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Clinical and Pharmacokinetic Phase I Trial with the Diethylaminoester of Flavone Acetic Acid (LM985, NSC 293015)*

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Abstract—*The diethylaminoester of flavone acetic acid (LM985) is a new anticancer agent with curative effects against slow growing murine tumors. Thirty-one adult patients with solid tumors received a total of 57 courses of LM985 given on days 1 and 8 every 4 weeks. The drug was given as a short infusion (1–2 hr) at doses ranging from 120 to 1900 mg/sq.m/day. The dose-limiting toxicity consisted of acute expressive aphasia; this neurotoxicity usually appeared at the end of the infusion and resolved spontaneously within a few minutes to 1 hr after the end of the infusion. In some patients, neurotoxicity was avoided by reducing the infusion rate. Neurotoxicity was observed in 5 out of 6 patients receiving 960 mg/sq.m over 1 hr and in 3 out of 3 patients receiving 1900 mg/sq.m over 2 hr. The drug did not induce any significant myelosuppression. Other side-effects were very mild and consisted mainly of occasional nausea and/or vomiting at all dose levels. One patient with breast cancer resistant to several hormonal and chemotherapy regimens had stable disease for 6 months. LM985 was detected in plasma in very small concentrations (0–2.5 μg/ml) but there was extensive formation of flavone acetic acid (peak concentration ranging between 8.3 and 64 μg/ml). A dose of 1500 mg/sq.m on days 1 and 8 every 4 weeks could be recommended for phase II studies with LM985; however, since LM985 is a prodrug of flavone acetic acid, phase II studies with LM985 should not be activated prior to the completion of the ongoing phase I trials with flavone acetic acid, which may be devoid of the acute toxicity of LM985.*

INTRODUCTION

SINCE 1975, most of the new anticancer agents have been discovered by screening new synthetic and natural compounds against a panel of murine tumors [1]. Only the agents active against the P388 murine leukemia receive further evaluation; the secondary screen consists of other murine tumors as well as 3 human tumor xenografts in the mouse. However, this screening technique has not been particularly effective in selecting active agents against human tumors. Possible reasons for this relative failure have been recently discussed [2]. One possible explanation is that murine tumors exhibit rapid growth whereas most human cancers follow a much slower pace.

Flavone acetic acid (LM975, NSC 347512) and its diethylaminoester derivative (LM985, NSC 293015) (Fig. 1) are 2 chromone derivatives whose antitumor activity was discovered by the National Cancer Institute Screening Program [3]. The mode of action of these compounds has not yet been clarified. However, their pattern of activity in experimental tumors suggests a unique mechanism of action. The 2 compounds have limited activity against the murine P388 leukemia and the murine CDF mammary tumor, and are totally inactive against the L1210 leukemia, the B16 melanoma and human xenografts [3]. However, activity in the colon 38 tumor was of particular interest. At a dose of 200 mg/kg given intraperitoneally 1 day after subcutaneous implantation of the tumor, there was 78% inhibition of tumor growth and a 213%

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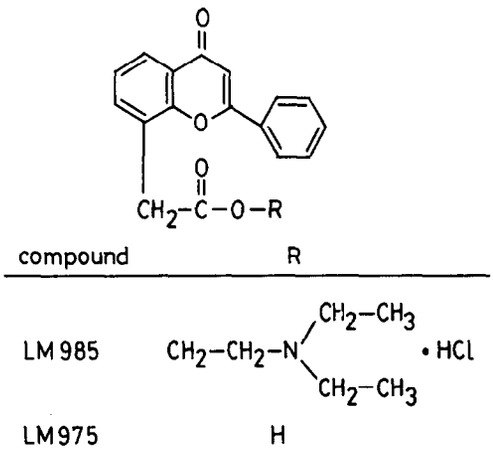

Fig. 1. Stucture of flavone acetic acid (LM975) and its diethylaminoester derivative (LM985).

increase in life span. With 2 injections 1 week apart, tumor growth was totally inhibited at a dose of 200 mg/kg and there were 10 long-term (20 days) survivors among 10 animals [4]. LM985 and LM975 appear to be equally active with a wide variety of schedules [4]. More recently, LM975 was shown to be active against a very broad spectrum of slow-growing tumors [5].

During preclinical evaluations, the toxicological profile of LM985 was investigated in mice and rats [6]. Acute side-effects included lethargy, tachypnea, ataxia, tremor and clonic convulsions; all these side-effects were transient. Other toxicities included mild histological evidence of hepatic toxicity but there was no detectable myelosuppression. At the time when our study was initiated, preliminary data were available from a phase I trial of LM985 in man [7]. When the drug was given by rapid i.v. injection, transient hypotension was observed at a dose of 200 mg/sq.m but this side-effect was eliminated by increasing the infusion time to 1 hr. No other toxicity has been detected.

