Phase I Clinical Study of N-[(4-Chlorophenyl)aminocarbonyl-2,3-dihydro-1H-indene-5-sulfonamide (LY186641)

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ABSTRACT

Between February 1987 and July 1988, 45 patients with advanced refractory cancer were treated with LY186641, a diarylsulfonylurea that has shown a broad spectrum of activity in preclinical testing. Patients received a weekly p.o. dose of LY186641 for 6 consecutive weeks; responding and stable patients continued weekly therapy until progression occurred. Using a standard phase I study design, the first three patients received LY186641 at 30 mg/m²/week; the dose was escalated in subsequent patients until dose-limiting toxicity occurred. Methemoglobinemia was the major toxicity observed and was dose related. Methemoglobin levels peaked approximately 24 h after LY186641 was administered and fell to low levels after 48 h. Six patients developed fatigue, cyanosis, and dyspnea associated with serum methemoglobinemia levels of >20%; four of these patients were subsequently removed from the study. Hemolytic anemia was also observed but was clinically significant in only 10 patients. Other side effects were mild and infrequent. The maximum tolerated dose of LY186641, when given at this schedule, was 2550 mg/m²/week. No objective tumor responses were observed.

INTRODUCTION

The diarylsulfonylureas have recently been identified as a new class of antineoplastic agents. These compounds have novel chemical structures, when compared to other cancer chemotherapeutic agents, and their biological and pharmacological properties are also distinctive. Preclinical evaluation revealed a wide spectrum of activity against murine solid tumors. Eight of nine solid tumors tested were sensitive to these drugs; in four of these tumors (6C3HED lymphosarcoma, adenocarcinoma 755, M-5 ovarian carcinoma, and 63H mammary carcinoma), greater than 90% shrinkage was achieved with no clinical toxicity (1). Good activity against CX1 colon and LX1 lung xenografts was also identified in nude mice. The mechanism of antitumor activity has not yet been identified but does not appear to involve interaction with DNA, RNA, or protein synthesis.

In addition to this broad spectrum of antineoplastic activity, many of the diarylsulfonylureas were found to be relatively nontoxic in preclinical testing. Because of its optimal activity spectrum and low toxicity, LY186641 was selected for phase I trials in humans. Side effects produced by this drug were dose-related hemolytic anemia and methemoglobinemia in mice, rats, dogs, and monkeys; no myelosuppression, hypoglycemia, or hepatotoxicity was observed in any of these animals. When administered p.o. to these animals, LY186641 was rapidly and completely absorbed from the gastrointestinal tract, was highly protein bound in the serum, and was excreted (as metabolites) in the urine. The half-life in preclinical studies ranged from 6 h in the rat to 203 h in the dog.

We report here the results of a phase I clinical and pharmacokinetic study using LY186641 in a weekly p.o. dosing schedule.

MATERIALS AND METHODS

Between February 1987 and July 1988, 45 patients at Vanderbilt University Medical Center entered this phase I study. To be eligible, patients were required to have a histological diagnosis of advanced cancer that was considered refractory or primarily resistant to all standard therapy. No patient had received anticancer therapy during the 3 weeks prior to entering this study (6 weeks if a prior nitrosourea or mitomycin C had been administered). In addition, all patients met the following criteria: (a) ECOG performance status 0, 1, or 2, (b) estimated life expectancy 12 weeks or more, (c) serum creatinine <1.5 mg/dl, (d) serum bilirubin level <2.0 mg/dl, (e) hematocrit >30% with no transfusions in the preceding 3 weeks, (f) WBC ≥3,000/mm³, and (g) platelets ≥100,000/mm³. Due to the fact that LY186641 is a sulfonamide, no patient with a history of diabetes mellitus that required treatment with either insulin or p.o. hypoglycemic agents was eligible for the study. Because of the preclinical toxicity in various animals, patients with a history of hemolytic anemia were excluded. All patients were required to give informed consent to participate in this study.

