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Phase I and pharmacological studies of the cryptophycin analogue LY355703 administered on a single intermittent or weekly schedule

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Abstract

LY355703 is a synthetic derivative of the marine cryptophycins, cytotoxic agents which induce mitotic arrest by binding at the microtubule vinca binding domain. Promising preclinical features of LY355703 were the 40–400 greater potency than paclitaxel or vinca alkaloids, the broad spectrum of antitumor activity in xenografts and the antitumour activity in multidrug resistant (MDR)-expressing murine tumours. Aims of this study were to define the maximum tolerated dose (MTD) and the dose recommended for Phase II, the pattern of toxicity, the pharmacokinetic profile and to document hints of antitumour activity of LY355703 given as 2-h infusion on day 1 every 3 weeks (Study 1) or, later on, on days 1, 8 and 15 every 4 weeks (Study 2). *The latter weekly regimen was selected because of the acute dose-related toxicity reported in Study 1.* The dose was escalated using a modified Continual Reassessment Method. Pharmacokinetic studies were performed on day 1 of cycle 1 in both studies; LY355703 plasma concentrations were assessed by liquid chromatography with tandem mass spectrometry. A total of 35 adult patients with solid tumours entered Study 1; the dose was escalated from 0.1 to 1.92 mg/m^2; at this dose 2 of 5 patients presented grade 3 neuropathy and myalgias; 1.48 mg/m^2 was then recommended for Phase II study. A total of 8 patients were treated in Study 2 at 1 mg/m^2; cumulative long-lasting neuroconstipation and neurosensory toxicity precluded the completion of the cycle in 9 out of 15 cycles; the clinical development of the weekly regimen was then discontinued. Other toxicities included cardiac dysrhythmia and mild alopecia. Pharmacokinetics of LY355703 appeared to be linear over the dose range studied. The administration of LY355703 on a 3-week schedule is associated with an acute dose-dependent peripheral neuropathy and myalgia of high interpatient variability for which possible risk factors and pharmacokinetic correlates could not be identified.

Keywords: Phase I; Cryptophycin; Analogue; Clinical pharmacology; New drugs

1. Introduction

Cryptophycins are macrocyclic depsipeptides isolated from blue-green algae, *Nostoc* sp [10] (Fig. 1). LY355703 or Cryptophycin-52 is a synthetic derivative of the naturally occurring cryptophycins with known antimitotic properties. LY355703 induces mitotic arrest by binding to microtubule ends with high affinity at the vinca binding domain [1]. The cytotoxic effects are concentration-dependent resulting in mitotic block in metaphase. Relatively little effect on the polymerisation state of microtubules was noted at low picomolar concentrations (3–30 pM), while higher concentrations (100–300 pM) or prolonged exposure times produced a progressive disorganisation of the mitotic spindle and, reduction in microtubule polymer mass. In contrast to

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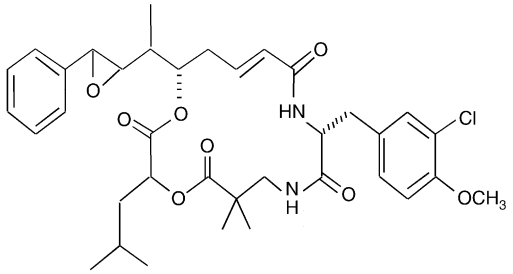

Fig. 1. Chemical structure of LY355703.

vinblastine, cryptophycin-induced microtubule depletion lasted for at least 24 h after removal of the compound [11]. In addition to the effects at high concentration, LY355703 suppresses microtubular dynamics at low picomolar concentrations [7]. LY355703 suppresses microtubular shortening rates to a greater extent than rates of microtubular growth unlike vinblastine which equally inhibits both rates of microtubule growth and shortening [7,8].

In vitro, LY355703 was 40–400 fold more potent than either paclitaxel or the vinca alkaloids in all tested cell lines with a similar, but not identical, pattern of cytotoxicity (data on file, Ely Lilly, 1997). LY355703 showed a broad spectrum of antitumour activity in murine and human xenografts with complete remissions in mammary and prostate xenograft models [15]; the antitumour activity was not schedule-dependent. In addition, significant antitumour activity was observed in tumours expressing the MDR phenotype, including models resistant to paclitaxel [13].