The purpose of this study was to characterize the toxicity and pharmacokinetics of LM985 after a weekly administration for 2 weeks.

MATERIALS AND METHODS

LM985 was supplied as a lyophilized powder by LIPHA (Lyon, France). The ampoules were reconstituted in 10 ml of sterile water and further diluted in 250 ml of 5% dextrose. The drug was given initially as a 1-hr infusion; due to the occurrence of neurologic side-effects, the infusion time was subsequently increased to 2 hr (see Results); a few patients received the drug as a 4-hr infusion. Treatment was given on days 1 and 8 every 4 weeks. The starting dose for this trial was based on an ongoing phase I trial with LM985 [7] and was equal to 50 mg/sq.m/day (100 mg/sq.m/course).

Thirty-one patients were entered into this study;

Table 1. Patient characteristics

No. of patients entered	31	
Median age (range)	61	(34–73)
Median PS-WHO (range)	1	(0–3)
Male/female	17/14	
Prior radiotherapy	0	
Prior chemotherapy	6	
Prior chemo- + radiotherapy	23	
No prior treatment	2	

all had histologically confirmed solid malignancy not amenable to any of the known effective or potentially effective treatments. The principal characteristics of these patients are listed in Table 1. All patients had a life expectancy of at least 6 weeks. Prior radiation therapy or chemotherapy had to be discontinued for at least 4 weeks before entry on this study; for prior treatment with mitomycin and nitrosoureas, this time was increased to 6 weeks. Recovery from side-effects induced by other treatments was required. The most common tumor types were colorectal cancers (9 cases), squamous cell carcinomas of the head and neck (6 cases), and malignant melanomas (4 cases); a wide variety of other tumors accounted for the 12 remaining patients. All patients had white blood cells higher than 4000/μl and platelets higher than 100,000/μl; they had normal renal (serum creatinine < 1.5 mg/dl) and hepatic (serum bilirubin < 1.5 mg/dl) functions. This study had been approved by the Protocol Review Committee of the Institute Jules Bordet and the patients gave their informed consent prior to being entered into this study.

The follow-up of the patients included a weekly history, physical examination, hematological examination and chemistry panel. The WHO guidelines were used to evaluate the antitumor response and toxicity [8].

Five patients underwent pharmacokinetic analysis. Blood samples were obtained prior to therapy, at mid-infusion, at the end of the infusion as well as at 5, 10, 15, 30 and 45 min and 1, 2, 4, 6 and 8 hr after the end of the infusion. Plasma was obtained by centrifugation. LM985 and its major metabolite, LM975 were assayed as previously described [9].

The LM985 plasma concentration time data obtained after i.v. administration were fitted to a biexponential equation. For the metabolites of the drug, the plasma concentration time data were fitted to a single exponential for the data following the peak concentration. All data fits were performed on MLAB, a non-linear fitting program using 1/precision square weighting function [10]. The area under the plasma concentration versus time curve (AUC) was calculated by trapezoidal rule until the last experimental time point and then by first-order

Table 2. Non-hematological toxicities

Dose (mg/sq.m)	Infusion time (hr)	Eval. pts.	Eval. courses	Toxic pts.	Nausea/ vomiting	Neuro-toxicity	Other side-effects
120	1	4	4	0			
240	1	6	6	1			1
480	1	7	8	4	1		3
960	1	6	9	5	1	5	2
960	2	3	4	2	1	1	1
1200	2	6	11	4	2	2	
1500	2	6	11	2	1	1	1
1500	4	1	2	1	1		
1900	2	3	3	3	1	3	
1900	4	1	1	0			

extrapolation to infinity using the experimentally determined terminal disposition half-life. The volume of distribution at steady state (VDSS) and the total body clearance (CLTB) for LM985 were calculated by means of non-compartmental techniques:

$$VDSS = (dose \times AUMC)/AUC^2$$

and $CLTB = dose/AUC$,

where the area under the moment curve (AUMC) was calculated according to a published method [11].

RESULTS

The drug did not induce any significant hematologic toxicity. Only 2 patients developed minor leukopenia at 1200 mg/m^2 over 2 hr. One of these patients had borderline counts prior to therapy. Similarly, thrombocytopenia was minimal; one patient with rapidly progressive disease treated at 120 mg/m^2 over 1 hr developed thrombocytopenia at 72,000/μl that was observed on day 20 with recovery on day 30. Another patient treated at 1500 mg/m^2 over 2 hr and who had received prior chemotherapy with triglycidyl urazol, at highly myelosuppressive dosage [12], developed thrombocytopenia at 88,000/μl on day 16 with recovery on day 26. There was no case of neutropenia.