Prior to the first dose of LY186641, all patients underwent the following laboratory and radiological tests: complete blood count, differential, reticulocyte count, methemoglobin level, hemoglobin level, SMA 18 chemistry screen, direct and indirect Coomb's test, glucose-6-phosphate dehydrogenase screen, urinalysis, chest roentgenogram, and electrocardiogram. In addition, patients had objective measurements of their tumors made either by physical examination or with appropriate radiological studies. Patients received weekly doses of LY186641 for 6 consecutive weeks. Before each weekly dose, the blood tests listed above, with the exception of the Coomb's test and glucose-6-phosphate dehydrogenase screen, were repeated. After receiving 6 doses of LY186641, patients were reevaluated for tumor response. Patients with objective evidence of tumor response or those with stable disease continued on a weekly schedule of LY186641 (same dose) until tumor progression was documented. Patients who had tumor progression at the time of reevaluation were withdrawn from the study.

The optimal starting dose for this phase I study was difficult to determine. Due to the relatively nontoxic nature of this drug, a single 10% lethal dose could not be found in the mouse. However, significant methemoglobinemia, weakness, and anorexia were observed in monkeys when 270 mg/m² were administered every 3 weeks. We, therefore, chose a starting dose approximately one-third of this dose, or 30 mg/m²/week. Three patients were entered at this initial dose level and were observed for toxic effects for a minimum of 3 weeks. Subsequent patients received higher doses; three patients were treated at each dose level. Initially, dose escalation was performed using a modified Fibonacci study using LY186641 in a weekly p.o. dosing schedule.

Pharmacokinetic studies were performed on the first two patients entered into this study, and on the first patient treated with each subsequent dose level. Plasma samples were obtained prior to administration of LY186641 and at 1, 2, 3, 4, 6, 12, 24, 36, 48, 55, and 168 h after dosing. In addition, sequential 12-h urine collections were obtained during the first 48 h after drug administration. LY186641

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was assayed using a reverse phase high pressure liquid chromatography system that was sensitive to 1 μg/ml. Individual elimination rate constants (kₑ) were calculated by linear regression analysis of data points on the terminal linear portion of the plot of the logarithm of the drug concentration versus time. Terminal half-life (tₑ) values were calculated using the equation: tₑ = 0.693 kₑ. The area under the concentration-time curve was calculated using the linear trapezoidal method or the logarithmic trapezoidal method extrapolated to infinity.

The clinical characteristics of the 45 patients in this clinical trial are detailed in Table 1. The majority of patients (64%) had ECOG performance status of 0 or 1; the remainder had ECOG performance status 2. Forty-one of 45 patients had received previous chemotherapy; however, only 14 of the patients (31%) had received two or more chemotherapeutic regimens. Forty-two % of our patients had advanced colorectal cancer; the remaining patients had a wide variety of malignancies.

The dose of LY186641 received by the 45 patients in this study are shown in Table 2. The initial dose escalation schedule was modified after the fourth dose level, when information became available from other concurrent phase I studies that much larger doses of this drug could be tolerated. The fifth and subsequent dose escalations were made at a fixed interval of 300 mg/m², rather than according to the Fibonacci scheme.

RESULTS

Toxicity. LY186641 was generally well tolerated over a wide dose range. No significant laboratory abnormalities were seen at doses lower than 1350 mg/m², and no patients had drug-related symptoms at doses <1950 mg/m². Methemoglobinemia and RBC hemolysis were the major toxicities observed, as predicted by the preclinical toxicity data. No myelosuppression, hypoglycemia, hepatoxicity, gastrointestinal toxicity, mucositis, or alopecia was observed with this drug.

