culture tubes, sodium fluoride (100 mg) was added and the solution was vortex-mixed for 1 min. This was followed by the addition of 100 μl of I.S. (100 μg/ml) and the resulting solution vortex-mixed for 30 s. Acetonitrile (200 μl) was added, the mixture vortex-mixed for 30 s, water (1.8 ml) was then added and the resulting mixture was shaken for 30 s. Ethylacetate (4 ml) was added and the mixture was vortex-mixed for 1 min, centrifuged at 4000 *g* for 10 min, the tubes placed in a dry ice–acetone bath for 20 min (until the aqueous phase was frozen) and the organic phase was decanted into a clean propylene tube. The ethylacetate was evaporated under airflow at room temperature, the residue reconstituted using 33 μl acetonitrile, then diluted with 167 μl of the mobile phase and analyzed.

2.6. Validation studies

2.6.1. Standard curve

Triplicate standard curves were prepared by spiking drug-free plasma with known amount of depsipeptide and I.S. prior to the extraction procedure. Concentration of depsipeptide were 50, 100, 200, 500, 1000 and 2000 ng/ml. Calibration curves plotting depsipeptide/I.S. peak area ratios as a function of depsipeptide plasma concentration were derived.

2.6.2. Recovery

The recovery of depsipeptide was determined

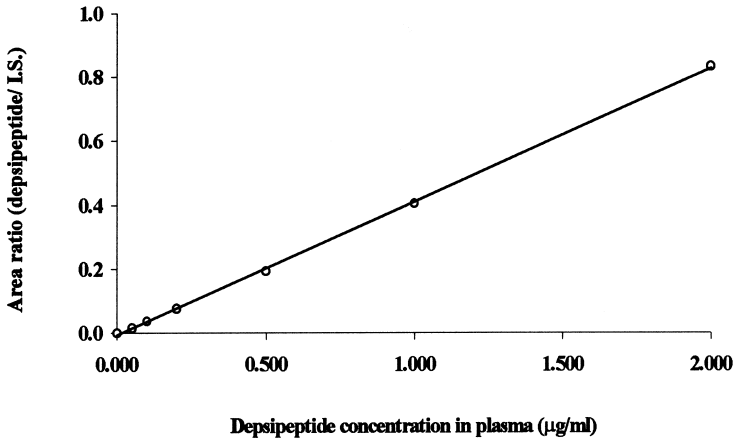

Fig. 3. Calibration curve plotting depsipeptide/I.S. peak area ratios as a function of depsipeptide plasma concentration.

using spiked plasma at 80, 800 and 1600 ng/ml. Recoveries were estimated by comparing the mean peak areas of extracted spiked plasma samples to the mean peak areas of equivalent aqueous standard solutions.

2.6.3. Accuracy and precision

Intra-day and inter-day variability studies were performed by spiking drug-free plasma with 80, 800 and 1600 ng/ml depsipeptide. Five samples at each concentration were extracted on each of the three consecutive days.

2.7. Clinical samples

A phase I trial of depsipeptide was carried out in patients with histologically incurable malignancy. The patients received depsipeptide by central intravenous (i.v.) infusion over 4 h on days 1, 8 and 15, sequence repeated every 28 days. The first three patients began with a dose of 1 mg/m^2 (level 1). Since no patient developed dose limiting toxicity, the next three patients were started at a dose of 2 mg/m^2 (level 2). The dose were then escalated with additional patients to 3.25, 5 and 7.5 mg/m^2 (levels 3, 4 and 5, respectively). Plasma samples were obtained

Table 1
Intra-day variabilities for the assay of depsipeptide in plasma

Depsipeptide level (ng/ml)	Mean observed concentration (ng/ml)	*n*	S.D.	C.V. (%)
80	87	5	0.006	7.269
800	818	5	0.052	6.412
1600	1706	5	0.083	4.876

Table 2
Inter-day variabilities for the assay of depsipeptide in plasma

Depsipeptide level (ng/ml)	Mean observed concentration (ng/ml)	*n*	S.D.	C.V. (%)
80	85	12	0.005	5.838
800	819	11	0.050	6.117
1600	1694	14	0.088	5.217

