# An Overview of Folate Metabolism: Features Relevant to the Action and Toxicities of Antifolate Anticancer Agents

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SINCE the observation of reduced folate levels in children with leukemia made by Farber et al[1] in the 1940s, the study of folic acid metabolism and the action of antifolate drugs has been intimately linked to the development of cancer therapeutics. Folic acid plays a role in a wide range of metabolic pathways in various species. In humans it is an essential vitamin and functions primarily in the processes involved in cellular proliferation and amino acid metabolism. This review will focus mainly on those aspects of mammalian folate metabolism relevant to cell proliferation since these are the most germane to the use of antifolates in cancer therapy. The textbook by R.L. Blakley[2] is a comprehensive work covering all aspects of folate metabolism.

## ASPECTS OF FOLATE METABOLISM

### *Folate Pathways Associated With Cell Proliferation*

Folic acid functions mainly in its fully reduced form, 5,6,7,8-tetrahydrofolate ($FH_4$; Fig 1). FH4 serves as a carrier for one-carbon moieties within the cell. These are obtained from a variety of sources that include serine. In this reaction, serine hydroxymethyl transferase forms 5,10-methylene tetrahydrofolate ($CH_2FH_4$) while converting serine to glycine (Fig 2). $CH_2FH_4$ may be converted within the cell to one-carbon carrying folate derivatives of various oxidation states. One of these, 10-formyl tetrahydrofolate, is the substrate for two enzymes involved in the de novo synthesis of purines. These are glycinamide ribonucleotide formyl transferase (GARFT) and aminoimidazole carboxamide ribonucleotide formyl transferase (AICARFT). Thus, two of the carbon atoms in the purine skeleton are derived from folate. The folate-dependent reactions of purine synthesis use the carbon atom from the 10-formyl group and release unsubstituted tetrahydrofolate as the folate product. Thus, the folate molecule can then acquire another carbon atom from serine and continue to cycle through GARFT and AICARFT, allowing continued purine synthesis without any overall consumption of folate. $CH_2FH_4$ is also the substrate for the enzyme thymidylate synthase (TS). Thymidylate synthase converts deoxyuridine monophosphate into thymidine monophosphate and is a key enzyme involved in cell proliferation because it is the rate-limiting step in the de novo synthesis of thymidylate, which is required exclusively for DNA synthesis. The folate product of TS is not tetrahydrofolate, but the oxidized form, dihydrofolate ($FH_2$). This product cannot continue to function in folate metabolism until it is converted back to $FH_4$ by the enzyme dihydrofolate reductase (DHFR).

### *The Role of Folate and Antifolate Polyglutamates*

Folic acid possesses a glutamate residue shown at the right-hand side of the folate structures in Fig 1. Naturally occurring folates within the cell are converted to polyglutamate forms by the addition of glutamate residues via a γ-peptide linkage. Antifolates that possess a glutamate residue (known as classical antifolates) are also frequently converted into their corresponding polyglutamate forms. The process of polyglutamation is accomplished by the enzyme folylpoly-γ-glutamate synthetase. This reaction is illustrated in Fig 3 using the antifolate LY231514 (MTA) as an example. The process is analogous for natural folates and many other classical antifolates. In Fig 3, the carboxylate groups of the glutamic acid residue are shown in their ionized form, carrying a negative charge, showing that polyglutamation increases the overall negative charge on the folate molecule by one unit for each additional glutamate. The negatively charged polyglutamates cannot cross the cell membrane and are therefore retained and concentrated within the cell. This is probably the major physiologic role of polyglutamation. Cells that are deficient in folylpoly-γ-glutamate synthetase are auxotrophic for the end products of

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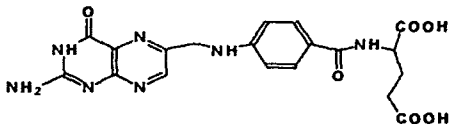
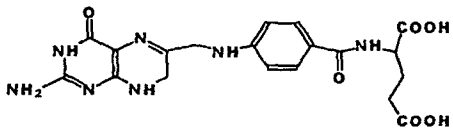
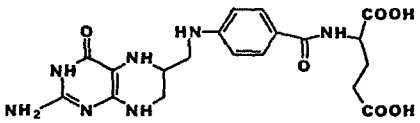
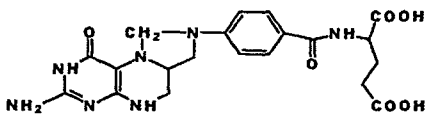
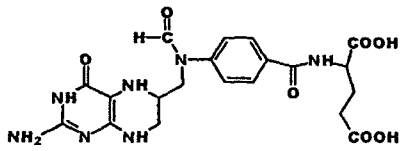


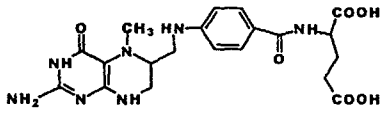




**Fig 1. Forms of folic acid.**

folate metabolism (thymidine, hypoxanthine, and glycine). In addition to being retained within the cells, the polyglutamate forms of natural folates also may be better substrates for the various folate metabolizing enzymes.

