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Phase I clinical and pharmacokinetic study of LY 195448

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Summary. LY 195448 is a phenethanolamine that has shown anti-tumour activity in a range of murine tumour models, although its mechanism of action is unknown. Pre-clinical studies have indicated the absence of "standard" side effects such as myelosuppression and gastrointestinal toxicity. The present phase I trial was carried out in nine patients at doses ranging up to 133 mg/m^2. The major toxicities up to that dose were mild, reversible hypotension, tachycardia and tremor. No haematological or biochemical toxicity was observed. Murine pharmacokinetics were assessed at a dose level that was effective in experimental tumours and compared with human pharmacokinetic parameters derived from this study. The results indicated the clinical possibility of reaching peak drug levels associated with experimental activity. However, no responses were seen at the doses used. This study was terminated prior to its completion due to an unexplained loss of activity against murine tumours since September 1987. No significant loss of the in vitro anti-mitotic activity originally reported by Boder et al. [3] was observed. Possible reasons for the apparent loss of in vivo activity have been intensively investigated, but no cause has been determined. Therefore, clinical trials with LY 195448 have been discontinued.

Introduction

LY 195448 is the R-enantiomer of LY 119123 (a racemic mixture) (Fig. 1). It is a phenethanolamine with weak beta-receptor antagonist activity that has shown anti-tumour activity against various murine tumour cell lines, including CA755 adenocarcinoma, C_3H mammary carcinoma, M5 ovarian carcinoma, Lewis lung carcinoma, colon 26 carcinoma and X5563 myeloma [3, 7]. The greatest cytotoxic activity has consistently been demonstrated by a rapid i.v. infusion (over 30 s) rather than a continuous-infusion schedule. The mechanism underlying the anti-tumour effect of this agent is unknown; however, interestingly, high doses of the beta-blocking drug propanolol can interfere with the therapeutic activity of this compound.

In addition, LY 195448 was found to be synergistic with several chemotherapeutic agents in vivo, including 5-fluorouracil (5-FU), mercaptopurine, cyclophosphamide and vindesine. This type of biological activity was observed in several murine tumour models, including adenocarcinoma 755, M5 ovarian carcinoma and Lewis lung carcinoma.

Pre-clinical studies in several species have indicated that the major toxicity was dose-dependent hypotension secondary to changes in total peripheral resistance (unpublished data). We report a phase I and clinical pharmacokinetic study of LY 195448 given as two 10-min infusions (6 h apart) on 1 day of each week. However, this study was terminated prior to its completion due to an unexplained loss of activity against murine tumours.

Materials and methods

Patient selection. Patients with histologically confirmed metastatic cancer refractory to conventional treatment were entered into the study after fully informed consent had been obtained. Eligibility criteria included adequate performance status (WHO grade 0–2), adequate pre-treatment bone marrow values (WBC of $>4\times10^9$ cells; platelet count of $>100\times10^9$) and hepatic (bilirubin levels of $<20\ \mu M$) and renal (creatinine values of $<120\ \mu M$) function. Patents with active cardiovascular disease or significantly abnormal ECGs were specifically excluded from the trial.

Nine patients were entered into the study; their characteristics are summarised in Table 1. Pre-treatment evaluation involved a complete physical examination, an ECG, an echocardiogram, a chest radiograph and the measurement of standard haematological and biochemical parameters. In addition, measurements of evaluable disease were made, including ultrasonographic examinations, computer-assisted tomography, and isotopic scans as appropriate. During therapy, patients were under constant observation and, with the patient in the recumbent position, pulse and blood pressure were measured (semi-automatic sphygmomanometer; Takeda Medical) every minute during infusion and for the first 5 min thereafter, then every 5 min for

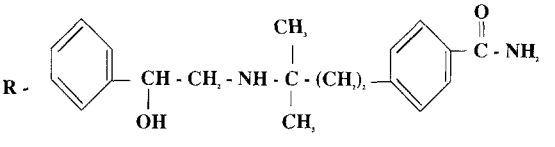

Fig. 1. Structure of LY 195448

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Table 1. Patient characteristics