The dose-limiting toxicity with LY186641 is methemoglobinemia. Fourteen patients developed methemoglobinemia (>5%); this toxicity was first seen at a dose level of 1350 mg/m², and became more frequent and severe with subsequent dose escalations. In most patients, methemoglobin levels peaked approximately 24 h after the dose of LY186641 and by 48 h were returning to normal. In most patients, methemoglobin levels had returned to normal prior to the subsequent dose (i.e., 7 days following a dose). Fig. 1 shows the time course of LY186641-induced methemoglobinemia in a typical patient.

The relationship of methemoglobinemia to dose level of drug administered is displayed in Table 3. Methemoglobinemia was asymptomatic at levels less than 20%; levels higher than 20% occurred only at the three highest doses of LY186641 (2250, 2550, and 2850 mg/m²). Four patients were removed from the study prematurely due to severe symptomatic methemoglobinemia. All four of these patients (two patients at 2850 mg/m², two patients at 2550 mg/m²) developed methemoglobin levels greater than 40%. These high levels were associated with cyanosis, severe weakness, and dyspnea. These patients were treated with supplemental oxygen and were closely observed, and symptoms resolved within 12 h in all cases. Methemoglobin levels had decreased to less than 20%, in all cases, by 48 h after the dose of LY186641. Of interest, only one of these patients developed methemoglobin levels of greater than 10% following the first dose of LY186641. Three patients became symptomatic with high methemoglobin levels after their second dose and were removed from the study; the remaining patient was removed following her fourth dose of LY186641.

In one patient who received 2250 mg/m²/week, the time course of methemoglobinemia was atypical, as illustrated in Fig. 2. In this patient, high levels of methemoglobin persisted 7 days after the LY186641 dose. With each dose, the level of abnormal hemoglobin became higher and resulted in discontinuation of the drug following three doses. This patient still had a methemoglobin level of 11.3% 4 weeks after his last dose of LY186641. Since the time course of this patient’s abnormal hemoglobin was much different than that of the other patients, specimens of his blood were sent for determination of sulfhemoglobin versus methemoglobin. At a time when his methemoglobin...
moglob" levels, as measured at Vanderbilt University, were 20.1%, the Mayo Clinic laboratory measured sulfhemoglobin as 9.1% and methemoglobin as 0.8%. Sulfhemoglobin levels were consistently low when measured in subsequent patients, and the predominant abnormal hemoglobin appeared to be methemoglobin. This one patient, therefore, appears to have had a distinct toxicity, perhaps related to a different interaction of the drug with the hemoglobin molecule, which resulted in his very prolonged duration of altered hemoglobin.

In contrast to the methemoglobinemia, hemolytic anemia was usually not an important clinical problem. Eighteen of 45 patients developed elevated reticulocyte counts (>5%) during the course of this study. Seventeen of these 18 patients were receiving doses of 1350 mg/m² or higher and, in general, elevations in reticulocyte counts became more frequent and severe with increasing dose. In 10 of the 18 patients with elevated reticulocyte counts, the hematocrit dropped more than 3% during the course of the 6-week study. Although decreasing hematocrits are difficult to interpret in patients with advanced malignancies, it is probable that drug-induced hemolysis contributed to the worsening anemia in these patients. In one patient, drug-induced hemolysis was severe and was definitely responsible for a falling hematocrit. This patient (who received 2550 mg/m²) had a peak reticulocyte count of 18.6% 1 week after dose 3, and her hematocrit dropped from a pretreatment level of 39% to a low of 26.7% 4 weeks later. The fourth dose after dose 3, and her hematocrit dropped from a pretreatment level of 39% to a low of 26.7% 4 weeks later. The fourth dose

Other side effects of LY186641 were uncommon. Two patients developed thrombocytopenia (platelets >1,000,000) while receiving the drug. Platelets rose from a pretreatment level of 638,000 to 1,298,000 in one patient and from 811,000 to 1,006,000 in another patient. Both patients were receiving dose levels of 2250 mg/m². The relationship of thrombocytopenia to the administration of LY186641 is unclear; however, in one patient platelet levels had decreased 8 weeks following his removal from this study. Three additional patients developed generalized fatigue which appeared to be related to LY186641; although all three patients had progressive cancer, they felt less fatigued after LY186641 was discontinued.