The formation of polyglutamates of those antifolates that are substrates for folylpoly-γ-glutamate synthetase also has profound effects on their activity. The polyglutamates may be retained within the cell for very long periods,[3] thus increasing the potency of the cytotoxic action of these compounds. In addition, the addition of glutamate residues frequently renders the compounds much more potent inhibitors of their target enzyme. For example, raltitrexed pentaglutamate is roughly 100-fold more potent as a TS inhibitor than the parent molecule.[4] The effects of polyglutamation on the potency of molecules such as these is so profound that they may be considered as prodrugs for their polyglutamate forms. Indeed, cellular resistance to antifolates can be caused by a reduction in the ability of the cell to form the polyglutamate derivatives.[5] A more complete and in-depth review of polyglutamation and its relevance to cancer therapy is given by Richard G. Moran elsewhere in this supplement.

*Cell Membrane Transport of Folates and Antifolates*

Folates do not cross the cell membrane to an appreciable extent by passive diffusion but require

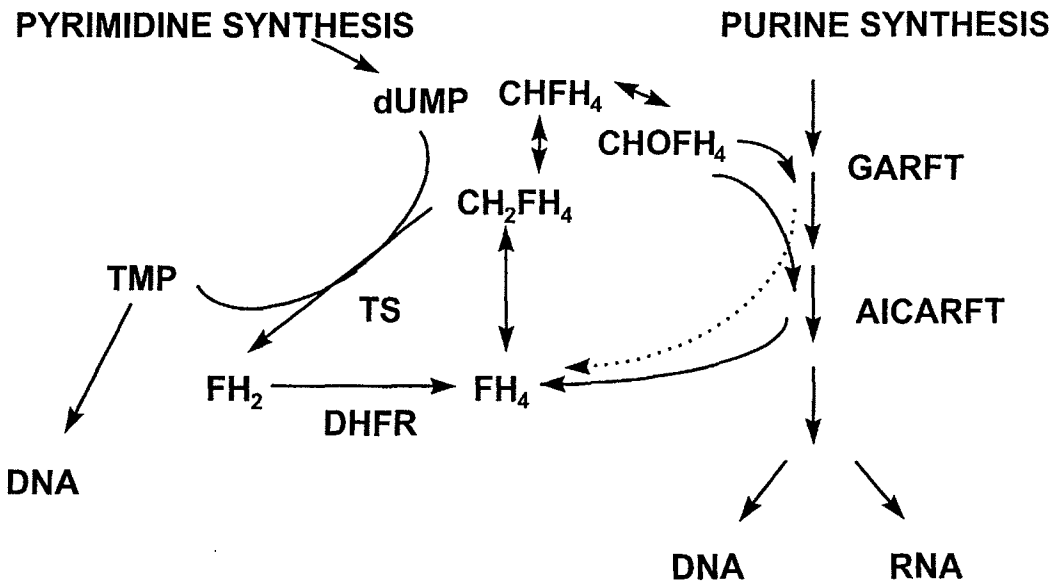



Fig 2. Metabolic pathways of folate metabolism.

specific transport mechanisms. There are several mechanisms that have been characterized; these are reviewed in depth by Sierra and Goldman in this supplement. Of these, the most extensively characterized mechanism involves the reduced folate carrier (RFC1). This is an anion exchange concentrative process and is known to be capable of transporting methotrexate and a number of other antifolates as well as tetrahydrofolate itself. Changes in this carrier that alter its relative affinity for antifolates have been shown to be a cause of drug resistance.[6] The reduced folate carrier has a relatively low affinity for natural folates (1 to 5 μmol/L) compared with their physiologic extracellular concentrations (typically in the nanomolar region). A second mechanism of folate transport, the folate receptor, has a much higher affinity for folates which, after binding, are internalized

