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PHASE I STUDIES

Pharmacokinetics and excretion of ^{14}C–Plitidepsin in patients with advanced cancer

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Summary Plitidepsin (Aplidin®) is a marine-derived anticancer compound currently investigated in phase III clinical trials. This article describes the distribution, metabolism and excretion of this novel agent and it mainly aims to identify the major routes of elimination. Six subjects were enrolled in a mass balance study during which radiolabelled plitidepsin was administered as a 3-h intravenous infusion. Blood samples were taken and urine and faeces were collected. Total radioactivity (TRA) analysis using Liquid Scintillation Counting (LSC) was done to determine the amount of radioactivity excreted from the body and plitidepsin concentrations in whole blood, plasma and urine were determined by validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays. In total, a mean of 77.4% of the administered radioactivity was excreted over a time period of 20 days, of which 71.3% was recovered in faeces and 6.1% was found in urine. The majority excreted in urine was accounted for by unchanged plitidepsin, with only 1.5% of the total administered dose explained by metabolites in urine. Faeces, on the other hand contained low levels of parent compound, which means that most of the TRA excreted in faeces was accounted for by metabolites. TRA levels were 3.7 times higher in whole blood compared to plasma. Plitidepsin was widely distributed and plasma clearance was low. This study shows that red blood cells are a major distribution compartment and that the biliary route is the main route of total radioactivity excretion.

Keywords Aplidin · Plitidepsin · Pharmacokinetics · ADME · LC-MS/MS · Total radioactivity · Mass balance

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Introduction

Plitidepsin (dehydrodidemnin B, Aplidin®; Fig. 1) is a novel antineoplastic drug currently under investigation in phase III clinical trials. The compound was originally isolated from the Mediterranean tunicate *Aplidium albicans* but it is now chemically synthesised by Pharma Mar [1, 2]. Plitidepsin was given orphan drug designation for the treatment of multiple myeloma in 2004 [3], and it has been proven to have anticancer activity in a wide variety of other cancer cells, including lung carcinoid, melanoma, neuroblastoma, leukaemia, myeloma and lymphoma [4–11]. Plitidepsin exerts its antitumor activity by targeting eEF1A2, one of the isoforms of the alpha subunit of the eukaryotic Elongation Factor 1, which is overexpressed in human tumours and is endowed with oncogenic properties, favouring tumour cell proliferation while inhibiting apoptosis [1]. Overall, it inhibits cell proliferation and survival. Previous research has shown that plitidepsin is extensively metabolised by cytochrome P450 3A4 (CYP3A4), with additional involvement of CYP2A6, 2E1 and 4A11 [12].

A mass balance study was carried out to characterise the distribution, metabolism and excretion of $^{14}C_1$-plitidepsin. Evaluable patients received a 3-h intravenous (i.v.) infusion containing radiolabelled plitidepsin. Plasma, whole blood,

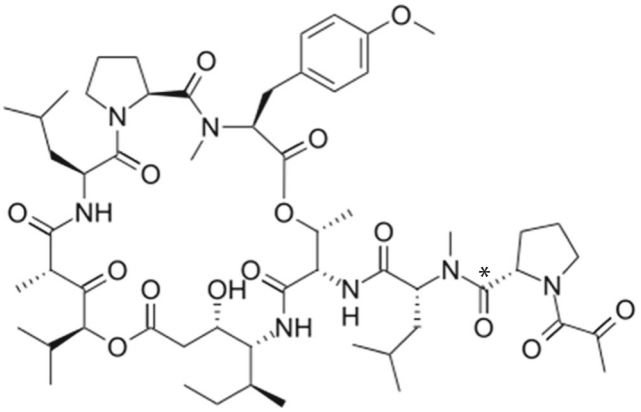

Fig. 1 Structural formula of $^{14}C_1$-plitidepsin. The asterisk (*) indicates the position of the radioactive label

urine and faecal samples were collected and analysed by Liquid Scintillation Counting (LSC) to determine the total radioactivity (TRA), and plasma, whole blood and urine were also analysed by liquid chromatography – tandem mass spectrometry (LC-MS/MS) to measure plitidepsin levels and to assess the pharmacokinetic profile of plitidepsin.

Methods

Study design and treatment

Six evaluable patients were enrolled in this phase I mass balance clinical study and remained hospitalised until Day 8. Plitidepsin was administered as a 3-h i.v. infusion on Days 1 and 15 in 4-week cycles (q4wk). This schedule was repeated for a maximum of six cycles. However, if continuing the treatment was deemed beneficial for the patient, the patient was considered for the compassionate use program. On Day 1, patients received a 3-h i.v. infusion containing 7 mg of radiolabelled plitidepsin with a radioactivity of 100 μCi. On Day 15, patients received an additional 3-h infusion containing 5 mg/m^2 of non-radioactive plitidepsin. The dose of 7.0 mg and 100 μCi of radiolabelled $^{14}C_1$-plitidepsin that was administered on Day 1 of Cycle 1 was based on the non-radiolabelled dose of 5.0 mg/m^2, multiplied by 1.4 m^2, which was the minimum body surface area (BSA) value required for inclusion into this study. The radiolabelled infusion was the only infusion administered using a central venous catheter. All subsequent infusions were administered i.v. using a peripheral line. Each infusion was preceded by prophylactic medication, including dexamethasone 8 mg, ondansetron 8 mg, clemastine 2 mg and ranitidine 50 mg.