Preclinical toxicology studies utilised a single intravenous (i.v.) infusion administered over 2 h on days 1 and 8 in rats and dogs. In rats, the dose-limiting toxicity (DLT) was neutropenia observed at 6 mg/m^2. The DLT in dogs was gastrointestinal (diarrhoea) as well as bone marrow toxicity (neutropenia) seen at 1 mg/m^2. No evidence of overt neurotoxicity or histopathological changes indicative of effects on the brain and peripheral nerves were reported in the dog or rat. At 1 mg/m^2, there was no significant change in the toxicity profile when LY355703 was administered as a 2-h infusion compared with an 8-h infusion. In contrast, at higher doses (2 mg/m^2), prolonged infusion resulted in more severe and unacceptable toxicity.

LY355703 was considered an excellent clinical candidate, in light of the high *in vitro* potency observed in cell lines including those with the MDR phenotype, the broad spectrum of antitumour activity observed in xenograft models and the potentially novel microtubular dynamic effects. Two schedules were investigated in the Phase I setting including a days 1 and 8 repeated every 21 days conducted in the United States and a day 1 every 21 days was conducted in Europe, the results of which are reported here.

The principal objectives of this study were to (1) define the toxicities of LY355703 given on a single every 3-week schedule in patients with advanced solid malignancies; (2) define the maximum tolerated dose (MTD) and identify the recommended phase II dose; (3) determine the preliminary pharmacokinetic profile of LY355703 in plasma; and (4) document antitumour activity in previously treated patients with advanced cancers.

2. Patients and methods

2.1. Patients

Adult patients aged 18 years or more with histologically- or cytologically-confirmed diagnoses of advanced solid tumours for which no proven effective therapy exists were eligible for this study. Eligibility criteria also included a performance status (PS) of $\leqslant 2$ on the Zubrod scale, no prior chemo- or radiotherapy for at least 4 weeks (6 weeks for mitomycin or nitrosoureas), adequate bone marrow reserve [absolute neutrophil count (ANC) $\geqslant 1.5\times10^9$/l, platelet count $\geqslant 100\times10^9$/l], hepatic [bilirubin $\leqslant 1.3\times$ upper limit of normal (ULN) and alanine transaminase/aspartate transaminase (ALAT/ASAT) $\leqslant 2.5\times$ ULN] renal function [creatinine $\leqslant 1.5\times$ ULN] and written informed consent of patients. Serious pre-existing medical conditions, pre-existing $\geqslant$grade 1 peripheral neuropathy, known significant hypersensitivity to Cremophor El® vehicle or polysorbate 80, symptomatic brain metastases were criteria for exclusion.

2.2. Drug administration

In the first part of the study (Study 1), LY355703 was administered as a 2-h i.v. infusion repeated every 3 weeks, from a starting dose of 0.1 mg/m^2 corresponding to 1/10 of the dog MTD. The dose was escalated according to the modified Continual Reassessment Method (mCRM) [5]. < At least one patient evaluable for toxicity (with a minimum of 3-week observation) had to be treated per dose level; at least three patients had to be treated per level in case of $\geqslant$grade 2 non-haematological or $\geqslant$grade 3 haematological toxicity; in case of DLT during the first cycle six patients had to be treated at the same dose level. The dose could be escalated further only if no more than one patient had experienced DLT during the first cycle. Toxicities were evaluated according to Common Toxicity Criteria (CTC version 1). The MTD was defined as the dose at which one-third of the patients suffered drug-related DLT during cycle 1. DLT were defined as grade 4 neutropenia of $\geqslant 5$ day-duration or febrile neutropenia (CTC criteria), grade 4 thrombocytopenia, any $\geqslant 3$ non-haematological

toxicity (excluding nausea, vomiting and alopecia). Intrapatient dose escalation at second cycle was allowed only if the patient had no toxicity in the first cycle and another patient had received the higher dose without toxicity. *A 25–50% of dose reduction* was applied in case of DLT at previous cycle. Patients were retreated on day 21 in case of ANC $\geqslant 1.5 \times 10^9$/l, platelets $\geqslant 10^9$/l and recovery to $\leqslant$grade 1 of non-haematological toxicities. Additional patients were to be enrolled at the dose selected for Phase II evaluation.

Once the MTD was defined in Study 1, a weekly regimen with dosing on days 1, 8, and 15 every 4 weeks (one cycle) was investigated in Study 2 in order to increase dose intensity. Eligibility criteria, MTD and DLT definitions were as in Study 1. Planned dose levels were 1 mg/m^2, 1.25 mg/m^2 and 1.5 mg /m^2 based on toxicity observed in Study 1. Days 8 and 15 doses were administered if ANC were $> 0.5 \times 10^9$/l, platelets $> 50 \times 10^9$/l and no haematological toxicity $\geqslant$grade 3 was present. If omitted, day 8 dose could be administered on day 15 while, if omitted, day 15 dose was deleted. Criteria for retreatment on day 28 were as on day 21 in Study 1; treatment could be delayed up to a maximum of 4 weeks after which, in case of no recovery, patients went off the study. *A 25%* decrease of the dose was applied at subsequent cycles in case of DLT. In case of $\geqslant$grade 3 neurological toxicity and/or myalgia, the decision of decreasing the dose by 50% or discontinuing the treatment was left to the discretion of the physician.