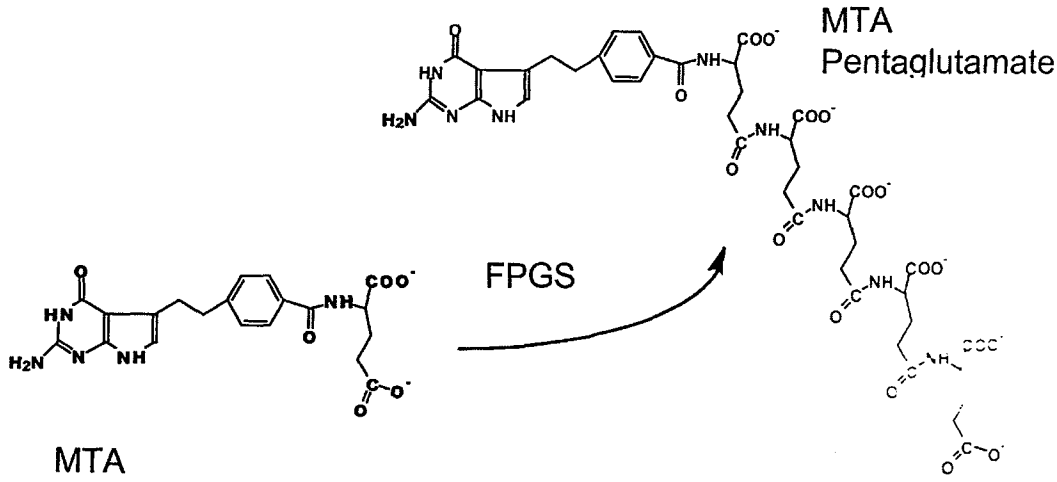
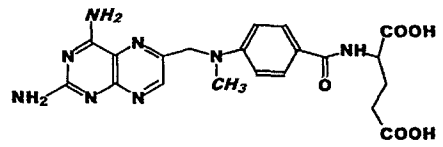



Fig 3. Formation of polyglutamates.

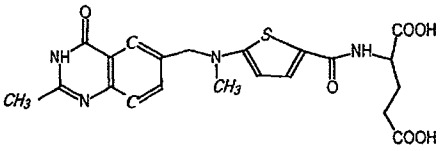
within a membrane vesicle and subsequently released into the cytoplasm. Three genes for folate receptors have been cloned (see Sierra and Goldman). Folate receptors may be responsible for the transport of some antifolates, for example, lometrexol. In addition to these two mechanisms, the level of folates and antifolates within the cell may be affected by an energy-dependent efflux pump and by a low pH transporter.

*Actions of Various Antifolates*

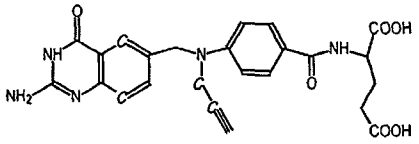
Having been introduced nearly 50 years ago, methotrexate (Fig 4) is the antifolate with the longest history. It acts mainly by inhibition of DHFR. The result of this inhibition is that intracellular folate accumulates in the form of dihydrofolate. There is a consequent inhibition of the de novo synthesis both of purines and thymidine. This may be due in part to a diminution in the intracellular pools of tetrahydrofolates, but additionally, methotrexate polyglutamates and the accumulated dihydrofolate polyglutamates are capable of inhibiting both TS and AICARFT directly.[7-9] Characteristically, the intracellular pools of dihydrofolate and deoxyuridine will increase following expo-



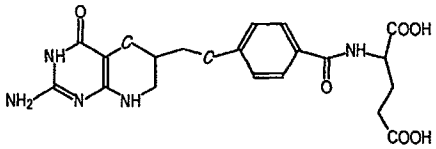
Methotrexate



Raltitrexed (Tomudex™, ZD 1694)

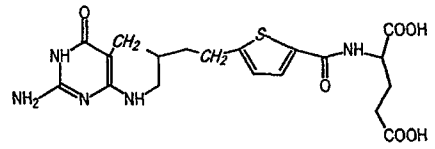


CB 3717, PDDF



Lometrexol

DDATHF



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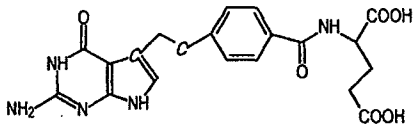

LY 231514

MTA

**Fig 4. Structures of various antifolates.**

sure to methotrexate[10]; these effects are illustrated in Fig 5.