Patient number	Age	Sex	Diagnosis	Previous therapy (excludes surgery)	Metastatic site(s)
1	50	M	Squamous cell cancer unknown primary site	XRT	Cervical nodes
2	64	M	Rectal cancer	TCNU	Liver and lung
3	41	M	Renal cancer	Vinblastine, bleomycin, interferon	Lung, left renal bed and liver
4	50	M	Colonic cancer	Nil	Liver and lung
5	40	M	Colonic cancer	XRT	Left iliac nodes
6	52	M	ACUP	Flavone acetic acid, 5-fluorouracil	Liver and SCF nodes
7	64	F	Breast cancer	Tamoxifen, epirubicin, vindesine, CMF	Lung
8	67	F	ACUP	Nil	Liver
9	49	M	Mixed SCLC/ adenocarcinoma	CAVE	Lung and neck nodes

XRT, external beam irradiation; ACUP, adenocarcinoma of unknown primary site; SCF, supraclavicular fossa; SCLC, small cell lung cancer; CMF, cyclophosphamide/methotrexate/fluorouracil; CAVE, cyclophosphamide/Adriamycin/vincristine/etoposide

a further 30 min, followed by every hour up to 24 h post-treatment. Continuous ECG monitoring was done throughout the infusion and for the first 30 min thereafter. Patients attended weekly for treatment, examination and repeat estimations of haematological and biochemical parameters. Evaluation of tumour response was carried out every 3 weeks by physical examination and plain radiology; more complex imaging studies were carried out if appropriate at 6-week intervals. Echocardiograms were done to assess cardiac contractility before and at the end of treatment.

Drug administration. LY 195448 was supplied by Eli-Lilly as a lyophilised powder (100 mg/vial), which was reconstituted with 0.9% sodium chloride. The drug was then diluted in 100 ml 0.9% sodium chloride and infused i.v. at a constant rate by volumetric infusion pump over 10 min. Two doses were given on each treatment day, with an interval of 6 h between doses. Treatment was repeated weekly for three courses; if there was no evidence of disease progression, a further three courses were given after a 1-week delay. The starting dose was 50 mg/m^2 and was escalated thereafter using a modified Fibonacci scheme, with three patients entered at each dose level. Dose escalation within patients was permitted after completion of the first 3 weeks of study if at least one other patient had previously tolerated treatment at the higher dose.

Clinical pharmacological studies. At entry into the trial, a pharmacokinetic study was carried out on each patient. Blood was collected into heparinised tubes during the infusions and for up to 24 h after administration of the drug. Samples were obtained at the following times after each infusion (i.e., two sets on each study day): 1, 3, 5, 15, 30 and 60 min and 1.5, 2, 3, 4, 6, 12 and 24 h. Each sample was centrifuged immediately (2,000 rpm for 5 min) and the plasma was frozen at −20° C until assayed.

Murine studies. There is some evidence relating murine pharmacokinetics to human data [4, 5]. In an effort to determine how close we had come to a therapeutically relevant dose level, we carried out a pharmacokinetic study in non-tumour-bearing mice (Swiss MFI/NuNu OLA/hsd). Initial murine tumour model experiments showed a dose of 10 mg/kg to be non-toxic and to produce inhibition of tumour growth in the test systems (e.g., 79% inhibition of adenocarcinoma 755). Synergy with 5-FU was also observed at this dose. Therefore, we selected a dose of 10 mg/kg b.d. to simulate an effective human dose level and schedule. Mice were given bolus i.v. tail-vein injections of LY 195448 dissolved in 0.9% sodium chloride. Groups of three animals were sacrificed by exsanguination at 5, 15, 30 and 60 min and 3, 6 and 24 h post-administration, and the plasma thus obtained was analysed by high-performance liquid chromatography (HPLC) (see below).