Prophylactic premedication against hypersensitivity reactions (HSR) was routinely given from the dose of 0.88 mg/m^2 onwards in Study 1 and at all dose levels in Study 2. Premedication was administered i.v. between 30 and 60 minutes before treatment and consisted of dexamethasone 20 mg, ranitidine 50 mg and clemastine 2 mg.

LY355703 was supplied by Eli Lilly in 20 ml vials containing LY355703 at the concentration of 1 mg/ml with polyoxyl 35 castor oil and absolute alcohol as solubilising agents. The drug was diluted by adding to one part per volume of the concentrated drug solution up to 19 parts per volume of normal saline and gently mixing. LY355703 was administered as a 2-h infusion with a volume pump through a separate i.v. line using non-poly vinyl chloride (PVC) containing tubing and i.v. bags.

3. Pretreatment and follow-up studies

Chemistry (including electrolytes bilirubin, ASAT/ALAT, lactate dehydrogenase (LDH), urea, creatinine, uric acid) and complete blood count (CBC) were repeated weekly, the latter more often in cases of grade 3 toxicity. Electrocardiogram (ECG) and neurological examination were performed at baseline and thereafter when clinically indicated. Tumour response was assessed after at least two cycles and classified according to World Health Organization (WHO) criteria [14]; patients with stable disease or tumour response continued treatment until tumour progression or until unacceptable toxicity.

4. Pharmacokinetic sampling and analysis

The pharmacokinetics of LY355703 were evaluated as part of a population pharmacokinetic analysis from data collected in two similar Phase 1 studies. Blood samples were collected for analysis of LY355703 in plasma from 32 patients in this study and from 25 patients in the study conducted in the United States. Blood samples (5 ml) were collected in heparinised tubes at specified times (up to 6 h) following the start of infusion on days 1, 8 and 15 from this study (cycle 1) and on days 1 and 8 from the other study (cycle 1). Additional blood samples were collected at the end of infusion following the first dose during the second and third administration cycles. Samples were centrifuged immediately at room temperature, and the plasma separated and stored at -80 °C. Batched samples were shipped on dry ice to the analytical site.

Plasma samples were assayed for LY355703 concentrations in plasma using a validated liquid chromatography with tandem mass spectrometry (LC/MS/MS) method over the concentration range 0.25–200 mg/ml [2]. The overall relative standard deviation (RSD), which is an expression of the precision, was $\leqslant 17.06\%$ at all concentrations. The overall relative error (RE), which is an expression of the accuracy and is defined as the maximum error observed when quality control samples of known concentrations (high, medium, low) were subjected to the assay used for clinical PK samples, was $\leqslant 12.35\%$ for all concentrations.

Plasma concentration–time data from both studies were combined (297 observations total) for a single pharmacokinetic analysis. Pharmacokinetic analysis was performed using population pharmacokinetic methods with the non-linear mixed-effects modelling program, NONMEM (Version 5, PREDPP 5) [6]. An open one-compartment model parameterised in terms of clearance and volume was determined to be the most appropriate structural model for the available data. A final population pharmacokinetic model from the combined data was developed previously. The effect of factors such as age, gender, body surface area, body weight, AST ALT, alkaline phosphatase, total bilirubin, albumin and calculated creatinine clearance with respect to clearance and volume were evaluated during model building.

Results from previous model development produced the following results. Plasma clearance (CL) was

demonstrated to be a function of ALT and body surface area (BSA) according to a multiplicative power function as follows:

$$CL = \Theta_1 \cdot \left(\frac{ALT}{22.8}\right)^{\Theta_2} \cdot \left(\frac{BSA}{1.84}\right)^{\Theta_3} \quad (1)$$

Volume of distribution (V) was determined to be only a function of body surface area:

$$V = \Theta_4 \left(\frac{BSA}{1.84}\right)^{\Theta_5} \quad (2)$$

The terminal elimination half-life ($t_{1/2}$) was calculated from CL and V by the following relationship:

$$t_{1/2} = \frac{\ln(2)}{(CL/V)} \quad (3)$$

The pharmacokinetic results presented here represent an analysis of plasma concentration-time data collected in this study, using the relationships produced from the final population model derived by combining data from the two studies.