Patients

Patients over the age of 18 years with histologically or cytologically confirmed diagnosis of advanced cancer refractory to standard therapy or for whom no standard therapy existed were enrolled in this clinical trial. Only patients with BSA above or equal to 1.4, with an Eastern Cooperative Oncology Group (ECOG) performance status between 0 and 2 were enrolled. Other inclusion criteria involved adequate bone marrow (platelet count ≥100 × 10^9/L, haemoglobin ≥5.58 mmol/L and absolute neutrophil count (ANC) ≥ 1.0 × 10^9/L), hepatic (aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) ≤ 3 x the upper limit of normal (ULN), and total bilirubin ≤1.0 x ULN), renal (calculated creatinine clearance (CrCl) ≥ 45 mL/min, creatine phosphokinase (CPK) ≤ 2.5 x ULN and albumin ≥30 g/L), and metabolic function. Moreover cardiac function needed to be sufficient with left ventricular ejection fraction (LVEF) ≥ 50%.

Patients were excluded when they had concomitant diseases or conditions, such as heart failure, active uncontrolled infections, myopathy or any clinical situation that caused significant and persistent elevation of CPK, and any other major illness that would substantially increase the risk associated with the patient's participation of the study. Patients were also excluded from the study when they had documented unstable brain metastases or leptomeningeal disease, had central nervous system (CNS) tumours or leukaemia or clinically relevant non-malignant disease. Patients who had had chemotherapy, radiotherapy, immunotherapy or molecular targeted cancer therapy within the previous four weeks prior to the start of the trial, or had major surgical procedures within the last eight weeks prior to the start of the trial were also excluded. More exclusion criteria included tumours or other conditions affecting the gastrointestinal tract that might be expected to induce occlusion of the gastrointestinal transit, inflammatory bowel disease or digestive tract fistulae, constipation and obstruction of the urinary tract. Patients were instructed to avoid consumption of red wine, Seville oranges, grapefruit (juice) as these are potent inhibitors of CYP3A4 [13]. Other inhibitors or inducers of CYP3A4 were not prohibited for the duration of the study, but usage of these compounds was monitored and registered. Informed consent was obtained from all individual participants included in the study.

Study medication

The solution of radiolabelled $^{14}C_1$-plitidepsin contained 7 mg of plitidepsin with a total radioactivity of 100 μCi and was prepared and supplied in a 250 mL infusion bag (Ecobag, NaCl 0.9% solution, B. Braun, Oss). The infusion bag was surrounded by an aluminium bag to ensure that the solution was not exposed to light. The solution was prepared by PRA Health Sciences (Zuidlaren, the Netherlands) by the addition of appropriate quantities of radiolabelled $^{14}C_1$-plitidepsin to non-radioactive plitidepsin. The solution was diluted with 250 mL of sodium chloride 0.9% for infusion. The infusion

set consisted of an infusion line coupled to an infusion filter (Codan B.V., Deventer, the Netherlands). The exact amount of radiolabelled and non-radiolabelled plitidepsin was calculated to give a final solution for administration that always contained 7.0 mg of plitidepsin and a total radioactivity of 100 μCi. Non-radiolabelled plitidepsin for subsequent administrations was supplied by Pharma Mar as a lyophilised powder for concentrate for solution for infusion in vials with a strength of 2.0 mg. Before use, the 2.0-mg vials were reconstituted by adding 4 mL of reconstitution solvent, obtaining a plitidepsin concentration of 0.5 mg/mL. Prior to administration, the reconstituted solution was further diluted with glucose 5% solution or sodium chloride 0.9% solution.

Chemicals

Plitidepsin and its stable isotopically labelled internal standard analogue (PM130461; $C_{52}{}^{13}C_5H_{87}N_6{}^{15}NO_{15}$) reference standards were supplied by Pharma Mar (Colmenar Viejo, Madrid, Spain). Acetonitrile (ACN) and water (Supra-Gradient grade) were obtained from Biosolve Ltd. (Valkenswaard, The Netherlands). Formic acid (≥98%; analytical grade), tert-butyl methyl ether (TBME), ammonia (25%), 2-propanol (>99.8%), hydrogen peroxide (30%), ethylenediaminetetraacetic acid (EDTA) 99% and sodium hydroxide (50%) were provided by Merck (Amsterdam, the Netherlands). Ammonium acetate (LC-MS grade) was purchased from Sigma Aldrich (Zwijndrecht, the Netherlands). Ultima Gold™ and Solvable™ originated from PerkinElmer (Groningen, the Netherlands). Water (non-HPLC grade) was obtained from B. Braun.

Safety

During the study period, patients were monitored and assessed for safety. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v.4.0. Safety assessment consisted of the evaluation of clinical and laboratory toxicities and changes from baseline in physical examination findings and vital signs.