Non-hematologic side-effects included minor nausea and/or vomiting in small numbers of patients at each dose level (Table 2). No patient required antiemetics. There was no obvious relationship between nausea/vomiting and dose. Three patients developed mild abnormalities of their liver function tests. In 2 patients, these abnormalities were limited to a mild elevation (< 1.5× the upper limit of normal values) of LDH. In 1 patient, LDH returned to normal within 8 days; for the other patient, the recovery could not be evaluated because of loss to follow-up. The third patient had a mild increase of alkaline phosphatase (< 1.5× the upper limit of normal values), of the transaminases (< 3.5× the upper limit of normal values) and of LDH (< 1.5× the upper limit of normal values). These abnormalities partially returned to normal at the time when the patient was removed from the study because of progressive disease. No subsequent follow-up was obtained. None of these 3 patients developed abnormalities of the bilirubin level. None of these 3 patients was retreated with LM985.

Neurotoxicity was the predominant and dose-limiting side-effect observed in the study. This neurotoxicity consisted of mild sedation and major expressive aphasia: the patients had difficulties in finding their words and in writing, but maintained a normal ability to understand conversation and orders. The result of their neurological examination including strength and sensitivity was normal. These side-effects usually started after completing 3/4 of the infusion; they were rapidly reversible, disppearing within a few minutes to 1 hr after stopping the infusion. In some patients, the neurologic side effect abated immediately after stopping the infusion or by slowing down the infusion rate. Neurotoxicity was observed in 5 out of 6 patients treated at 960 mg/m^2 over 1 hr and in 3 out of 3 patients treated at 1900 mg/m^2 over 2 hr; these dosages are considered to represent the maximum tolerated dose. There was no long-term sequella and all patients completely recovered normal neurological function. Therefore, examinations such as cerebrospinal fluid examination, brain scan and CT-scan were not performed. In 3 patients treated at 1500 mg/m^2 over 2 hr (1 patient) and 1900 mg/m^2 over 2 hr (2 patients), respectively, continuous electroencephalograms were obtained before, during and after the infusion. The tracings did not show any specific abnormalities. Neurotoxicity was observed sporadically at doses ranging between 960 and 1500 mg/m^2 over 2 hr: 1 patient developed mild sedation without linguistic difficulties at 960 mg/m^2; 1 patient had expressive aphasia at 1200 mg/m^2 as well as another patient treated at 1500 mg/m^2. For 2 patients who developed neurotoxicity with the 2 hr infusion, retreatment was

given as a 4 hr infusion with complete disappearance of the neurologic side-effects.

Other side-effects were infrequent and not necessarily drug-related. Two patients (1 at 240 mg/m^2 and 1 at 480 mg/m^2) complained of dryness of the mouth. One patient treated at 480 mg/m^2 complained of fatigue 2 days after the administration of the drug. Two patients treated at 960 mg/m^2 over 1 hr developed acute diarrhea at the end of the infusion; diarrhea was concommittant with neurologic side effect of the drug observed in these 2 patients. One patient developed fever up to 39.5°C, 2 hr after to the end of his second infusion; the fever resolved spontaneously and the patient was not rechallenged with LM985. The only other major side-effect observed in this trial was severe hypertension; this phenomenon occurred in 1 patient 45 min after the start of his second infusion at 480 mg/m^2 over 1 hr. The blood pressure was 140/90 before therapy, increased to 185/130 45 min after the start of the treatment and to 220/130 60 min after the start of the treatment; the blood pressure dropped to 160/100 spontaneously 105 min after the start of the treatment. This patient was not retreated with LM985.

No objective antitumor response was observed in this trial. However, 1 patient with breast cancer pretreated with radiation therapy, hormonotherapy (tamoxifen, medroxyprogesterone, aminoglutetimide, trilostane) and chemotherapy (doxorubicin, vincristine, methotrexate, fluorouracil, cyclophosphamide, PALA, vindesine, cisplatin and iproplatin) had stable disease for 6 months.

PHARMACOKINETIC DATA

LM985 was detected only in 3 patients. In these 3 patients, the peak plasma concentration was achieved during the infusion (range 0.5–2.5 μg/ml). LM985 disappeared from plasma with a mean harmonic terminal half-life of 2.8 hr (range 1.6–9.2 hr); for the 3 patients where LM985 was detectable, the mean area under the plasma concentration versus time curve was 4.6 μg/ml × hr (range 1.1–11.1 μg/ml × hr); the mean total body clearance was 11.7 l/sq.m/min (range 1.4–19.1 l/sq.m/min) and the mean volume of distribution at steady state, 835 l/sq.m (362–1132 l/sq.m). In all 5 patients, another compound with the same chromatographic properties as LM975 was detected. The concentrations of this metabolite were higher than the concentration of parent drug in all 5 patients with a peak ranging between 8.3 and 64.0 μg/ml; the peak was observed between 0 and 1 hr after the end of the infusion. Following the peak plasma concentration, the concentration of LM975 decreased with a mean harmonic terminal half-life of 3.3 hr (range: 4–5.8 hr). The mean area under the plasma concentration versus time curve was 80.3 μg/ml × hr (range 29.4–172.7).

