ANTIFOLATE DRUGS IN CANCER THERAPY

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12 Preclinical and Clinical Evaluation of the Glycinamide Ribonucleotide Formyltransferase Inhibitors Lometrexol and LY309887

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1. INTRODUCTION

The importance of the purine *de novo* pathway in providing DNA precursors for cancer cell growth led to the hypothesis that novel antifolate inhibitors of glycinamide ribonucleotide formyltransferase (GARFT), the first folate-dependent enzyme in this pathway, might have utility in the treatment of cancer. In 1987, clinical investigations were initiated with lometrexol (6R-dideazatetrahydrofolic acid, 6R-DDATHF), a novel "tight-binding" inhibitor of GARFT with potent antitumor activity in a number of murine and human xenograft solid tumors. Unexpected observations of delayed cumulative toxicity in phase I clinical trials prompted extensive preclinical investigations of the dynamics of folate status on the efficacy and toxicity of GARFT inhibitors and other antifolates *(1)*. In addition, structure-activity studies have led to the identification of a second generation GARFT inhibitor, LY309887 (2′,5′-thienyl-dideazatetrahydrofolic acid), which is more potent than lometrexol and has greater antitumor efficacy in vivo *(2)*. Biochemical and pharmacological differences between LY309887 and lometrexol with respect to potency to inhibit GARFT, differential transport and storage in liver, and polyglutamation suggest that LY309887 may have greater antitumor efficacy and more manageable toxicity in the clinic than lometrexol. A murine model of the delayed cumulative toxicity seen with lometrexol has been refined and characterized to provide greater understanding of the pharmacokinetics and pharmacodynamics of these events. In concert with recently published nutritional data on the folate status of humans and more sophisticated methods of assessing and modulating antifolate toxicities through vitamin supplementation, antifolate therapy may be poised to enter a new phase of clinical success. In this report, we describe LY309887, a GARFT inhibitor with unique biochemical and pharmacological properties that has antitumor activity against a broad panel of human xenograft tumors, and greater potency than lometrexol both as an inhibitor of GARFT and as an inhibitor of tumor growth in vivo. An overview of the phase I clinical results with lometrexol and the design of the phase I clinical trial with LY309887 will be presented.

2. INHIBITION OF GARFT AND POLYGLUTAMATION BY FOLYLPOLYGLUTAMATE SYNTHETASE

The natural forms of folic acid and "classical" inhibitors of folate-dependent enzymes are polyglutamated intracellularly by the enzyme folylpolyglutamate synthetase. Polyglutamylation enhances both intracellular retention and affinity of folates and antifolates for many of the folate-utilizing enzymes *(3)*. Table 1 summarizes the inhibition of GARFT by lometrexol, compound LY254155 (6R,S-2′,5′-thienyl-DDATHF, a diastereoisomeric mix of LY309887 and LY309886, respectively) and their polyglutamates. Monoglutamated LY254155 was approx 30-fold more potent than lometrexol. Polyglutamation enhanced inhibition by both compounds: lometrexol-triglutamate (LY235337) was approx 4.5-fold more potent than parent compound; a 10-fold increase in inhibition was seen with the triglutamated thiophene (LY314209). These data demonstrate that the thiophene was inherently more potent as a GARFT inhibitor and that in the polyglutamated state it achieved picomolar affinity for GARFT.

The kinetic constants (K_m, V_{max}, and first-order rate constant [V_{max}/K_m]) for activa-

Table 1
Inhibition of hGARFT
by Lometrexol, 254155, and Polyglutamates

Compound No.	*Compound Name*	*hGARFT K_i (nM)*
LY249543	lometrexol	59.7 ($n = 2$)
LY235540	diglu	15.4 ($n = 2$)
LY235337	triglu	13.3 ($n = 2$)
LY266978	tetraglu	7.1 ± 2.2 ($n = 4$)
LY235542	pentaglu	5.3 ($n = 2$)
LY254155	thienyl-DDATHF	2.1 ± 0.2 ($n = 5$)
LY314565	diglu	1.2 ($n = 1$)
LY314209	triglu	0.25 ($n = 2$)

The potency of antifolate analogs to inhibit monofunctional human GARFT was assessed spectrophometrically using the Morrison equation, which is appropriate for determining the affinity of "tight-binding" compounds that produce stoichiometric inhibition (*2,4*).

Table 2
Kinetic Constants for Activation of GARFT Inhibitors by FPGS

Compound	*K_m (μM)*	*Vmax (μmoles/h/mg)*	*k′ (V_m/K_m)*
lometrexol	16.4 ± 1.0	977 ± 128	60
LY309887	6.5 ± 1.1	686 ± 116	43
MTA	1.9 ± 0.5	725 ± 95	381

tion of lometrexol and LY309887, determined using hog liver FPGS are summarized in Table 2 (*5*). Lometrexol and LY309887 had similar K_m values as FPGS substrates. However, lometrexol had a significantly higher V_{max}. The relative efficiencies of substrate utilization by an enzyme can be determined by comparing first-order rate constants, k′ (V_{max}/K_m). The data suggest that despite equal K_m values, lometrexol was a better substrate, which would be more extensively polyglutamated in vivo. For comparison, data obtained with the multitargeted antifolate inhibitor, LY231514 (MTA), is shown. With a first-order rate constant of 381, it clearly had the greatest affinity and efficacy as an FPGS substrate. In other experiments, polyglutamated products formed during a 24-h incubation of lometrexol, LY309887 or MTA with FPGS were separated by quantitative HPLC. At low substrate concentrations, i.e., below the K_m (1 μ*M*), polyglutamation of all substrates was more extensive and a higher percentage of the total product was converted to tetra- and pentaglutamated forms than at high substrate concentrations (20 μ*M*) in which over 70% of each antifolate was present as the triglutamate analog. These observations are consistent with the known substrate inhibition of FPGS that occurs in vivo at high intracellular folate concentrations (*6*).

An important inference from these data is that folate-deficient patients may accumulate and retain greater amounts of highly polyglutamated "classical antifolates," particularly in liver, a known folate depot, than patients who are folate-replete. Continuous cycling of stored antifolate through the enterohepatic pathway may explain the phenomenon of delayed and cumulative toxicity in cancer patients on lometrexol and in

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