Pharmacokinetics

Concentration-time profiles were constructed for plitidepsin in plasma and in whole blood. Additionally, TRA curves in plasma and whole blood were prepared. Non-compartmental analysis was done using R (v.3.0.1) [14] to calculate the following pharmacokinetic parameters for plitidepsin and TRA, if possible, in both matrices: the area under the curve (AUC), using the linear-log trapezoidal rule with extrapolation to infinity, the maximum concentration (C_{max}), the time to reach C_{max} (t_{max}) the terminal half-life ($t_{1/2}$), total body clearance (CL), and apparent volume of distribution (V_z).

Sample collection

Blood samples were drawn from the arm contralateral to the arm for i.v. infusion at the following time points: pre-infusion, 1.5 h post-start of the infusion, just before the end of infusion (EOI), 15 min (min), 30 min, 1 h, 1.5 h, 2 h, 4 h, 9 h, 24 h, 48 h, 72 h, 96 h, 120 h and 192 h after EOI. For each time point, two Vacutainer® blood collection tubes (4 mL/6 mL) were used (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The 6 mL tube was centrifuged at 3200 rpm, 4 °C for 15 min to obtain plasma. The 4 mL tube was used to collect whole blood and was therefore not centrifuged after sampling. Plasma and whole blood samples were aliquoted and all samples were stored at −80 °C.

For the purpose of the mass balance analysis, urine and faecal samples were collected for the duration of the study. From Day 1 until Day 8 collection took place at the clinical site. Once discharged, patients were instructed to continue the collection of excreta at home, which was delivered to the clinic via a daily courier service. Urine was collected at predetermined intervals: pre-infusion, during infusion, from EOI to 2 h after EOI, from 2 h to 4 h after EOI, from 4 h to 6 h after EOI, from 6 h to 8 h after EOI, from 8 h to 10 h after EOI, from 10 h to 12 h EOI and from 12 h to 24 h after EOI. On Day 2 urine was collected in 8-h portions. From Day 3 onwards until Day 10, urine was collected over periods of 24 h. From this day onward, collection of urine was stopped when less than 1% of the radioactive dose had been recovered over 24 h for two consecutive days. These samples were stored at −80 °C as well.

Faeces were collected and weighted per stool. Collection of faeces also continued until less than 1% of the total administered dose was excreted for two consecutive days. Sample pre-treatment involved diluting and homogenising the faeces by adding water (1:3 *w*/*v*). A T25 basic Ultra Turrax (IKA Works, Staufen, Germany) was used to homogenise the samples before aliquoting. The total amount of faeces collected and the time of each defecation relative to the time of drug administration were recorded. Aliquots of faecal homogenate samples were stored in polypropylene containers at −80 °C.

Administered dose

To be able to calculate the mass balance, the amount of radioactivity administered to each patient was recorded. The remaining radioactivity in the infusion set was determined by weighing the infusion set before and after administration. Weight was considered equal to volume (i.e. 1 mg = 1 mL).

An additional vial containing $^{14}C_1$-plitidepsin from the same batch was provided, from which an aliquot was used to measure the amount of radioactivity by LSC to assess the specific activity of the batch. The specific activity was then multiplied by the difference in weight before and after administration and was used to calculate the administered dose, correcting the mass balance equation for losses in the administration system.

Total radioactivity analysis

The mass balance of plitidepsin was calculated from the sum of total recovery of radioactivity in urine and faeces, compared to the administered radiolabelled dose, and was expressed as a percentage of the administered dose. TRA analysis was performed on a Liquid Scintillation Counter Tri-Carb 2910 (PerkinElmer, Waltham, MA, USA). Aliquots of 200 μL of whole blood, plasma and faeces (all in duplicate), and 1 mL of urine (in singular) were transferred to scintillation vials. Prior to analysis, tissue and fibres in whole blood and faecal samples were solubilised and decolourised. To achieve this, 1 mL Solvable™, 0.4 mL hydrogen peroxide and 1 mL of isopropanol were added to faeces homogenates, and 1 mL Solvable™, 0.5 mL hydrogen peroxide and 0.1 mL NaEDTA were added to whole blood samples. These samples were placed in a water bath (40–45 °C; GF1086, Salm en Kipp, Breukelen, The Netherlands) to facilitate the chemical processes. Finally, 10 mL of Ultima Gold Cocktail™ (PerkinElmer) was added and the samples were analysed by LSC either for 60 min or until the 2-sigma error was less than or equal to 5%, whichever came first.

Bioanalysis

The analysis of plasma, whole blood and urine samples was performed using a validated bioanalytical assay. In summary, 40 μL of 1 M ammonium hydroxide was added to 200 μL of biological matrix and plitidepsin was extracted by liquid-liquid extraction using TBME. Separation was carried out using an HPLC Acquity I Class pump (Waters, Milford, MA, USA) coupled with a SunFire C18 column (50 mm × 2.1 mm, 5 μm, Waters). Gradient elution was applied (0.1% formic acid in 5 mM ammonium acetate (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B)). The flow rate was set at 400 μL per minute and the column oven was heated to 60 °C. The elution gradient was set as follows: 50% B (0.0–0.3 min), 95% B (0.3–1.8 min), 50% B (1.8–2.0 min). The divert valve was set in place to direct the eluent flow to the mass spectrometer from 0.5 to 1.6 min and to the waste for the remainder of the acquisition time.