DISCUSSION

The aim of this study was to characterize the toxic effects of LM985 after administration to man and to define a dose suitable for phase II investigation. The spectrum of toxic effect of LM985 is unusual in comparison to most anticancer drugs. The drug did not induce myelosuppression, alopecia or mucositis. Nausea and vomiting were very mild; no patient required antiemetics. In addition, in our population of heavily pretreated patients, nausea and vomiting may correspond to anticipatory vomiting induced by prior chemotherapy. The major toxicity observed in this trial consisted of expressive aphasia and mild sedation; these side-effects were acute and rapidly reversible with no permanent neurologic deficit. Several patients did not complain of any trouble and aphasia was detected only by questioning the patient during and after treatment. In several patients, neurotoxicity could be rapidly reversed by slowing down the infusion rate. Neurotoxicity was obviously dose-related since almost all patients treated at 960 mg/sq.m. over 1 hr and 1900 mg/sq.m over 2 hr developed aphasia whereas this side-effect was a very unusual event at lower doses. The fact that neurotoxicity is observed at a dose twice as high when the infusion time is multiplied by a factor of 2 suggests that neurotoxicity is related to the peak plasma concentration of the drug.

On a chemical basis, LM985 contains an ethylaminoester bond (Fig. 1), which is also a basic feature of local anesthetics such as procaine and xylocaine [13]. When given by i.v. infusion, the administration of procaine is limited by cardiovascular and neurologic side effects [13, 14]. The neurologic side effects of local anesthetics consists mainly of excitation and convulsions. The effects on blood pressure are quite variable. The major toxicity of LM985 was neurotoxicity; in addition, when LM985 is given by rapid i.v. injection, hypotension is very common [7]. Finally, in our study, 1 patient developed acute hypertension which may have been caused by LM985. The acute side effects of local anesthetics are rapidly reversible; this reversibility is a function of the speed of hydrolysis of the local anesthetic to the corresponding acid [13]. Our pharmacokinetic data show that LM985 is also rapidly hydrolyzed to its corresponding acid, flavone acetic acid. The rapid hydrolysis of LM985 may explain the quick disappearance of the neurologic side effects observed in our study. This constellation of findings highly suggests that the mechanism of the acute side-effects of LM985 is similar to that of local anesthetics.

The pharmacokinetic behavior of LM985 is characterized by a rapid and extensive conversion of the parent drug to flavone acetic acid. Similar findings were reported by Kaye *et al.* [7, 9]. In both studies, the terminal half-life of LM985 and its metabolite, flavone acetic acid, was very short, being less than 3 hr. Extensive conversion of LM985 to flavone acetic acid was also observed in both studies. In our hands, the ratio of peak concentration between LM985 and flavone acetic acid was 0.05 (range 0–0.16); the corresponding ratio for the area under the plasma concentration versus time curve was 0.06 (range 0–0.24). These data indicate that LM975 may have a major role in the biological activity of LM985. It is also important to note that plasma concentrations of LM985 are much lower than the plasma concentrations of LM985 observed in mice at therapeutic doses [6].

Although one patient with heavily pretreated breast cancer had stable disease for 6 months, no major antitumor response was observed in this trial. Absence of major antitumor response in a phase I trial is common and related to the design of the study itself: most patients have been heavily pretreated; some of them lack evaluable lesions; many patients are treated at suboptimal doses and for relatively short periods of time. However, as mentioned earlier, the parent drug plasma concentrations in our patients were much lower than the plasma concentrations in mice at therapeutic doses. It may well be that man is much more sensitive than the mouse to the toxic effects of LM985 and that the therapeutic index is much lower in man.

A dose of 1500 mg/sq.m on days 1 and 8 every 4 weeks could be recommended for phase II trial with LM985. However, we do not currently recommend phase II studies with this agent since LM985 acts clearly as a prodrug of LM975. In addition, if our hypothesis regarding the mechanism of the acute toxic effects of LM985 is correct, these acute side effects could not be observed with flavone acetic acid since this compound lacks the ethylaminoester characteristic of the local anesthetics. Preliminary data from an ongoing phase I trial with flavone acetic acid show indeed that much higher doses of LM975 can be given without toxicity; in addition, much higher plasma levels of LM975 have been achieved [15, 16]). Other phase I studies with LM975 have been activated in the United States. We feel that a decision regarding phase II studies with LM985 should be withheld pending the completion of the phase I studies with LM975.

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