It has been argued that the effects of methotrexate on reduced folate pools, and consequently, the indirect inhibition of purine de novo synthesis and amino acid interconversions, may be detrimental to its main antiproliferative action, namely, the inhibition of the synthesis of thymidylate that is required exclusively for DNA synthesis.[11] For this reason, many researchers have developed antifolates designed to inhibit TS directly while not affecting other folate enzymes. The first of these to be used clinically was CB 3717,[12] but this has been superseded by raltitrexed (Tomudex, ZD 1694; Zeneca Pharmaceuticals, Cheshire, England), which is licensed for the treatment of colon cancer in some countries. These specific TS inhibitors produce the elevation of the deoxyuridine pool in a manner similar to that observed following methotrexate but, importantly, dihydrofolate pools do not increase and purine synthesis is unaffected (Fig 6).

Both direct TS inhibitors (such as raltitrexed and CB 3717) and drugs that inhibit TS indirectly (such as methotrexate) lead to a marked increase in the intracellular pool of deoxyuridine monophosphate. The reduction in thymidine nucleotides caused by these drugs leads to activation of the pyrimidine synthetic pathways producing deoxyuridine and, thus, to a disproportionate increase in the concentration of deoxyuridine. It has been shown that this increase in the intracellular pool of deoxyuridine monophosphate is mirrored by a corresponding increase in the extracellular pool of deoxyuridine,[13] presumably due to intracellular phosphatases allowing the release of deoxyuridine from the cells (Fig 7). This provides a useful surrogate for in vivo TS inhibition. The plasma deoxyuridine levels can be monitored and an elevation compared with baseline indicates the inhibition, in vivo, of TS.[14]

In addition, selective inhibitors of GARFT, the first folate-dependent enzyme involved in the pathway of de novo purine synthesis, have been developed. Examples of these are lometrexol and LY309887 (Fig 4). These compounds have good antitumor activity in preclinical systems with the suggestion that their activity may be preserved in tumor cells that have a nonfunctional p53 pathway. The clinical toxicity of many antifolates is, not surprisingly, affected by the pretreatment folate status of the patient. In the case of the GARFT inhibitors, the effect of the folate status is particularly marked, with the maximum tolerated dose being at least 10-fold higher in patients who have received folate supplementation compared with those who have not.[15]

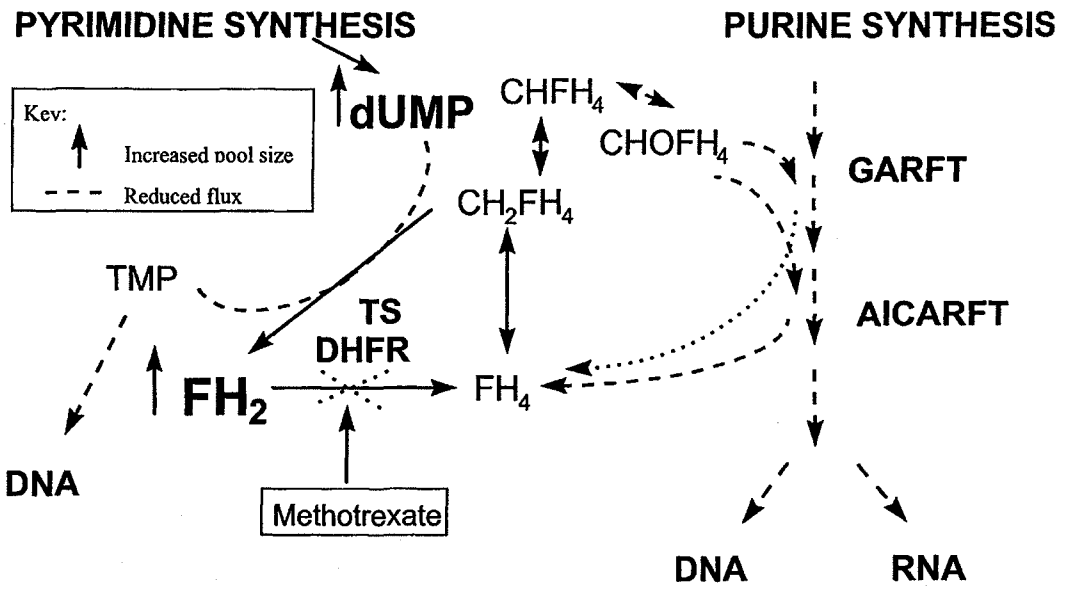


**Fig 5. Effects of DHFR inhibition.**

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