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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Sponsored by Eli Lilly and Company.

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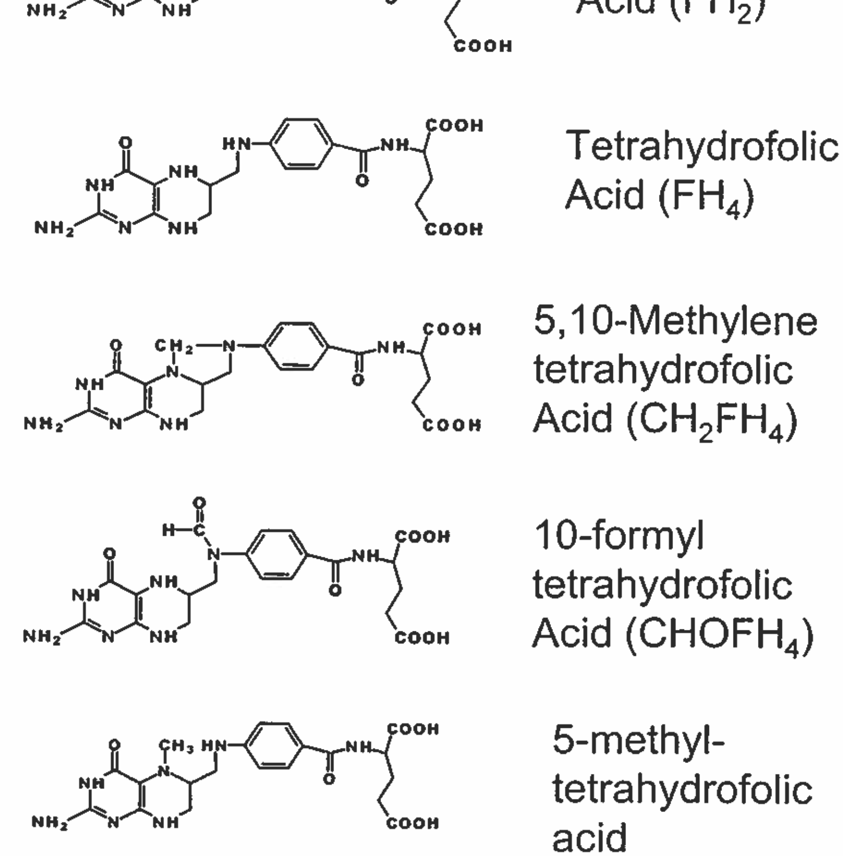


Fig 1. Forms of

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 inhibit parent molecule.[4] The effects of polyg on the potency of molecules such as profound that they may be considered for their polyglutamate forms. Indeed, sistance to antifolates can be caused by in the ability of the cell to form the po derivatives.[5] A more complete and i view of polyglutamation and its releva cer therapy is given by Richard G. M where in this supplement.

Cell Membrane Transport of Folates and

Folates do not cross the cell memb appreciable extent by passive diffusion

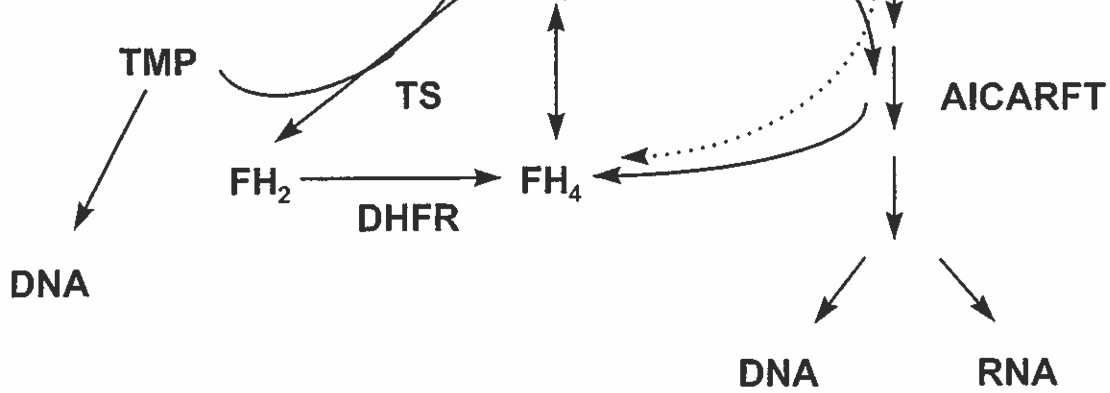


Fig 2. Metabolic pathways of folate metabolism.