For spectrometric analysis, an API5500 tandem mass spectrometer (Sciex, Framingham, MA, USA) was used. Data acquisition was performed using Analyst software (version 1.5.2. Sciex). Analyses were performed in the positive ion mode by multiple reaction monitoring (MRM), selecting precursor ion *m/z* 1110.8 and product ion *m/z* 295.1 for plitidepsin, and precursor ion *m/z* 1116.8 and product ion *m/z* 301.1 for the stable isotopically labelled internal standard PM130461.

Results

Patients

Six patients were successfully enrolled in this study between March 2015 and July 2016, of which four were female and two were male. Median age was 62 years (range, 47–70 years). Median BSA was 1.9 m^2 (range, 1.6–2.2 m^2). At diagnosis four patients had metastatic disease and two had locally advanced disease. Primary tumours comprised ovarian adenocarcinoma (n = 2 patients), colon adenocarcinoma, non-small cell lung cancer (NSCLC), pancreas adenocarcinoma, and squamous cell carcinoma in the floor of the mouth. An overview of all patient baseline characteristics can be found in Table 1.

Table 1 Baseline characteristics for the patients enrolled in the mass balance study

Characteristic		n
Gender	Male	2
	Female	4
Age at entry (years)	Median	61
	Range	47–69
	18–49	1
	50–69	5
Race	Caucasian	6
ECOG PS	0	6
BSA (m^2)	Median	1.9
	Range	1.6–2.2
Height (cm)	Median	176.0
	Range	157.0–185.0
Weight (kg)	Median	69.7
	Range	60.0–100.0
BMI (kg/m^2)	Median	23.6
	Range	20.7–29.2
ECG	Normal	3
	No significant abnormalities	3
LVEF (%)	Median	65.0
	Range	55.0–79.0

Data shown are n of patients, except for median and range

Abbreviations: *BSA* body surface area, *ECOG* Eastern Cooperative Oncology Group, *PS* performance status, *BMI* body mass index, *ECG* electrocardiogram, *LVEF* left ventricular ejection fraction

Table 2 Calculated administered dose for each patient enrolled in the mass balance study

Patient #	Dose administered (mg)	Radioactivity administered (μCi)
001	6.1	79.4
002	6.1	84.2
003	5.9	92.2
004	6.2	74.8
005	6.0	75.0
006	5.8	72.5

Median time on treatment for the six patients treated in this study was 6.0 weeks at the cut-off date (range, 4.6–12.0 weeks). A total of 12 plitidepsin cycles were administered prior to cut-off, with a median of 2 cycles per patient (range, 1–4 cycles). Patients received one ($n = 2$ patients), two ($n = 3$) or four ($n = 1$) cycles each. Four out of six patients treated with plitidepsin discontinued treatment prior to cut-off: three patients discontinued due to disease progression, and one due to a treatment-related AE (grade 3 myalgia).

Administered dose

The administered dose, both the plitidepsin dose and the radioactivity, was calculated for each patient. Results are presented in Table 2. The administered dose varied for each patient, with the largest deviations from the planned dose (7 mg; 100 μCi) in the last three patients. Two batches were made, which explains the differences between administered doses. The specific activity in the second batch (patients 004 – patient 006) was lower compared to the first batch.

Table 3 Cumulative excretion of total radioactivity in urine and faeces after a single i.v. dose of 5.8–6.2 mg $^{14}C_1$-plitidepsin to six patients with advanced cancer

Patient #	Urine (%)	Faeces (%)	Total (%)
001	9.7	70.5	80.2
002	7.1	70.1	77.2
003	4.6	76.2	80.8
004	4.9	68.8	73.7
005	6.0	71.9	77.9
006	4.3	70.1	74.4
Mean	6.1	71.3	77.4
SD	2.1	2.6	2.9

Mass balance

Figure 2 shows the cumulative recovery of ^{14}C after administration of 7 mg of $^{14}C_1$-plitidepsin containing 100 μCi as a 3-h i.v. infusion. Table 3 shows the percentage of total ^{14}C content that was excreted in faeces and urine in each patient. The excretion profile of radiolabelled product are similar for all individual patients. Most of the radiolabelled material excreted was recovered in the faeces (71.3% ± 2.6%), and smaller amounts were recovered in urine (6.1% ± 1.5%) over a collection period of up to 20 days and 10 days, respectively. Faeces contained the majority of the radioactivity with the biliary route considered as the major route of excretion, while urinary excretion played a much smaller role.

