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Editorial correspondence should be addressed to George P. Canellos, MD, *Journal of Clinical Oncology,* 454 Brookline Ave, Suite 28, Boston, MA 02115. Telephone: (617) 632-5150.

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Initial Phase I Evaluation of the Novel Thymidylate Synthase Inhibitor, LY231514, Using the Modified Continual Reassessment Method for Dose Escalation

By D.A. Rinaldi, H.A. Burris, F.A. Dorr, J.R. Woodworth, J.G. Kuhn, J.R. Eckardt, G. Rodriguez, S.W. Corso, S.M. Fields, C. Langley, G. Clark, D. Faries, P. Lu, and D.D. Von Hoff

Purpose: **To determine the toxicities, maximal-tolerated dose (MTD), pharmacokinetic profile, and potential antitumor activity of LY231514, a novel thymidylate synthase (TS) inhibitor.**

Patients and Methods: **Patients with advanced solid tumors were administered LY231514 intravenously over 10 minutes, weekly for 4 weeks, every 42 days. Dose escalation was based on the modified continual reassessment method (MCRM), with one patient treated at each minimally toxic dose level. Pharmacokinetic studies were performed in all patients.**

Results: **Twenty-five patients were administered 58 courses of LY231514 at doses that ranged from 10 to 40 mg/m^2/wk. Reversible neutropenia was the dose-limiting toxicity. Inability to maintain the weekly treatment schedule due to neutropenia limited dose escalation on this schedule. Nonhematologic toxicities observed included mild fatigue, anorexia, and nausea. At the 40-mg/m^2/wk dose level, the mean harmonic half-life, maximum plasma concentration, clearance, and apparent volume of distribution at steady-state were 2.02 hours, 11.20 μg/mL, 52.3 mL/min/m^2, and 6.64 L/m^2, respectively. No major antitumor responses were observed; however, minor responses were achieved in two patients with advanced colorectal cancer.**

Conclusion: **The dose-limiting toxicity, MTD, and recommended phase II dose of LY231514 when administered weekly for 4 weeks every 42 days are neutropenia, 40 mg/m^2, and 30 mg/m^2, respectively.**

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LY231514 (N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo, 3-d]pyrimidin-5-yl) ethyl]benzoyl]-L-glutamic acid disodium salt) is a novel compound representative of a new class of folate antimetabolites. It has a pyrrole ring that replaces the pyrazine ring in the pterine portion of folic acid, and a methylene group that replaces the benzylic nitrogen in the bridge portion (Fig 1). The primary mechanism of antitumor effect of LY231514 is via inhibition of the enzyme thymidylate synthase (TS), which is the only de novo source of thymidylate for the cell.[1-3] This enzyme catalyzes the reductive methylation of deoxyuridine monophosphate (dUMP), in the presence of a reduced folate cofactor, 5,10-methylene tetrahydrofolate, to deoxythymidine monophosphate (dTMP) and dihydrofolate. Deoxythymidine monophosphate is the precursor of deoxythymidine triphosphate (dTTP), one of the deoxyribonucleotides necessary for DNA synthesis.[4,5] LY231514 undergoes extensive intracellular polyglutamation,[1-3] which with other chemotherapeutic agents, converts the drug from a form that readily effluxes from the cell, to a form that is retained intracellularly for a prolonged period. This produces a more sustained drug effect.[5]

In preclinical models, LY231514 has demonstrated activity against a wide spectrum of tumor types. In vitro, it is highly cytotoxic against CCRF-CEM human leukemia cells in culture, with a 50% inhibitory concentration (IC50) of 0.007 μg/mL. This activity was reversed by the addition of thymidine to the medium.[1-3] LY231514 has also demonstrated substantial in vitro activity against human tumor colony-forming units obtained from patients with colon cancer, renal cancer, hepatoma, carcinoid tumor, and both non–small-cell and small-cell lung cancer (Von Hoff DD, personal communication, August 1995).[6] In animal studies, LY231514 was able to suppress tumor growth completely at doses $\geq$ 10 mg/kg in mice with two types of transplanted human colon xenografts (VRC5 and GC3) resistant to methotrexate (MTX).[1]