5. Results

Thirty-five patients entered Study 1, and 8 patients entered Study 2 (Table 1). Chemotherapy had been previously administered in all cases,with 13 patients having previously received platinum and/or a taxane. All patients were evaluable for toxicity. The dose escalation schema along with the number of patients per dose level is summarised in Table 2. The median number of cycles was 3 in Study 1 and 4 for Study 2. In total, only 4 patients received > 4 cycles; all other

Table 1
Patient characteristics

	No. of patients	
	Study 1	Study 2
Total	35	8
Female/male	18/17	4/4
Median age (years)	57 (42–78)	51.5 (34–66)
Performance status (Zubrod)		
0	20	6
1	15	2
Tumour type		
Sarcoma	8	2
Colorectal	5	0
Ovary	5	2
Kidney	5	0
Other	12	4
Prior treatment		
Chemotherapy only	27	6
Chemo- and RT[a]	8	2
No prior	0	0
Median (range) chemotherapies	2 (1–5)	2 (1–4)

[a] RT, radiotherapy

Table 2
Dose escalation scheme

Dose (mg/m2)	Dose escalation Increment (%)	No. of patients				No. of pts. With DLT at cy 1
		New	Increased to this dose	Total	Total courses	
Study 1 (d 1 q 3wks)						
0.1	–	1	–	1	2	0/1
0.2	100	1	1	2	4	0/1
0.4	100	3	0	3	7	0/3
0.52	30	3	0	3	18	0/3
0.68	30	6	2	8	19	1/6 grade 3 cardiac dysrhythmia
0.88	30	3	0	3	10	0/3
1.14	30	3	0	3	6	0/3
1.48	30	8	0	8	17	1/8 grade 3 cardiac dysrhythmia
1.92	30	5	0	5	8	2/5 grade 3 neuropathic pain grade 3 myalgia
1.71	−11	2	0	2	2	2/2 grade 3 myalgia
Study 2 (d 1, 8, 15 q 4 wks)						
1	–	8	0	8	15	1/8 grade 3 hypertension

d,day; q, every; wks, weeks; cy, cycle; DLT, dose-limiting toxicity; pts, patients

patients received less than 4 cycles. A single, new patient was treated in each of the first 3 dose levels. At the 0.4 mg/m^2 dose level, 3 patients were enrolled due to a grade 2 skin reaction at the injection site observed with the first patient. Three patients were enrolled at 0.52 mg/m^2 with 1 patient experiencing grade 2 neuromotor toxicity. One of the first 3 patients at 0.68 mg/m^2 developed a DLT with grade 3 cardiac dysrhythmia from bradycardia and grade 2 hypertension associated with HSR. Two additional patients presented with grade 3 HSR at cycle 2 prompting the introduction of prophylactic premedication in all subsequent patients. An additional 3 patients were enrolled at 0.68 mg/m^2 without further DLT. The dose was escalated by a 30% increase with 3 patients per level up to 1.48 mg/m^2, at which point a total of 8 patients were treated, 2 during the phase of dose escalation and 6 in the definition of the recommended dose.

Two of 5 patients treated at 1.92 mg/m^2 developed DLT, consisting of grade 3 neuropathy in one case and grade 3 myalgia in the other. The subsequent dose level of 1.7 mg/m^2 was chosen in part due to available data from the companion study in the United States, which identified neuromotor toxicity at 1.84 mg/m^2 and 2.2 mg/m^2. The observation of grade 3 reversible myalgia in the first 2 patients treated at 1.7 mg/m^2 prompted accrual to be stopped at this dose and expanded at 1.48 mg/m^2, which was defined as the recommended Phase II dose.

In Study 2, 8 patients were enrolled at 1 mg/m^2 with 15 cycles administered in total. Nine of the 15 cycles could not be completed on time due to a variety of toxicities including grade 2 neuroconstipation (3 cycles), grade 2 neurosensory (2 cycles), grade 2 cardiac dysrhythmia (1 cycle), myalgia (1 cycle), thrombosis (1 cycle), grade 3 hypertension (1 cycle). Study 2 was discontinued, in the absence of MTD, because a decrease of the dose would provide a lower dose intensity than the one recommended in Study 1 for Phase II evaluation.