Based on urine LC-MS/MS data and TRA data it was concluded that about three-quarters of the radioactivity recovered in urine was excreted as the unchanged drug. Thus, about 1.5% of the total dose administered was excreted as metabolites in the urine. Plitidepsin parent drug

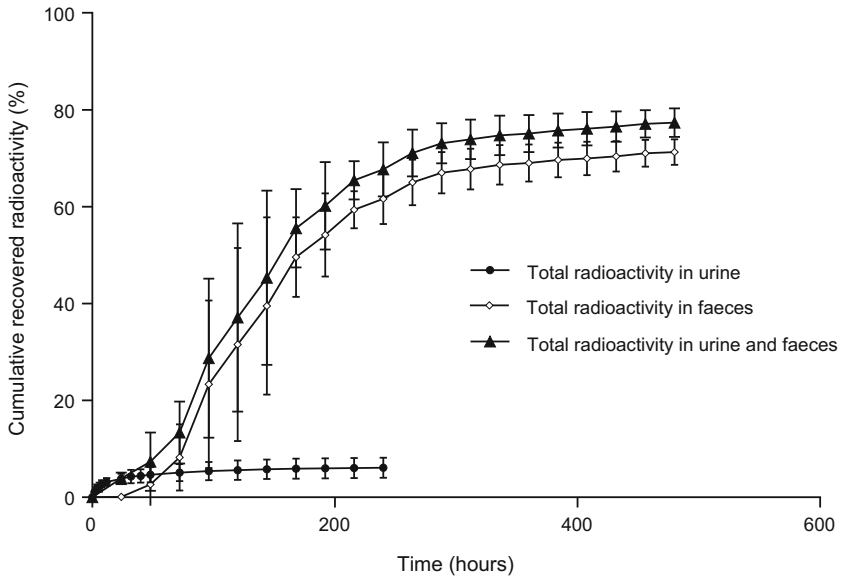

Fig. 2 Mean (±SD) cumulative recovered radioactivity in excreta after a single i.v. dose of 5.8–6.2 mg $^{14}C_1$-plitidepsin to patients with advanced cancer ($n = 6$)

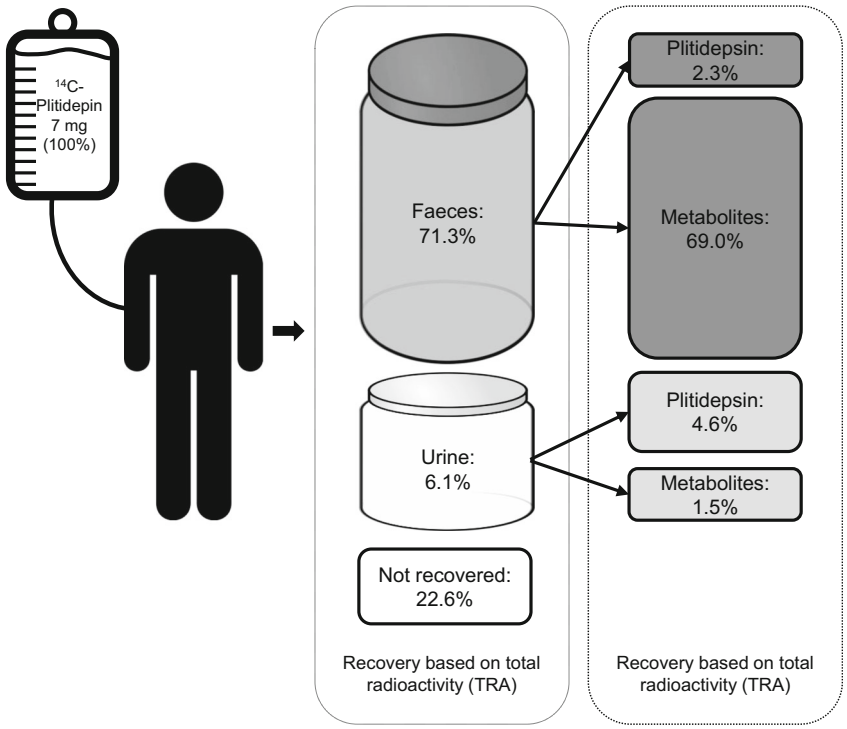

Fig. 3 Summary of TRA excretion after administration of $^{14}C_1$-plitidepsin

concentrations in faeces were determined based on TRA observed at the retention time of plitidepsin in LC-MS/MS data, and was confirmed by comparison with the retention time of the reference standard. Of the total administered radioactive dose, approximately 2.3% was accounted for by unchanged parent drug. This indicates that the majority of the TRA excreted in faeces can be attributed to plitidepsin metabolites. Plitidepsin thus undergoes metabolism to some extent, as was seen in preclinical studies [12]. A summary can be found in Fig. 3.

Pharmacokinetic analysis

Concentration-time curves for TRA and LC-MS/MS results for plitidepsin only and dose-normalised PK parameters for plitidepsin and TRA in whole blood and plasma are presented