Toxicology studies of LY231514 in mice (CD-1 strain), using daily intraperitoneal doses of up to 150 mg/kg for 2 weeks, were associated with minimal toxicity. There was a dose-related decrease in body weight, reaching a maximum of 20% at the 150-mg/kg level. Moderate decreases in WBC and platelet counts, as well as mild decreases in RBC counts, were also observed. Weekly

From the Institute for Drug Development, Cancer Therapy and Research Center; Brooke Army Medical Center, Fort Sam Houston; The University of Texas Health Science Center at San Antonio, San Antonio, TX; and Eli Lilly and Co, Indianapolis, IN.

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The opinions or assertions herein are the private views of the authors and are not to be construed as reflecting the views of the Department of the Army or the Department of Defense.

Address reprint requests to David A. Rinaldi, MD, Cancer Therapy and Research Center, 8122 Datapoint Dr, Suite 1000, San Antonio, TX 78229.

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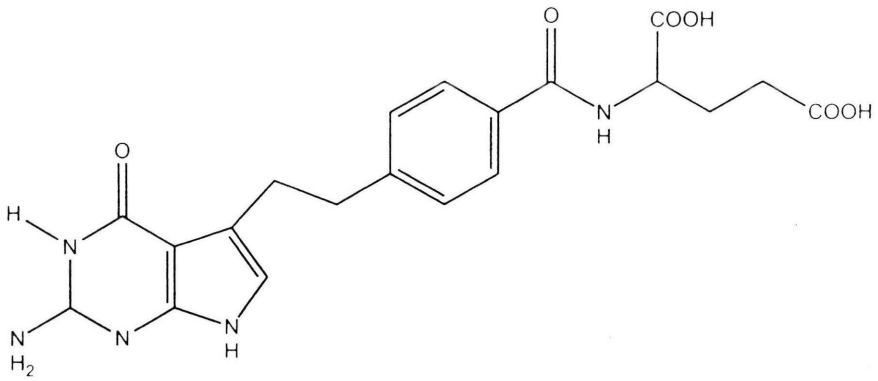
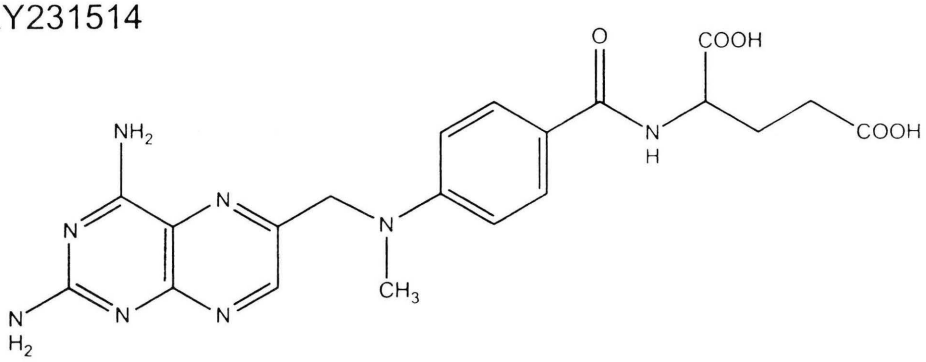


LY231514



Methotrexate

Fig 1. Structure of LY231514 and methotrexate.

doses of 315 mg/kg (944 mg/m^2) for 6 weeks was also minimally toxic in mice. The 50% lethal dose (LD_{50}) of LY231514 given as a single intravenous dose was more than 1,574 mg/kg.[1]

Beagle dogs were treated on various intravenous dosing schedules to determine toxicity. The weekly schedule began at 105 mg/kg, but the two dogs died after two doses. The dosing was then reduced to 105 mg/kg for one dose, followed by 26.24 mg/kg/wk for five doses. The major toxicities observed were anorexia, emesis, diarrhea, oral mucositis, and weight loss. Neutropenia, lymphopenia, and mild anemia were also observed. By 6 weeks, two of the four dogs died of sepsis, secondary to mucositis in one case and pneumonia in the other. Plasma concentrations of LY231514 increased in a linear fashion with increasing doses. The terminal half-life was approximately 2.3 hours. When comparing the toxicity of the various schedules, modest toxicity was observed in dogs treated with 100 mg/kg as a single dose, 5 mg/kg, twice weekly, and 0.5 mg/kg/d.[1]