6. Laboratory toxicities

In both studies, the most frequent haematological toxicity was grade 3 or 4 lymphopenia, which was observed at all dose levels and was neither cumulative nor associated with infections. Grade 3 lymphopenia occurred in 17 patients on Study 1, starting at the 0.52 mg/m^2 dose level, and in 7 out of 8 patients in Study 2 on 1.0 mg/m^2. Grade 3 thrombocytopenia or neutropenia were not reported; grade 3 hyperbilirubinaemia occurred in 2 patients, possibly related to tumour progression.

7. Peripheral neuropathy and myalgias

Peripheral neuropathy and myalgia were the primary toxicities of LY355703. Neuropathy was dose-limiting with both schedules, but of a higher grade, more acute in onset and more prolonged in Study 1 compared with the schedule in Study 2, where neurotoxicity appeared to be cumulative and more rapidly reversible. First observed at 0.52 mg/m^2, neurotoxicity was manifested as neurosensory and neuromotor toxicity as well as neuroconstipation (Table 3). At higher doses, neurosensory toxicity appeared approximately 2 days after treatment, and represented a DLT in 1 patient treated at 1.92 mg/m^2. This 54-year old lady with liver metastases from a gastrointestinal stromal tumour, pretreated with doxorubicin and ifosfamide, presented severe neuropathic pain in the distribution of cranial nerve V, unresponsive to non-steroidal anti-inflammatory drugs (NSAID), 24 h after the first administration

Table 3
Peripheral neuropathy and myalgia of Ly355703

Dose (mg/m2)	No. of pts	No. of patients with CTC toxicity											
		Neurosensory			Neuromotor			Neuroconstipation			Myalgia		
		1	2	3	1	2	3	1	2	3	1	2	3
Study 1 (d1 q. 3wks) (No. of patients with CTC toxicity)													
0.52	3	2				2					1		
0.68	6	3			1	1							
0.88	3	1				1					1	1	
1.14	3					2							1
1.48	8	2	1		1			2	1			4	
1.92	5	1	2			1		1	1	1	2	2	1
1.7	2	2				1			2				2
Study 2 (d1, 8, 15 q 4 wks)													
1	8	3	2					3	4		2	3	1

of LY355703. Jaw pain was also present along with grade 2 neuromotor toxicity and grade 2 neuroconstipation. One year after treatment, toxicity persisted with paresthesias, dysesthesia of the legs, and absent deep tendon reflexes in the lower extremities. Electromyography, nerve conduction studies, evoked tibial nerve potentials, spinal cord magnetic resonance imaging (MRI) were all normal, supporting a clinical diagnosis of sensory neuropathy due to axonal degeneration.

The other important symptom of peripheral neuropathy in Study 1 was neuroconstipation, first observed at 1.48 mg/m^2. Typically, neuroconstipation appeared 1 week after treatment, with a clinical picture of paralytic ileus accompanied by diffuse abdominal pain. Myalgia, more frequently localised to the lower extremities, appeared 24–48 h following treatment and persisted for approximately 72–96 h. Myalgia was universal at 1.92 mg/m^2 and 1.7 mg/m^2 and of longer duration than observed at the lower dose levels. At 1.48 mg/m^2, myalgias were present in half of the patients and were moderate in intensity. NSAIDs were ineffective in controlling myalgias once they appeared and strong opioids could achieve only a partial effect. Prophylactic premedication was administered at cycle 2 in 3 patients who suffered from myalgias at cycle 1 and were retreated at 1.48 mg/m^2; mild opioids, but not NSAID could prevent the appearance of toxicity.

In Study 2, at a comparable dose intensity, neuroconstipation and myalgia were still the primary toxicities and cumulative in nature. The incidence of myalgia and neuroconstipation precluded a regular weekly treatment in 1 of 8 patients at cycle 1 and in 2 of 6 who received $\geqslant$2 cycles. One of the 2 patients at 1.7 mg/m^2 presented with grade 2 neuromotor and neurosensory toxicity with a vulvar vestibulitis syndrome [4].

8. Other non-haematological toxicities

Alopecia occurred in 18 patients in Study 1. Approximately half of the patients treated at dose levels of 1.48 mg/m^2 and below experienced mild alopecia whereas all patients developed alopecia at dose levels above 1.48 mg/m^2.