Table 3
Depsipeptide recovery from plasma

Depsipeptide level (ng/ml)	Mean observed recovery (%)	*n*	S.D.	C.V. (%)
80	68.704	12	1.957	2.849
800	84.431	11	7.739	9.166
1600	81.894	14	11.244	13.730

just prior to the infusion and then 3:30, 3:45 and 4:00 h into the infusion followed by 5, 10, 15, 30 and 45 min, 1, 2, 3, 4, 6 and 8 h after the end of the infusion. The plasma was frozen at −20°C until assayed.

3. Results and discussion

3.1. Chromatographic results

On the basis of the lipophilic nature of depsipeptide, it was assumed that the compound could be extracted from the plasma using an organic solvent. Ethylacetate and methylene chloride were evaluated as extraction solvents for depsipeptide and the former was found to be more adequate. Several methods for initial protein precipitation were also investigated including the use of methanol, trichloroacetic acid, saturated ammonium phosphate solution and acetonitrile. Protein precipitation by acetonitrile or methanol was found to be most suitable since an improvement of the depsipeptide recovery was observed. Finally, acetonitrile was chosen because it resulted in a lower chromatographic background. The remaining task was to select an appropriate internal standard. The *tert*.-boc-D-glutamic acid α-benzyl ester was found to be eluted with a good peak shape in a region without interference. Sodium fluoride was used to prevent enzymic hydrolysis of the depsipeptide and the internal standard.

The aim of this work was to design a simple analytical method easily reproducible in laboratories that were not necessarily skilled in chromatographic techniques. Since gradient profiles change according to the HPLC pump used, the method transfer to another apparatus may require some modifications. As a result, an isocratic system was preferred. Although a temperature increase diminished considerably the analysis length, it also dramatically reduced sensitivity by increasing the background noise. As a consequence, the assay was carried out at ambient temperature. Under the chromatographic conditions used in this study, the depsipeptide and the internal standard were resolved with a retention time of 34.8 and 75.1 min, respectively (Fig. 2A). Chromatograms resulting from the analysis of blank plasma and plasma spiked with depsipeptide and internal standard are shown in Fig. 2B and C.

Since the patients only received depsipeptide during the treatment, there was no need to check if interference occurred with other drugs. Standards curves for depsipeptide were linear over the range investigated with the regression equation and correlation coefficient of: $y=0.3941x-0.0073$, $r=0.999$. A calibration curve plotting depsipeptide/I.S. peak area ratios as a function of depsipeptide plasma concentration is presented in Fig. 3.

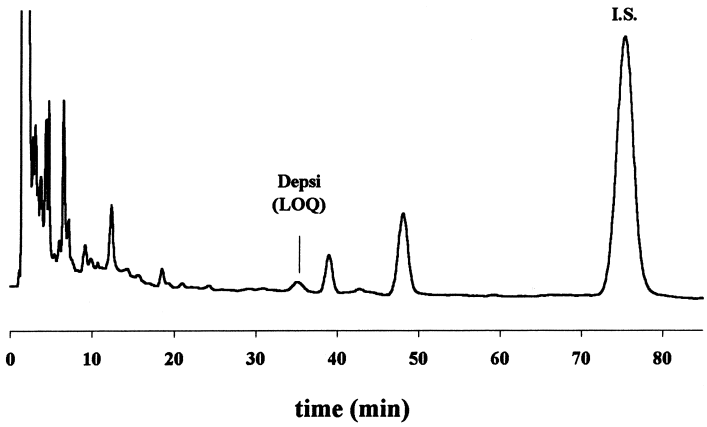

Fig. 4. Chromatogram of the analysis of plasma spiked with 50 μg/ml internal standard and a concentration of depsipeptide (50 ng/ml) corresponding to its limit of quantification.