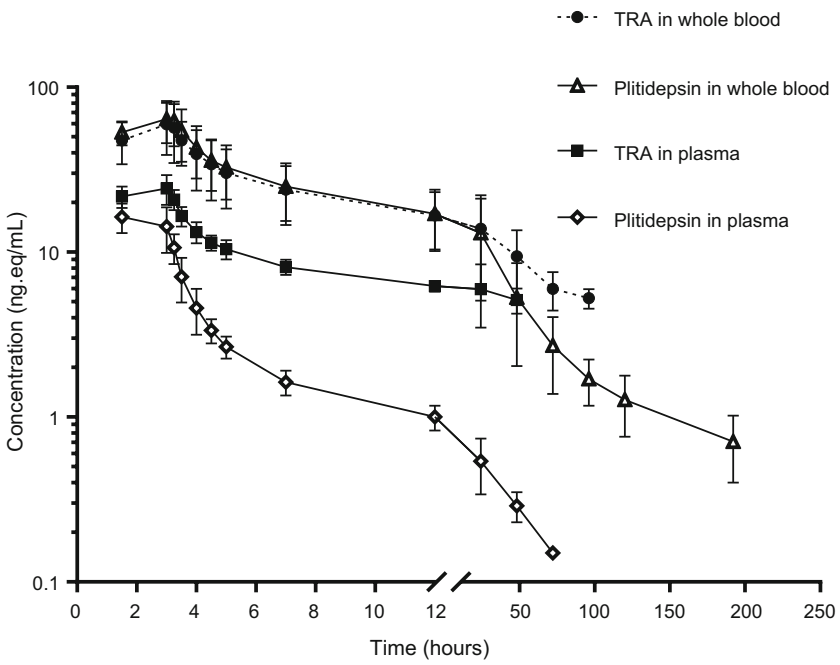

Fig. 4 Mean (±SD) log-linear concentration-time curves for total radioactivity and plitidepsin in whole blood and plasma after a single i.v. dose of 5.8–6.2 mg $^{14}C_1$-plitidepsin to patients with advanced cancer (n = 6)

Table 4 Summary of pharmacokinetic parameters of ^{14}C radioactivity in plasma and whole blood, and unlabelled plitidepsin in plasma and whole blood

Parameter		Total ^{14}C Radioactivity Plasma ($n = 6$)[c]	Total ^{14}C Radioactivity Whole Blood ($n = 6$)[c]	Unlabelled Plitidepsin in Plasma ($n = 6$)[d]	Unlabelled Plitidepsin in Whole Blood ($n = 6$)[e]
C_{max} [a] (ng/mL)	Mean	25.4	59.8	17.6	65.7
	CV%	15.8	33.6	19.3	25.9
t_{max} (h)	Mean	2.5	2.8	2.0	2.8
	CV%	32.0	25.0	35.0	25.0
t_{last} (h)	Mean	37.8	69.9	61.7	196.0
	CV%	67.7	53.0	28.2	4.5
$AUC_{0\text{-}48h}$ [b] (ng*h/mL)	Mean	NA	NA	78.9	816.0
	CV%	NA	NA	10.8	42.2
$AUC_{0\text{-}last}$ [b] (ng*h/mL)	Mean	281.7	975.6	81.1	1061
	CV%	48.5	62.7	12.1	42.1
$AUC_{0\text{-}inf}$ [b] (ng*h/mL)	Mean	570	1080	88.2	776
	CV%	NA	NA	13.6	41.3
$t_{1/2}$ (h)	Mean	20.3	40.2	22.1	60.9
	CV%	NA	NA	17.7	4.5
CL (L/h)	Mean	11.4	5.63	68.2	8.23
	CV%	NA	NA	14.9	40.3
V_z (L)	Mean	315	321	2180	751
	CV%	NA	NA	13.8	45.5

[a] C_{max} unit for ^{14}C-radioactivity is ng equivalent/mL

[b] Unit for AUCs for total ^{14}C-radioactivity is ng equivalent*h/mL

$AUC_{0\text{-}48h}$ (ng*h/mL) = area under the concentration-time curve from time 0 to 48 h; $AUC_{0\text{-}inf}$ = area under the concentration-time curve from time 0 to infinity; $AUC_{0\text{-}last}$ = area under the concentration-time curve from time 0 to the last quantifiable concentration; CL = clearance; C_{max} = maximum observed concentration; CV = coefficient of variation; NA: not applicable; t_{max} = time to reach maximum observed concentration; t_{last} = time of last quantifiable concentration; $t_{1/2}$ = terminal half-life; V_z = volume of distribution

[c] Parameters $AUC_{0\text{-}inf}$, $t_{1/2}$, CL and V_z were calculated based on 1 out of the 6 patients, since the elimination rate constant could not be determined for the other patients. Therefore, no CV could be calculated

[d] Parameters $AUC_{0\text{-}inf}$, $t_{1/2}$, CL and V_z were calculated based on 5 out of the 6 patients, since the elimination rate constant could not be determined for the other patient