Cardiac dysrhythmias were reported in 5 patients (14%) in Study 1, and were a DLT in 2 patients., In Study 2, one patient (13%) experienced a cardiac dysrhythmia. The first DLT was reported at 0.68 mg/m^2 in a patient with head and neck cancer, who presented 1 h after the initial infusion with hypertension, sweating, chills and nausea; the infusion was stopped and steroids and metoclopramide were given. The patient became somnolent, the heart rate decreased to 38 beats per minute (bpm) and the blood pressure was unmeasurable. The ECG showed sinus bradycardia and i.v. atropine was administered with recovery within 15 min. This event reappeared at the second cycle in spite of premedication against HSR. The second DLT related to cardiac dysrhythmia occurred in a patient with small cell lung cancer during the first infusion at 1.48 mg/m^2. The patient presented with severe acute neuropathic pain (possibly at the tumour site) for which he received subcutaneous (s.c.) and i.v. morphine; he then developed bradycardia with an ECG diagnosis of sick sinus syndrome, possibly pre-existent, but not evident in the presence of a normal heart rate.

HSR occurred in 4 patients in Study 1, was of grade 3 in 3 patients at 0.68 mg/m^2 without premedication, and of grade 1 in 1 patient at 1.14 mg/m^2 with premedication. In 1 patient at 0.68 mg/m^2 HSR was a DLT, it appeared during the first infusion as well as during the second, the latter with premedication. Patient went off study. Grade 3 HSR at 0.68 mg/m^2 occurred in 2 other patients at cycle 2; 1 patient went off study because of tumour progression, the other refused premedication at cycle 3 and presented again HSR.

HSR of grade 2 severity occurred in 1 patient in Study 2 during the first infusion, in spite of premedication. The patient refused any further LY355703. HSR appeared during the first or second infusion and consisted of a combination of hypertension, bradycardia and hypotension, hypertension, sweating, flushing and shortening of breath.

A total of 8 patients with peripheral i.v. access experienced local skin irritation. In Study 1 there were 7 patients with local injection site reactions. Grade 2 injection site reactions occurred in one patient at 0.4 mg/m^2, 2 patients at 0.52 mg/m^2 and 2 patients at 0.68. Grade 1 local toxicity was reported in 1 patient at 1.70 mg/m^2 and 1 patient at 1.92 mg/m^2. In Study 2, 1 patient experienced grade 1 local toxicity.

9. Antitumour activity

Complete or partial responses were not observed in either study. In Study 1, 11 patients achieved stable disease, lasting for 8 cycles in 2 of them.

10. Pharmacokinetic results

Plasma concentrations (Fig. 2) reached a maximum at the end of infusion and generally fell below the minimum quantitation limit of the assay (0.25 ng/ml) within 10 h after the start of infusion. The rapid decline in plasma concentrations after termination of infusion is suggestive of a short terminal elimination half-life. Plasma concentrations collected at the end of infusion during subsequent cycles (2 and 3) were consistent with

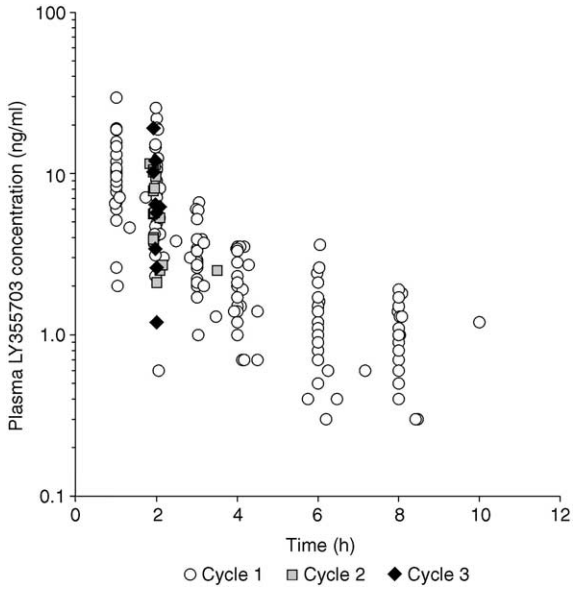

Fig. 2. Individual plasma concentrations as a function of time.

those during the first cycle suggesting a lack of time dependency.

Pharmacokinetic parameter estimates are presented in Table 4. Plasma clearance was 54.9 l/h for a typical patient with an ALT of 22.8 U/l and a BSA of 1.84 m^2 and ranged from 37.7 to 99.8 l/h. Volume of distribution was 139 l for a typical patient with a BSA of 1.84 m^2 and ranged from 89.7 to 225 l. Inter-patient variability with respect to CL and V were low at 13 and 12%, respectively. The terminal elimination half-life was short ranging from 1.1 to 2.2 h. Area under the curve and C_{max} values were shown to be linear with dose (Fig. 3). This is consistent with modelling results which showed that dose was not a statistically significant covariate with respect to CL. Therefore, the pharmacokinetics of LY355703 appeared to be linear over the dose range studied.