**DISCUSSION**

In this phase I study, we have established that LY186641 can be safely administered to humans over a wide dose range. The maximum tolerated dose, when the drug is administered once per week, is 2550 mg/m². The dose-limiting toxicity is symptomatic methemoglobinemia; the only other significant toxicity using this schedule is hemolytic anemia, which was not a major clinical problem. LY186641 produced none of the side effects commonly encountered with other antineoplastic agents, including myelosuppression, nausea, vomiting, alopecia, mucositis, and hepatotoxicity. In addition, LY186641 does not share the hypoglycemic effects of other sulfonylureas.

Both of the significant side effects caused by LY186641 are probably related to its oxidant capacity. Several drugs, including sulfonamides, have been shown to increase the rate of hemoglobin oxidation from the ferrous to the ferric state, resulting in the production of methemoglobinemia (2). Most drug-induced methemoglobinemia is clinically unimportant due to the low levels produced; however, levels greater than 20% have been associated with cyanosis. Higher levels produce the symptoms of severe anemia, and levels of greater than 60% have been associated with vascular collapse, coma, and death.

### Table 3 Incidence of methemoglobinemia as related to dose of LY186641

<table>
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<th>Dose (mg/m²/wk)</th>
<th>&gt;5%</th>
<th>&gt;10%</th>
<th>&gt;20%</th>
<th>&gt;30%</th>
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<tr>
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<td>2/2</td>
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</table>

*LY186641 discontinued prematurely due to symptomatic methemoglobinemia.*
patients who developed methemoglobin levels of greater than 20% had symptoms; these included mild to moderate dyspnea and cyanosis, which resolved in 12–24 h. With this schedule of drug administration, peak levels of methemoglobinemia occurred approximately 24 h after drug administration and declined rapidly during the next 48 h. Although serial peak methemoglobin levels varied in a single patient with repetitive doses, the trend was toward higher peak levels with repeated doses. This was probably related to the relatively long half-life of the parent drug and/or its metabolites.

The hemolytic anemia observed was probably also due to the oxidative nature of LY186641. Heinz body hemolytic anemia, or “bite cell” anemia, has been described with a variety of oxidative drugs including phenazopyridine hydrochloride (pyridium), salicylazosulfapyridine (azulfidine), N-2-pyridylsulfanilimide (sulfapyridine), diazoxide, and others (3–6). These drugs share an oxidative potential capable of overriding the protective role of the hexose monophosphate shunt in normal RBC. In addition to changing the oxidative state of hemoglobin, this results in the denaturation of globin chains and precipitation of these chains as Heinz bodies (7). The presence of the relatively rigid Heinz body within the RBC may cause part of the cell to be pinched off when it traverses the spleen, resulting in the bite cell (8, 9). The subsequent survival time of the bite cell is greatly shortened, resulting in the development of anemia. As with methemoglobinemia, this type of anemia is rapidly reversible when the offending agent is withdrawn; this was observed in our patients treated with LY186641.

We recommend that LY186641 undergo phase II testing against a variety of solid tumors. Daily dosing schedules of this drug may be preferable to this weekly schedule in allowing a greater total dose of LY186641 to be given. Daily schedules may also avoid high peak levels of drug, which are probably related to its toxic effects. In addition, chronic exposure to LY186641 with a daily dosing schedule had the greatest anti-neoplastic activity in preclinical testing. Other phase I studies testing LY186641 on daily dosing schedules are nearing completion, and phase II testing will soon be initiated.

REFERENCES
Phase I Clinical Study of \(N\)-[(4-Chlorophenyl)amino]carbonyl-2,3-dihydro-1\(H\)-indene-5-sulfonamide (LY186641)


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