[e] Parameters $AUC_{0\text{-}inf}$, $t_{1/2}$, CL and V_z were calculated based on 2 out of the 6 patients, since the elimination rate constant could not be determined for the other patients

in Fig. 4 and Table 4, respectively. In whole blood, total radioactivity levels could be measured for up to 96 h after dosing, while plitidepsin concentrations could be quantified for all patients for up to 192 h with the sensitive LC-MS/MS method (with a lower limit of quantification (LLOQ) of 0.1 ng/mL). In plasma, total radioactivity levels were much lower and could not be detected in plasma beyond the 48-h time point. The LC-MS/MS plitidepsin concentrations in plasma were quantified for up to 72 h. Following non-compartmental analysis, plitidepsin was found to be widely distributed, with a normalised apparent V_z of about 2180 L calculated from plasma and 751 L calculated from whole blood concentrations. This indicated that blood cells are an important distribution compartment. Plitidepsin displayed low clearance in whole blood (8.46 L/h), but it displayed much higher clearance in plasma (69.2 L/h). Mean maximum plasma concentrations were reached after 2 h and 2.5 h (t_{max}) for plitidepsin and TRA, respectively. Plitidepsin C_{max} concentrations were 3.7 fold higher in whole blood than in plasma (65.7 and 17.6 ng/mL, respectively), the mean whole blood-plasma $AUC_{0\text{-}48h}$ ratio was 10.3, and differences between the compartments were also reflected in elimination $t_{1/2}$ values; plitidepsin had a $t_{1/2}$ of 22.1 h in plasma and 60.9 h in whole blood. The $t_{1/2}$ for TRA in plasma and whole blood, could only be determined for 1 patient (20.3 and 40.2 h, respectively). The TRA curve in whole blood was comparable to the plitidepsin curve from 0 to 24 h after dosing, indicating that the presence of plitidepsin metabolites in this compartment is limited during the first 24 h after dosing. After 24 h, TRA concentrations are higher than plitidepsin concentrations, which means that metabolites have been formed. Comparison of the TRA curve and the plitidepsin curve in plasma suggested that the drug is metabolised and the absolute differences in concentrations are maximal at the end of infusion.

Table 5 Adverse events related to plitidepsin or with unknown relationship (worst grade per patient)

Category/MedDRA code	NCI-CTCAE grade				
	Grade 1	Grade 2	Grade 3	All grades	
	n	n	n	n	%
Gastrointestinal disorders					
Abdominal pain	1		.	1	16.7
Diarrhea	.	1	.	1	16.7
Dry mouth	1		.	1	16.7
Nausea	2		.	2	33.3
Vomiting	2		.	2	33.3
General disorders and administration site conditions					
Fatigue	2	1	.	3	50.0
Influenza like illness	1	.	.	1	16.7
Pyrexia	1	.	.	1	16.7
Investigations					
Oxygen saturation decreased	1	.	.	1	16.7
Musculoskeletal and connective tissue disorders					
Arthralgia	1	.	.	1	16.7
Back pain	1	.	.	1	16.7
Myalgia	1	.	1	2	33.3
Nervous system disorders					
Dysgeusia	1	.	.	1	16.7
Headache	1	.	.	1	16.7
Respiratory, thoracic and mediastinal disorders					
Dyspnea	1	.	.	1	16.7
Skin and subcutaneous tissue disorders					
Erythema	1	.	.	1	16.7

Abbreviations: *MedDRA* Medical Dictionary for Regulatory Activities, *NCI-CTCAE* National Cancer Institute Common Terminology Criteria for Adverse Events

Safety

Adverse events (AEs) were reported for all six patients in the study. An overview can be found in Table 5 and Table 6. The most common plitidepsin-related AEs or with unknown relationship were myalgia (33.3%), fatigue (33.3%), and nausea (33.3%). Most plitidepsin-related AEs were grade 1. The only severe plitidepsin-related AE was an episode of grade 3 myalgia, which happened four days after administration of Cycle 2. This was considered a serious adverse event (SAE) and resulted in treatment discontinuation. The other plitidepsin-related musculoskeletal disorder was grade 1 back pain, which was reported in one patient and had no effects on treatment. Other plitidepsin-related AEs were grade 1 and only affected one patient each. They comprised of dysgeusia, headache, decreased oxygen saturation, dyspnea, erythema and dry mouth. Regardless of relationship, the only haematological abnormalities reported prior to cut-off were anaemia (five patients and 11 cycles) and lymphopenia (three patients and six cycles). All haematological abnormalities were grade 1/2, and none had any effects on treatment. Of note, both patients with grade 2 anaemia already had anaemia at baseline. Other biochemical abnormalities were grade 1 only. They comprised ALAT increase (two patients and four cycles), ASAT increase (one patient and two cycles) and CPK increase (one patient and one cycle). The most common biochemical abnormality was gamma-glutamyltransferase (GGT) increase, which occurred in all patients and nine cycles. Most GGT increases were grade 1/2, and grade 3 episodes were found in two patients and three cycles. Of note, both patients with grade 3 GGT increase while on treatment already had grade 2 GGT increase at baseline. No cycle delays, dose reductions or deaths occurred due to plitidepsin-related AEs in this study. Two patients died before cut-off. Both deaths were due to progression of the malignant disease. In conclusion, plitidepsin administered as a 3-h i.v. infusion on Days 1 and 15 every four weeks was well tolerated in the six patients with solid tumours treated in this mass balance study.