11. Discussion

Cryptophycins are potent new antimitotic agents of marine origin which bind to microtubule ends with high affinity at the vinca binding domain. LY355703 satisfied the criteria for an ideal chemotherapeutic agent with a mechanism of action against a validated target, high potency in the low nanomolar range of concentrations, non-schedule-dependent antitumour activity, and activity against drug-resistant cells.

This Phase I and pharmacokinetic study was designed to evaluate the feasibility of administering LY355703 as a 2-h infusion initially as a single dose every 3 weeks (Study 1), then as weekly lower doses for 3 consecutive weeks, followed by a 1 week pause (Study 2). Preclinical toxicology studies identified the dog as the most sensitive animal species with DLTs at 1 mg/m^2 including both gastrointestinal and bone marrow toxicity.

The main DLTs in our studies were peripheral neuropathy and myalgia. In Study 1 they were acute, appearing within 24–48 h after the first infusion. The MTD was identified in the dose range of 1.7–1.92mg/m^2, with 2 of 2 patients at 1.7 mg/m^2 and 2 of 5 patients at 1.92 mg/m^2 experiencing grade 3 myalgia or grade 3

Table 4
Summary of mean LY355703 pharmacokinetic parameters

Parameter description	Population[a] estimate (%SEE)	Inter-patient[b] variability (%SEE)
Clearance		
Parameter for CL (l/h)	54.9 (5.9)	13% (41)
Effect of alanine transaminase on CL[c]	−0.227 (24)	–
Effect of body surface area on CL[c]	1.32 (37)	–
Volume of distribution		
Parameter for V (l)	139 (7.3)	12% (62)
Effect of body surface area on V[d]	1.92 (32)	–
Residual error		
Proportional[e]	44% (9.9)	

SEE = standard error of the estimate.

[a] Pharmacokinetic database: 32 subjects; 179 observations.

[b] Exponential error model; results represented as % coefficient of variation

[c] $$CL = 54.9 \cdot \left(\frac{ALT}{22.8}\right)^{-0.227} \cdot \left(\frac{BSA}{1.84}\right)^{1.32}$$

[d] $$V = 139 \cdot \left(\frac{BSA}{1.84}\right)^{1.92}$$

[e] The difference between model predicted cryptophycin plasma concentration and observed concentration was modelled as $C_{ij} = C_{sij} \cdot (1 + (\epsilon_{ij}))$. The value includes intra-subject variability, model mis-specification and any errors in blood sampling (phlebotomy) times.

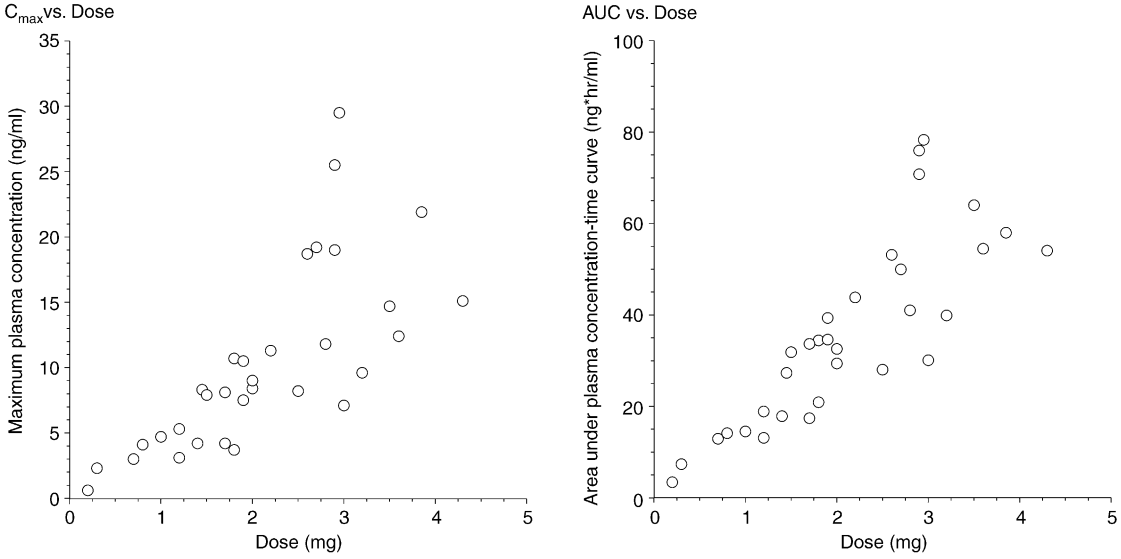

Fig. 3. Maximum plasma concentration. Area under the plasma concentration–time curve as a function of dose.