Drug analysis. An HPLC assay was developed using LY 104119 as an internal standard (supplied by Eli-Lilly). The mobile phase was composed of 90% mobile phase A and 10% mobile phase B. Mobile phase A comprised 0.005 *M* 1-heptane sulphonic acid sodium salt (BDH) and 0.005 *M* potassium dihydrogen orthophosphate (BDH) with pH adjusted to 3.5 with phosphoric acid; this was then diluted 80:20 (vol/vol) with acetonitrile. Mobile phase B was made up of the same chemicals but diluted 50:50 (vol/vol) with acetonitrile. Double-distilled, deionised water from a quartz glass still was used throughout.

The extraction of LY 195448 involved the addition of 10 µg internal standard to 1 ml plasma. After vortexing, 1 ml 1 *M* sodium carbonate was added, followed by 6 ml ethylacetate. Each sample was then vortexed for 5 min; the precipitate and aqueous phase were then sedimented by centrifugation for 5 min at 1,000 rpm. The drug-containing organic layer was then removed and evaporated at ambient temperature using a Buchler vortex evaporator. The residue was redissolved in 0.2 ml mobile phase. The extraction efficiency of LY 195448 was approximately 85%, and that of the internal standard was 80%.

The HPLC system (Hewlett-Packard 1090) delivered a flow rate of 2 ml/min. The stainless steel column

(4.6 × 150 mm internal diameter) was packed with (5-μm) Ultrasphere I. P. (Beckman). Samples were injected onto the column via a manual injection port containing a 100-μl loop (Reodyne). The eluant was freshly prepared and degassed daily. A variable-wavelength UV detector (Hewlett-Packard 1040A) was set at 233 nm for detection of the eluting compounds; the output was recorded and integrated by a computer (Hewlett-Packard 85B). All separations were carried out at ambient temperature using isocratic elution. A standard curve was prepared by plotting the ratio of peak heights of LY 195448 to internal standard against the drug concentration; this curve was linear in the range from 0.1 to 15 μg/ml. The assay was sensitive to a drug concentration of 50 ng/ml, the lowest reliable peak height being taken as 3 times greater than the height of the baseline noise. LY 195448, the internal standard and the hydroxy metabolite of LY 195448 chromatographed as single peaks, with respective retention times of 8, 6 and 3 min. Both inter- and intra-assay coefficients of variation were < 10%.

Pharmacokinetic calculations. The AUC was calculated from time 0 to the last measured time point using the log trapezoidal rule, and then extrapolated from the last time point to infinity. The terminal half-lives were found by calculating the significance of the regression using the determination coefficient by the least-squares method. Both one- and two-compartment open models corrected with the appropriate infusion models were evaluated for goodness of fit to the patient plasma concentration-time data at all dose levels. The data set for each patient was fitted by non-linear least-squares analysis using an in-house program based on the Marquhandt algorithm [1]. The best fits were obtained using a two-compartment open model. It was possible to calculate the drug clearance and steady-state volume of distribution from the microscopic rate constants [6].

Results

Nine patients were entered into the study and all were considered evaluable for assessment of toxicity. A total of 45 courses of LY 195448 were given at doses ranging from 50 to 133 mg/m^2. Three patients were treated at each dose of 50, 75 and 100 mg/m^2 and two, at 133 mg/m^2.

Toxic effects of LY 195448

All toxic effects seen in this phase I study were noted during or within a few hours after the drug infusion and are summarised in Table 2. At the weekly assessments before each treatment, there was no evidence of any haematological or biochemical disturbance attributable to the drug.

Nausea and vomiting were seen at all dose levels but were not protracted, and individual patients seemed to be more susceptible to this side effect. The drop in blood pressure that was observed in some patients was in most cases temporally related to drug administration, and in all cases it resolved spontaneously. Problems were encountered in grading blood pressure changes because of the inherent variability throughout the day, changes with activity, and changes attributed to anxiety associated with patients' first exposure to chemotherapy and the intensive monitoring procedures related to the study. Similarly changes in pulse rate were difficult to assess, being subject to wide fluctuations throughout the course of the day. For example, the one instance of WHO grade 3 tachycardia noted in Table 2 is based on one isolated reading of pulse rate.