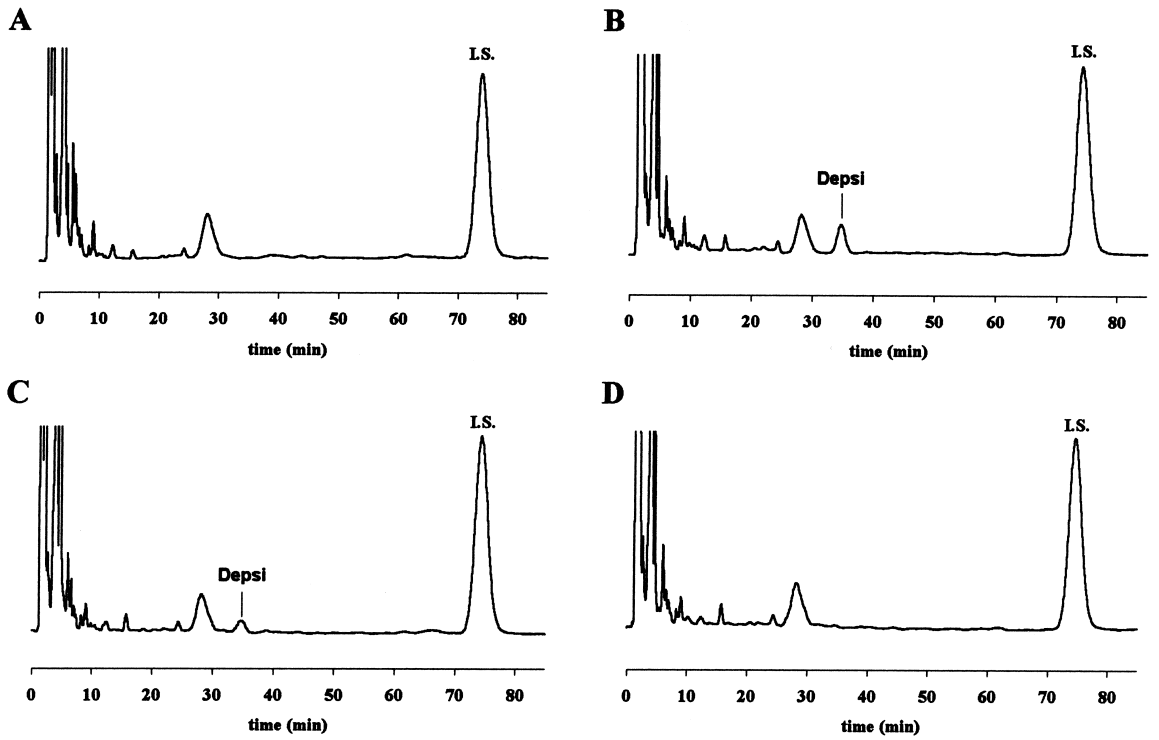

Fig. 5. Chromatograms from the analysis of plasma samples from a patient with advanced cancer receiving 2 mg/m^2 of depsipeptide per day as a 4-h i.v. infusion: (A) pretherapy (0 h); (B) end of the infusion (4 h); (C) post completion of the infusion (4:30); (D) post completion of the infusion (8 h).

The intra-day and inter-day variabilities are presented in Tables 1 and 2. In all cases, the coefficients of variation (C.V.s) were less than 8%. Recovery for depsipeptide exceeds 68% (Table 3).

3.2. Limits of detection and quantification

The limit of detection (LOD) of depsipeptide in human plasma was 20 ng/ml. The limit of quantification (LOQ) of depsipeptide was 50 ng/ml (Fig. 4).

3.3. Assay of patient samples

Chromatograms of plasma samples obtained from a patient with advanced cancer who received a 2 mg/m^2 day dose of depsipeptide administered as 4-h i.v. infusion are presented in Fig. 5A to D. The plasma concentration–time profiles for five patients representative of each dose level are presented in Fig. 6. At this time, depsipeptide is still under clinical trial at the Lombardi Cancer Center in order to determine dose-limiting toxicity and maximum tolerated dose.