neuropathic pain. The 4 patients who presented DLT had been pretreated with ifosfamide and anthracyclines only. At the lower dose of 1.48 mg/m^2, only 1 of 8 patients had DLT with cardiac dysrhythmia and, less severe peripheral neuropathy was present. In Study 2, only 2 of 8 patients treated at 1 mg/m^2 could complete 2 cycles of treatment because of cumulative neuroconstipation and myalgia. The development of the weekly regimen was discontinued because a dose below 1 mg/m^2 would achieve a dose intensity lower than that achieved in Study 1; the schedule recommended for Phase II was 1.48 mg/ m^2 given every 3 weeks.

Comparable results were achieved in the study conducted in the United States, where LY355703 was given on days 1 and 8 every 3 weeks. The MTD was identified at 2.2 mg/m^2 due to acute neurotoxicity and the recommended Phase II dose was 1.48 mg/m^2. The most common toxicities reported were abdominal pain, allergic reactions, arthralgia, myalgia, asthenia, neuroconstipation and hypertension [12]. This schedule was selected for the Phase II programme, which was initiated in Europe and USA in a variety of tumor types sensitive to taxanes and vinca alkaloids (breast), or in which hints of antitumour activity had been observed in Phase I (NSCLC).

The main toxicities of LY355703, peripheral neuropathy and myalgias, not predicted by preclinical studies, are typical of vinca alkaloids and taxanes. LY355703-induced neuropathy had a predominant neurosensory component with minor motor signs; neuroconstipation was also present similar to the vinca alkaloids, which induce neuroconstipation via autonomic neuropathy. At doses higher than 1.48 mg/m^2 peripheral neuropathy appeared within 48 h in 3 of 7 patients after the first infusion, mainly in the form of dysesthesia of the extremities and neuroconstipation. Risk factors for neuropathy such as concomitant disease, previous neurotoxic treatments, site of disease, concomitant medications and patient characteristics could not be identified. The interpatient variability of peripheral neuropathy indicates that other factors, still to be clarified, affect the development of severe neurotoxicity; patients with severe neurotoxicity did not have a pharmacokinetic profile different from that of patients without neurotoxicity. In the weekly regimen, peripheral neuropathy, consisting mainly of neuroconstipation, was cumulative and precluded the day 15 administration.

Peripheral neuropathy of cytotoxic drugs is becoming a common DLT. Different mechanisms of neurotoxicity and nerve injury have been defined according to the mechanism of action of the cytotoxic agent. Vinca alkaloids and paclitaxel act at the microtubule level, causing mitotic arrest and axonal degeneration, affecting small sensory fibres at low doses while at higher doses motor and large sensory fibres are affected [9]. The alkylating agent cisplatin, on the contrary, accumulates in dorsal root ganglia causing sensory, mainly proprioception, and autonomic neuropathy [3]. The limited, clinical experience with LY355703 supports a combined neurotoxic effect, intermediate between vinca alkaloids and taxanes with both neurosensory and neuromotor toxicity, possibly related to the different effects against microtubules.

The pharmacokinetics of LY355703 appeared to be linear over the dose range studied with a short terminal elimination half-life. As previously stated, this initial study could not establish a relationship of pharmacokinetic parameters, either C_{max} or AUC, with neurotoxicity. This lack of relationship could be the result of limited pharmacokinetic sampling, limited number of severe neurotoxic events or simply reflects either a long intracellular half-life with a prolonged target effect. The

lack of information on metabolic features and relevance of possible metabolites contributes further to the apparent lack of a pharmacokinetic-pharmacodynamic relationship. Although no overt neurotoxicity was observed in preclinical animal models, DLTs in both rats and dogs occurred at exposure levels (both C_{max} and AUC) three to five fold less than those achieved in this Phase I study.

In conclusion, the results of this Phase I and pharmacological study demonstrate that the administration of LY355703 given on a schedule of once every 3 weeks is associated with a unique toxicity profile, dominated by acute dose-dependent peripheral neuropathy and myalgias with inter-patient variability at the dose recommended for Phase II. The administration on a weekly regimen did not improve tolerability and resulted in a lower dose intensity due to accumulation of toxicities with dose omissions. The failure of preclinical models to identify neurotoxicity in this study underscores the need to develop preclinical models which will predict clinical neuropathy.

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