Ventricular ectopic beats of a simple unifocal nature were observed in five patients. In each case this was noted before, during and after drug administration, with no change in frequency; therefore, it was considered to be a normal rather than drug-induced phenomenon [8]. No changes were noted in echocardiographic indices of ventricular contraction. One patient experienced an exacerbation of pre-existing diarrhoea following the administration of LY 195448, but no change in bowel habit was observed in any other patient. A coarse tremor was observed in three patients. This was not clinically typical of catecholamine or anti-dopaminergic effects and was generally of a mild and transient nature; however, in one patient it was a factor in the discontinuation of therapy.

Therapeutic responses

None of the nine patients showed an obejctive response according to WHO criteria.

Pharmacokinetic studies

The mean pharmacokinetic parameters for the patient group are summarised in Table 3. A representative human plasma concentration vs time plot after an i.v. dose of 50 mg/m^2 is given in Fig. 2.

Murine studies

The murine plasma concentration vs time plot is shown in Fig. 3. The peak plasma concentration in mice following a dose of 10 mg/kg was 2.7 μg/ml, total AUC was 5 μg·h/ml and the terminal-phase half-life was 0.5 h. These parameters are included in Table 3 for comparison with the human data.

Table 2. Toxic effects of LY 195448[a]

Dose level (mg/m^2)	Number of courses	N/V				BP				Pulse				Dry mouth				Tremor				Flushing				Drowsiness			
		0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
50	10	8	1	1	–	5	4	1	–	5	5	–	–	8	2	–	–	10	–	–	–	9	1	–	–	8	2	–	–
75	14	13	–	1	–	11	3	–	–	5	6	3	–	8	6	–	–	10	4	–	–	14	–	–	–	14	–	–	–
100	15	9	1	5	–	5	8	2	–	5	7	2	1	13	2	–	–	7	4	4	–	15	–	–	–	13	2	–	–
133	6	1	–	5	–	–	6	–	–	2	4	–	–	5	1	–	–	6	–	–	–	6	–	–	–	6	–	–	–

[a] Maximal grade of toxicity for each course. For nausea and vomiting (N/V), blood pressure (BP) and pulse, WHO grades were used. The following grading system was used for other toxicities: grade 0, none; grade 1, mild; grade 2, moderate; grade 3, severe

