Antifolate Drugs in Cancer Therapy

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Humana Press
Totowa, New Jersey

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Cover illustration:

Cover design by Patricia F. Cleary.

Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

8 Preclinical Pharmacology Studies and the Clinical Development of a Novel Multitargeted Antifolate, MTA (LY231514)

Chuan Shih and Donald E. Thornton

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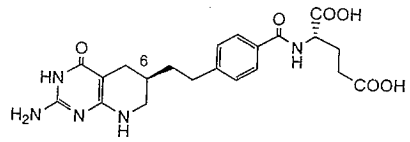
1. INTRODUCTION

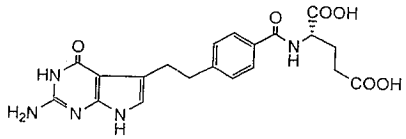
Since the early 1950s, extensive research efforts have been devoted to the discovery and development of antifolate antimetabolites as chemotherapeutic agents for the management of neoplastic diseases. However, it was only in the last 10–15 yr, because of the rapid advances of medicinal chemistry, X-ray protein crystallography, molecular biology, pharmacology, and clinical medicine, that a significant number of new generation antifolates were brought forward for clinical development. Several folate-based antimetabolites are currently being investigated in clinical trials. These include lometrexol (6R-5,10-dideazatetrahydrofolic acid) *(1–3),* LY309887 *(4),* and AG2034 *(5),* which are potent and selective inhibitors of glycinamide ribonucleotide formyltransferase (GARFT), an enzyme in the purine *de novo* biosynthetic pathway; trimetrexate *(6),* edatrexate *(7,8),* and PT523 *(9)* which act on dihydrofolate reductase (DHFR); raltitrexed *(10,11),* AG337 *(12),* BW1843U89 *(13),* and ZD933 *(14)* which specifically target the enzyme thymidylate synthase (TS) involved in pyrimidine biosynthesis.

N-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5 -yl)ethyl]-benzoyl]-L-glutamic acid, LY231514, is a structurally novel antifolate that possesses a unique 6-5 fused pyrrolo[2,3-d]pyrimidine nucleus instead of the more common 6-6 fused pteridine or quinazoline ring structure. LY231514 was discovered through struc-

From: *Anticancer Drug Development Guide: Antifolate Drugs in Cancer Therapy*
Edited by: A.L. Jackman © Humana Press Inc., Totowa, NJ



Lometrexol
(6R-DDATHF)



MTA (LY231514)

Fig. 1. The structures of lometrexol (6R-5,10-dideazatetrahydrofolic acid, DDATHF) and MTA (N-[4-[2-(2-amino-3,4-dihydro-4-oxo-7H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]-benzoyl]-L-glutamic acid).

ture activity relationship (SAR) studies of the novel antipurine antifolate lometrexol series, by eliminating the C5 methylene of lometrexol and converting the sp3 center at C6 to sp2 geometry (Fig. 1) *(15,16)*. These modifications give rise to a very potent cytotoxic agent (IC_{50} = 15 n*M*) against human CCRF-CEM leukemia cells in culture. However, the end-product reversal pattern of this new pyrrolopyrimidine-based antifolate was completely different to the GARFT inhibitor lometrexol. The purine precusor hypoxanthine (100 μ*M*) or aminoimidazole carboxamide (AICA) (300 μ*M*) was incapable of protecting the cells from the cytotoxicity of LY231514. In contrast, thymidine (5 μ*M*) was able to provide partial protection to the cells up to 10X IC_{50} concentrations of LY231514. The replacement of the tetrahydropyridine ring of lometrexol with a pyrrole moiety caused a major loss of activity in the inhibition of purine biosynthesis and shifted the major site of action of LY231514 to the inhibition of pyrimidine biosynthesis (thymidylate cycle). As a "classical" antifolate, LY231514 was found to be one of the best known substrates for mammalian folylpolyglutamate synthetase (FPGS) *(17)* and it is believed that polyglutamation and the polyglutamated metabolites of LY231514 play profound roles in determining both the selectivity and antitumor activity of this novel agent. Recent studies have shown that the polyglutamates of LY231514, (e.g., the triglutamate glu_3 and the pentaglutamate glu_5) potently inhibit several key enzymes of the folate metabolism, including TS, DHFR, GARFT, and aminoimidazole carboxamide ribonucleotide formyltransferase (AICARFT) *(18)*. As a result of this activity against several enzymes, LY231514 has become known as MTA, multitargeted antifolate.