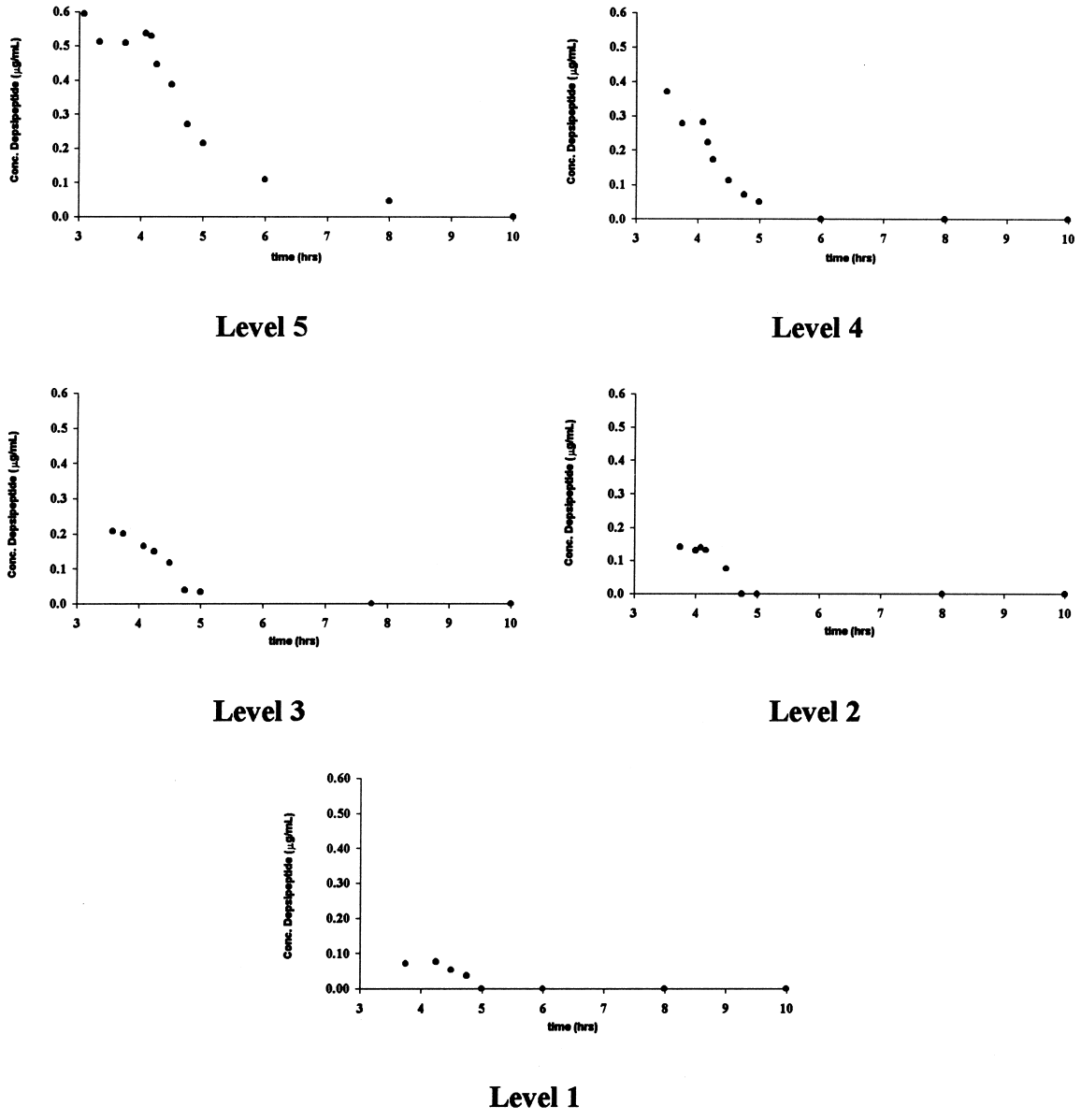

Fig. 6. Plasma concentration–time profiles for five patients with advanced cancer receiving 1, 2, 3.25, 5 or 7.5 mg/m^2 of depsipeptide per day as a 4-h i.v. infusion.

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