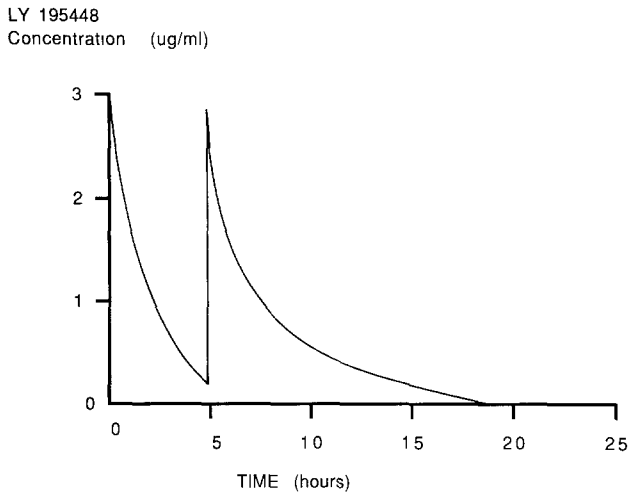

Fig. 2. Representative human plasma concentration vs time plot

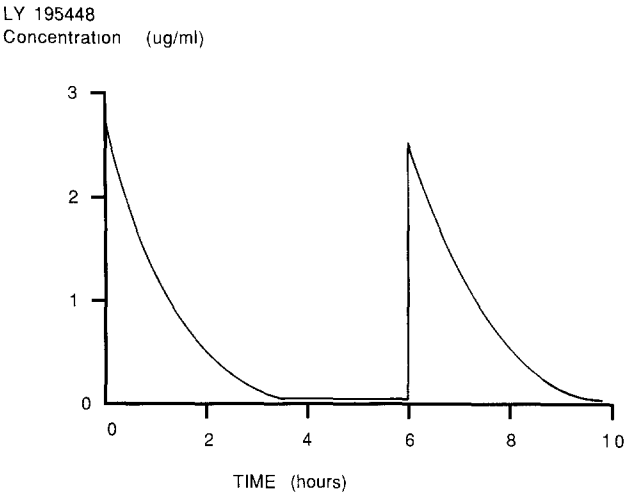

Fig. 3. Murine plasma concentration vs time plot

Table 3. Individual pharmacokinetic parameters

Patient number	Dose level (mg/m^2)	Maximum concentration (μg/ml)	Terminal phase half-life (h)	AUC (μg · h/ml)	Total clearance (l/h per kg)	Apparent volume of distribution (l/kg)
1	50	1.73	3.5	5.25	0.51	0.21
2	50	2.77	9.9	6.15	0.40	0.15
2	75	5.50	2.8	13.22	0.27	0.24
3	50	2.20	11.5	4.10	0.66	0.37
4	75	1.36	0.5	1.90	1.97	0.90
5	75	1.57	1.7	4.12	0.94	0.24
6	100	3.66	3.3	13.13	0.41	0.21
6	133	8.38	5.3	32.44	0.21	0.29
7	100	4.26	3.5	12.75	0.48	0.43
8	100	6.85	1.4	14.78	0.45	0.18
9	133	7.03	1.9	17.00	0.42	0.16
Murine		2.70	0.5	5.00	2.0	1.52[a]

AUC = area under the plasma concentration-time curve from time zero to infinity
[a] Data derived from the murine study described in *Materials and methods* (dose, 10 mg/kg)

Discussion

In early studies, LY 195448 showed a promising and wide range of activity in experimental tumour systems [2, 3, 7]. However, it showed little activity against the more traditional P388 and L1210 leukaemia models, suggesting a novel mechanism of action. Pre-clinical toxicology accurately predicted the lack of expected cytotoxic adverse effects such as myelosuppression and alopecia and gave some indication of the cardiovascular nature of the likely dose-limiting toxicity.

In the present study we did not reach a maximum tolerated dose. However, although there are limitations to extrapolation from animal to human pharmacokinetics, from our murine data it is clear that an expected therapeutic range is clinically achievable in humans (Table 3). The pattern of side effects thus far demonstrated (Table 2) show that cardiovascular effects and nausea and vomiting are the most frequent events; it seems likely that these effects would eventually have been dose-limiting.

Our particular problem in defining toxicity in this study is noteworthy. We had difficulty in defining resting values of blood pressure and pulse rate on the 1st day of drug exposure. Our practice was to use the mean value of a series of three readings taken over 30 min prior to the first infusion; this value was then taken as the normal for comparison until the time of the second infusion, when we invariably had to "reset" our normal value by a similar procedure. Consequently, changes over time, particularly those not closely related to the time of infusion, became difficult to interpret. In a previous phase I trial of flavone acetic acid in our department [9], we found hypotension to be dose-limiting, but the longer duration of infusion (up to 6 h) enabled the easy identification and interpretation of a slowly progressive drop in blood pressure. The short duration of infusion in the present trial and the transient nature of the observed changes in blood pressure and pulse made interpretation much more difficult. As more novel agents become available for phase I testing, more drugs are likely to be found to cause cardiovascular changes. Therefore, there is a need for a standardised approach to this problem.

During the course of this phase I study, repeated testing of batches of clinical material indicated a loss of antitumour activity in tumour-bearing mice compared with the initial data. We thought that changes in drug formulation would possibly be required, and since pharmacological

data indicated that target concentrations had been reached, we decided to terminate the study. Despite extensive investigation, no satisfactory explanation for this loss of anti-tumour activity has been identified since that time. No further clinical studies are currently planned with this compound.

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