The phase I clinical evaluation of MTA began in late 1992. Objective tumor responses were observed in patients with colorectal cancer and pancreatic cancer, some of whom had failed treatment with other TS inhibitors such as 5FU and raltitrexed *(19–21)*. Phase II studies have shown activity in a range of solid tumors, including colorectal, breast and nonsmall-cell lung cancers *(22–27)*. The purpose of this chapter is to comprehensively review the unique biochemical and pharmacological modes of action, and the recent phase I and II clinical findings of this novel multitargeted antifolate, MTA.

Table 1
Inhibitory Activity of MTA, Methotrexate and Their Polyglutamates Against rhTS, rhDHFR, rmGARFT, and rhAICARFT (Ki [mean ± SE, n*M*])

Compound	*rhTS*	*rhDHFR*	*rmGARFT*	*rhAICARFT*
MTA	109 ± 9	7.0 ± 1.9	9300 ± 690	3580
MTA-glu_3	1.6 ± 0.1	7.1 ± 1.6	380 ± 92	480
MTA-glu_5	1.3 ± 0.3	7.2 ± 0.4	65 ± 16	265
MTX	13,000	0.004	80,000	143,000
MTX-glu_5	47	0.004	2500	56

2. PRECLINICAL PHARMACOLOGY STUDIES OF MTA

2.1. Folate Enzyme Inhibition Studies

The inhibition of recombinant human (rh)TS, rhDHFR, recombinant mouse (rm)GARFT, and rhAICARFT by MTA and its polyglutamates (glu_3 and glu_5) *(18)* is summarized in Table 1. The parent monoglutamate MTA inhibited rhTS with a K_i of 109 ± 9 n*M*. It has been well documented that mammalian TS shows a strong preference for polyglutamated folate substrates. The longer chain γ-glutamyl derivatives of MTA had significantly enhanced affinity toward rhTS. The addition of two extra γ-glutamyl residues (glu_3) to MTA resulted in 68-fold reduction of the K_i value (K_i = 1.6 n*M*). Further extension of the glutamate tail (MTA-glu_5) only slightly increased the affinity toward rhTS (K_i = 1.3 n*M*). MTA was also found to be a very potent inhibitor of human DHFR (K_i = 7.0 n*M*). In contrast to rhTS, attachment of additional γ-glutamyl residues to MTA had little effect on the inhibition of DHFR; MTA-glu_3 and MTA-glu_5 exhibited identical K_i valués against rhDHFR, 7.1 n*M*. Tight-binding analysis showed that MTA-glu_n inhibited both TS and DHFR competitively. When MTA was tested against the enzymes along the purine *de novo* biosynthetic pathway, it only demonstrated moderate inhibition toward rmGARFT (K_i = 9.3 μ*M*). The triglutamate and pentaglutamate of MTA had significantly enhanced inhibitory activity against GARFT, with K_i values of 380 n*M* (24-fold) and 65 n*M* (144-fold), respectively. The pentaglutamate of MTA also inhibited human AICARFT with a K_i of 265 n*M*. Kinetic analysis confirmed the competitive inhibition pattern of MTA polyglutamates against both GARFT and AICARFT. Finally, MTA and its polyglutamates were competitive inhibitors of both the dehydrogenase and synthetase domains of C1 tetrahydrofolate synthase. The K_i values for the mono-, tri- and pentaglutamyl derivatives of MTA were 9.9, 3.9, and 4.7 μM, respectively, for dehydrogenase and 329,25,4 and 1.6 μM for synthetase. MTA was a relatively less potent inhibitor of C1 tetrahydrofolate synthase than other enzyme targets such as TS, DHFR, and GARFT. However, cell-culture experiments have suggested that the intracellular drug concentration of MTA can reach levels of 50 μ*M* (RM Schultz, unpublished observation), and at these concentrations the activity of C1 tetrahydrofolate synthase can also be greatly suppressed by MTA polyglutamates. The important role of TS in serving as a rate-limiting enzyme in folate metabolism, as well as the relative order of inhibitory potency toward TS by MTA-glu_n indicate that TS is a major site of action for MTA. Inhibition of DHFR and other enzymes in the *de novo* purine biosynthetic pathway may also contribute significantly to the overall antiproliferative effect of MTA

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