Table 6 Haematological and biochemical abnormalities: worst grade per patient

	NCI-CTCAE grade				
	Grade 1	Grade 2	Grade 3	All grades	
	n	n	n	n	%
Haematological abnormalities					
Anaemia	3	2	.	5	83.3
Lymphopenia	4	.	.	4	66.6
Biochemical abnormalities					
ALAT increase	2	.	.	2	33.3
ASAT increase	1	.	.	1	16.7
CPK increase	1	.	.	1	16.7
GGT increase	3	1	2	6	100.0

No patients had episodes of leukopenia, neutropenia or thrombocytopenia of any grade

No patients had episodes of bilirubin increase or creatinine increase of any grade. Abbreviations: *ALAT* alanine aminotransferase, *ASAT* aspartate aminotransferase, *CPK* creatine phosphokinase, *GGT* gamma-glutamyltransferase, *NCI-CTCAE* National Cancer Institute Common Terminology Criteria for Adverse Events

Discussion

The total mean recovered radioactivity in the six patients enrolled in the mass balance study was 77.4%. Even though recoveries of a least 90% are generally desired, lower recoveries are often observed for compounds (and metabolites) that have a long $t_{1/2}$ [15]. The majority of the radioactive dose was recovered in the faeces (71.3%), with only a small portion of 6.1% retrieved in urine. Lower urinal recoveries were also found in a study by Izquierdo et al. [16]. They reported a mean recovery of 4.3% over the first 48 h after administration and a maximum of <11% in all patients. Similar results were found by Faivre et al. [17], who also concluded that a mean of 4.16% was recovered within the first 48 h after administration, with maximum recoveries of 15% overall. These studies suggest that renal excretion is a minor elimination route of plitidepsin, and will therefore unlikely cause any problems in patients with renal dysfunction. The biliary route is therefore likely the main route of elimination. However, since plitidepsin is a substrate for poly-glycoprotein (p-gp), the role of this protein in plitidepsin (intestinal) efflux cannot be ruled out.

Based on combined results from TRA and LC-MS/MS, only a small portion of the total administered radioactive dose was accounted for by unchanged plitidepsin in faeces. The majority of the TRA excreted in faeces is therefore accounted for by metabolites. This indicates that plitidepsin undergoes metabolism to some extend and these metabolites are mainly excreted through the biliary route.

Only 6.1% ± 1.5% of the total administrated radioactivity was recovered in urine, the majority as unchanged drug. Very low amounts of metabolites were found in urine, approximately 1.5% of the total administered radioactive dose.

In terms of pharmacokinetics, many similarities were found between this mass balance study and pharmacokinetic studies published before. Overall, plitidepsin levels were higher in whole blood as compared to plasma [10, 17, 18], due to the fact that plitidepsin is present within red blood cells. Moreover, the volume of distribution and $t_{1/2}$ were larger and shorter in plasma than in whole blood [10, 16, 17]. Shorter $t_{1/2}$ values for plitidepsin in whole blood were reported in previous trials [4, 10, 16, 17]. However, in these cases administration schedules as well as sampling schedules were different from the current trial. The pharmacokinetic study by Mateos et al. [10] used the same dosing schedule and sampling schedule as the current trial and reported a $t_{1/2}$ of 44 h based on 41 patients. However, the mean $t_{1/2}$ of 60.9 h reported in this trial, was based on 2 patients only (59.0 and 62.0 h, respectively). The differences observed between plitidepsin and TRA parameters in the compartments can be explained by circulating metabolites. As expected, TRA concentrations are higher than plitidepsin concentrations at all sampling points, and the difference between the two increases over time, meaning that metabolism takes place early after dosing and the formation of metabolites increases over time.

The large volume of distribution indicates that large amounts of the drug are distributed into peripheral tissue, as can be expected for lipophilic drugs [6, 19]. Plitidepsin's lipophilicity is in this case also reflected in the fact that only a very small portion of plitidepsin was excreted via the urine. The tissue binding and lipophilicity could have contributed to plitidepsin's incomplete recovery [20].

Conclusion

Six patients were enrolled in this clinical mass balance study, which investigated the pharmacokinetics and the main routes of excretion of plitidepsin and metabolites. A mean of 71.3% of the administered radioactive dose was recovered in faeces, whereas only 6.1% was eliminated via the urine. This proves that the hepatic/biliary route is the major route of excretion and the renal route plays only a minor part. Non-compartmental analysis showed that plitidepsin had a large distribution volume, and maximum plitidepsin concentrations were on average 3.7 times higher in whole blood compared to plasma. Metabolite concentrations are low in whole blood, which was indicated by the similarities in total radioactivity curves and plitidepsin curves in whole blood. However, differences between TRA and plitidepsin concentrations in whole blood were larger after 24 h after infusion, meaning that some metabolites have been formed. Larger differences were observed between plasma TRA levels and plitidepsin levels, which indicates the presence of metabolites.

Plitidepsin has an adequate safety profile when administered as a 7 mg i.v. dose on Day 1 and Day 15 every four weeks. Only one plitidepsin-related SAE was reported which led to patient hospitalisation and drug discontinuation. In short, TRA is mainly excreted via faeces and hardly via urine. Plitidepsin is metabolised, which is especially apparent in faeces and to a much lesser extent in urine, whole blood and plasma.

Compliance with ethical standards

Conflict of interest SF, SM, IG are currently employees, and BM-L was a former employee at Pharma Mar, S.A. LA, HR, MMT, NV, AHMVS, JHMS and JHB are employed at the Netherlands Cancer Institute.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the participating institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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