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

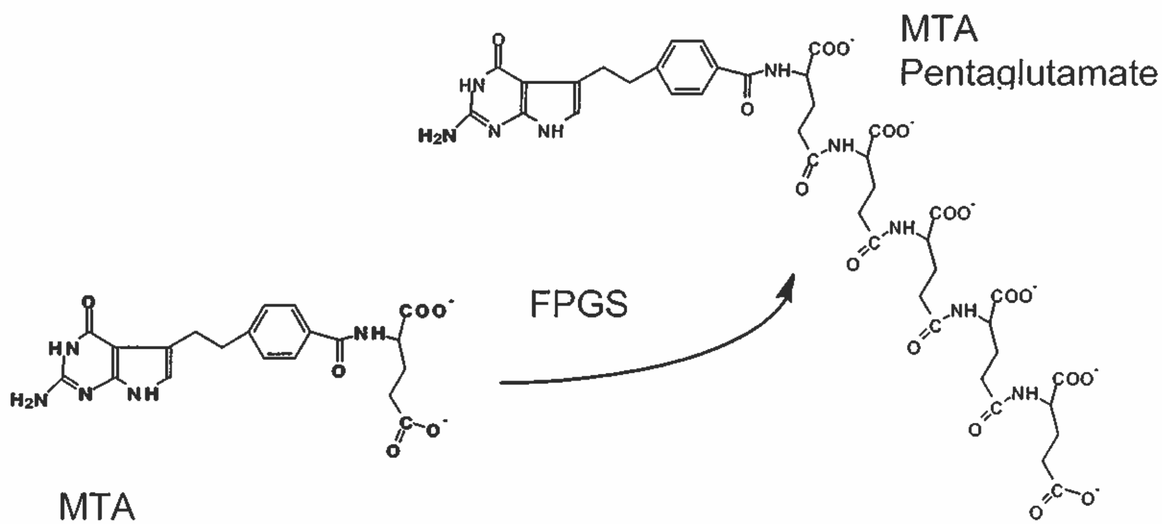


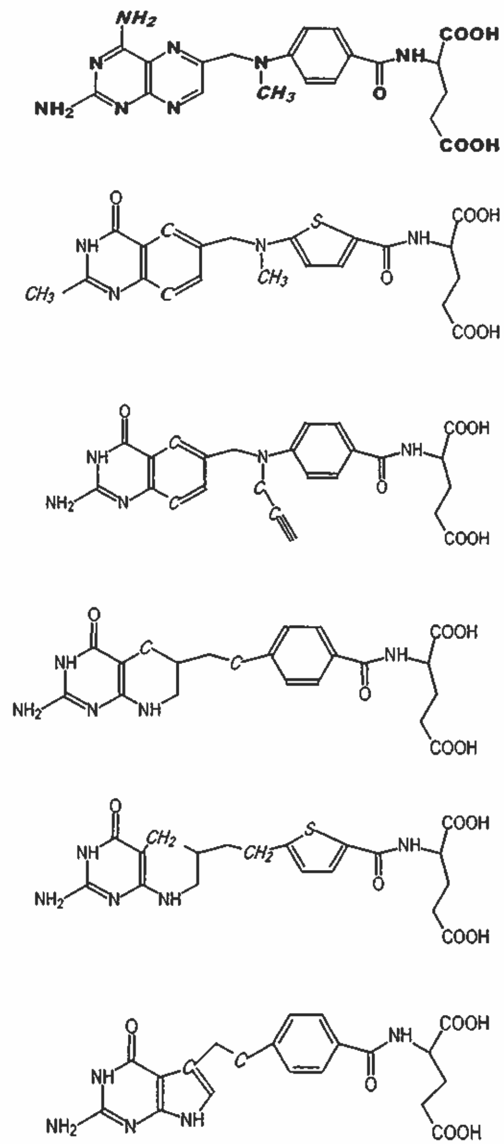
Fig 3. Formation of polyglutamates.

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

tamates and the accumulate polyglutamates are capable of TS and AICARFT directly.[7-] cally, the intracellular pools and deoxyuridine will increase



Methotrexate

Raltitrexed (Tomudex™, ZD 1694)

CB 3717, PDDF

Lometrexol

DDATHF

LY 309887

LY 231514

MTA

Fig 4. S antifolates.

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 de-

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]

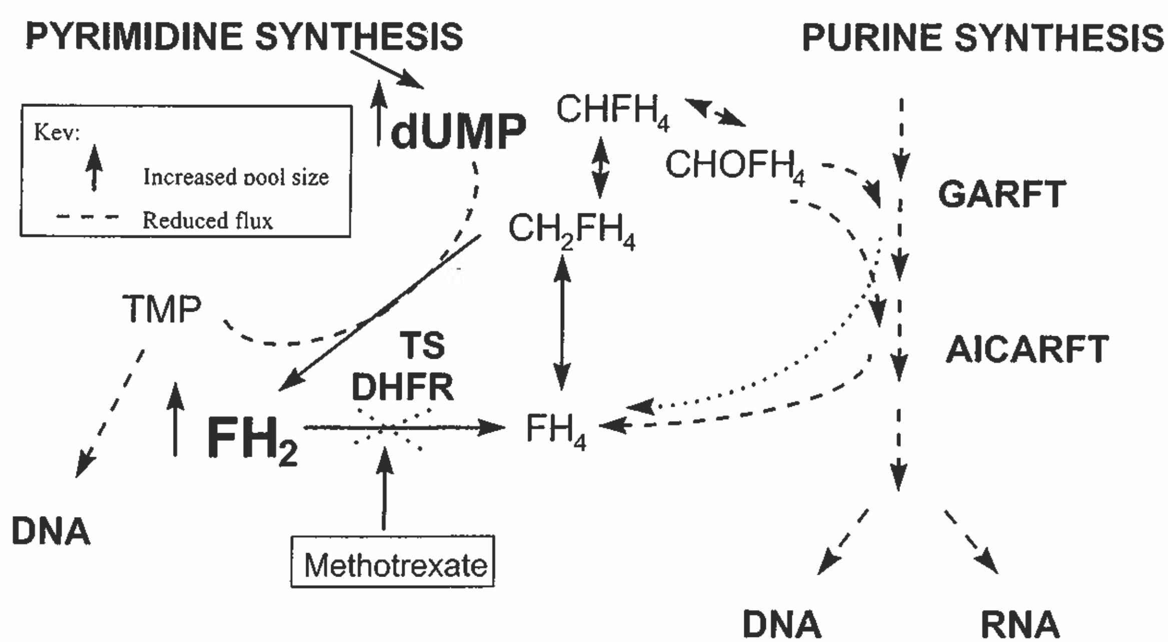


Fig 5. Effects of DHFR inhibition.

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