In vitro and in vivo, folinic acid has been shown to antagonize the antitumor effect of other TS inhibitors currently undergoing clinical evaluation. This effect appears to be mediated via a competitive inhibition for transport of the agent into the cell and/or intracellular polyglutamation.[7,8] Folinic acid was evaluated as a rescue agent for LY231514. Four beagle dogs were administered potentially lethal intravenous doses (50 mg/kg for two doses, 3 days apart) of LY231514. All dogs developed signs of toxicity characterized by oral mucositis, anorexia, diarrhea, and a decrease in the leukocyte count by 50% to 80% beginning the day after the second dose of LY231514. Folinic acid was administered parenterally for 7 days with total daily doses of 150 mg initially, then tapering to 20 mg/d. The clinical signs resolved within 4 days and the hematologic abnormalities resolved within 6 days of the initiation of folinic acid rescue. At the termination of the study, one dog had a residual healed oral ulcer. The other animals had no gross pathologic evidence of residual tissue damage following folinic acid rescue.[1]

The starting dose of a phase I investigational drug trial is generally one third the toxic-dose-low in the most sensitive large animal species tested, or one tenth the 10% lethal dose (LD_{10}) in mice. LY231514 was only minimally toxic in mice. In dogs, deaths occurred in those that received 26.24 mg/kg (525 mg/m^2) per week, so one third of this, 175 mg/m^2/wk, was not felt to be a safe

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starting dose. An initial dose of 10 mg/m^2/wk was used to enhance safety. The dose escalation format was based on the modified continual reassessment method (MCRM) proposed by Faries.[9] Using this scheme, a single patient is treated at each minimally toxic dose level and more patients are added to a level when significant toxicity is observed. This dose-escalation format reduces the number of patients treated with lower, possibly less effective doses, while increasing the proportion treated at dose levels closer to the maximal-tolerated dose (MTD). The objectives of this study were to determine the qualitative and quantitative toxicities, the MTD, pharmacokinetic profile, and antitumor effect of LY231514 when dosed weekly for 4 weeks. This schedule will be repeated every 42 days to allow for resolution of toxic effects.

PATIENTS AND METHODS

Patient Selection

All patients underwent a complete history, physical examination, chest x-ray, and laboratory evaluation. Eligibility criteria included the following: (1) histologic evidence of solid tumor refractory to conventional therapy and other investigational agents of higher priority; (2) at least 18 years of age; (3) World Health Organization (WHO) performance status $\leq$ 2; (4) life expectancy $\geq$ 12 weeks; (5) off previous anticancer therapy for at least 3 weeks (at least 6 weeks for nitrosoureas or mitomycin); (6) adequate bone marrow function (WBC count $\geq$ 3,000/μL or granulocyte count $\geq$ 1,500/μL, platelet count $\geq$ 100,000/μL, hemoglobin level $\geq$ 9 g/dL), hepatic function (bilirubin level $\leq$ 1.5 mg/dL, AST $\leq$ two times the upper limit of normal, albumin level $\geq$ 2.5 g/dL, normal prothrombin/partial thromboplastin time), renal function (creatinine concentration $\leq$ 1.5 mg/dL or creatinine clearance $\geq$ 60 mL/min), cardiac function (no dysrhythmias requiring therapy and no myocardial infarction in the previous 6 months), and metabolic function (electrolytes within normal limits unless due to cancer, blood glucose $<$ 200 mg/dL); and (7) written, informed consent. Exclusion criteria included the following: (1) clinical evidence of brain metastases, (2) serious preexisting medical conditions that would prevent full compliance with the study, (3) pregnancy, (4) concomitant anticancer therapy, (5) use of aspirin, or (6) presence of pleural or peritoneal effusions. Patients who required chronic aspirin therapy and those with effusions were excluded due to the structural similarities of LY231514 and MTX (Fig 1). MTX may be displaced from albumin and its renal secretion may be impaired by the concurrent use of aspirin, thereby increasing its cytotoxic effect.[10] MTX also is retained in effusions and released slowly into plasma, causing potentially substantial toxicity.

Pharmacokinetics

Plasma samples for pharmacokinetics were planned for all patients during their first treatment course. A reverse-phase high-performance liquid chromatography (HPLC) assay was developed to determine the concentration of LY231514 in plasma. A quantity of 0.5 to 1 mL of plasma was subjected to a preconditioned solid-phase extraction (SPE) cartridge (Bond Elut Certity II, part no. 1210-2080; Varian, Harbor City, CA). The SPE cartridges were preconditioned with 2 mL of HPLC grade methanol, followed by 2 mL of a pH 7.0 phosphate buffer. Immediately following the addition of the sample, the column was washed with 2 mL of the pH 7.0 phosphate buffer, and then with 2 mL of methanol. The absorbed LY231514 was eluted with 2 mL of 40% acetonitrile and 60% buffer solution. The eluate was evaporated to dryness under nitrogen. The residues were reconstituted with 200 μL of distilled, deionized water, and then filtered with 0.1-μm Ultrafree-MC centrifuge filters (Millipore Inc, Bedford, MA). The extraction efficiency of LY231514 from plasma was 60%. The chromatographic procedure consisted of injecting 150 μL of the filtrate onto an octadecyl column (YMCbasic, 25 cm $\times$ 4.6 mm; YMC Inc, Wilmington NC) preceded by a YMCbasic precolumn (23 cm $\times$ 4 mm). The mobile phase consisted of 14% acetonitrile and 86% pH 3.0 phosphate buffer solution, pumped at a flow rate of 0.8 mL/min, and monitored by UV detection at 250 nm. The internal standard used was dideazatetrahydrofolate (Lometrexol; Eli Lilly, Indianapolis, IN), with a retention time of approximately 13 minutes, The retention time for LY231514 was approximately 17 minutes. Two calibration curves were used in the assay of the plasma samples. A low concentration range (10 to 400 ng/mL) was used for the 1-mL plasma sample, and a high concentration range (400 to 20,000 ng/mL) for the 0.5-mL plasma sample. Both concentration curves were linear over their respective ranges, with a correlation coefficient more than 0.96. The lower limit of quantitation of LY231514 was 10 ng/mL.

Drug Administration

LY231514 disodium was supplied as a lyophilized powder in 100-mg vials and reconstituted in 10 mL of normal saline. The appropriate dose was then withdrawn and diluted in normal saline to a total volume of 50 mL. This was administered intravenously over 10 minutes. weekly for 4 weeks, repeated every 42 days. To be eligible to receive subsequent weekly doses, all toxicity must have been $\leq$ grade I at the time of treatment. Toxicity was assessed according to the WHO toxicity criteria. Patients were evaluated by a physician weekly during therapy for signs and symptoms of toxicity. The initial patient treated at each dose level was observed for a minimum of 4 weeks before decisions regarding dose escalation were made. Folinic acid would be considered, based on animal rescue data, for grade IV myelosuppression that persisted for 7 days or for grade III/IV nonhematologic side effects. The planned dosing of folinic acid was 50 mg/m^2 intravenously every 6 hours for 2 days, then 40 mg/m^2 intravenously every 6 hours for 6 additional days. All serious adverse events were reported to the institutional review board and the study sponsor, Eli Lilly and Co, Indianapolis, IN.

Dose Escalation

Dose levels to be studied were 10, 20, 40, 75, 150, 225, 375, . . . to 1,000 mg/m^2/wk. Dose escalation was planned based on the MCRM, with one patient treated at each minimally toxic dose level. Before each new patient was treated, an estimated MTD was calculated based on the toxicity experienced by all previously treated patients. The dose level selected for a new patient was based on the following criteria: at least three patients would be treated at the initial dose level of 10 mg/m^2; the dose level for a new patient could not be more than one level above the level assigned to the previous patient; the dose level could not be greater than the estimated MTD; a minimum of three patients would be treated at a level before dose escalation when moderate reversible toxicity (grade III hematologic or grade II nonhematologic toxicity, excluding nausea, vomiting,

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