Synergistic cell growth inhibition by combination of antifolates

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Due to selective antitumor activity of folate analogues their use has been of a major importance in experimental and clinical therapeutics for the past four decades [1 - 5]. The initial by developed compounds, among them aminopterin and methotrexate (MTX)\(^1\), were folate analogues having a 4-amino group in place of 4-hydroxy and both were the common feature of being tight inhibitors of dihydrofolate reductase (EC 1.5.1.3) with \(K_i\) about \(10^{-9}\) M (Fig. 1,2). These compounds possess a glutamate moiety in their structure and can be converted intracellularly to \(\gamma\)-polyglutamate forms [4, 6], which besides dihydrofolate reductase inhibit also thymidylate synthase (EC 2.1.1.45), glycaminamide ribonucleotide and aminomimidazolecarboxamide ribonucleotide formyltransferases (EC 2.1.1.21 and EC 2.1.1.22) [7 - 9]. To avoid polyglutamylation, a number of structural changes of folate analogues have been made resulting in new classes of compounds, e.g. metoprine and trimetrexate (TMTX) (Fig. 2). Their biochemical and therapeutic properties are in process of evaluation [10, 11].

Two other structural modifications: in the pteridine ring and in the substituent of the 10 nitrogen, yielded derivatives which directly inhibited enzymes catalyzing one carbon transfer rather than reduction of dihydrofolate. The first of these, 10-propargyl-5,8-dideazafolic acid (PDDF) (Fig. 2), has the 5 and 8 nitrogen replaced in the pteridine ring by carbon and a propargyl group bound to nitrogen at the 10 position. The resulting compound is a potent inhibitor of thymidylate synthase (\(K_i\) about \(10^{-6}\) M [12, 13]) and its polyglutamate derivatives are approximately two orders of magnitude more effective than the monoglutamate [14, 15]. Recently, a more modified form of this inhibitor has been generated, which is 100 times more effective against tumor cells than PDDF [16].

The third structural modification in pteridine ring has resulted in a unique folate derivative that inhibits glycaminamide ribonucleotide formyltransferase catalysing the folate-dependent step in purine biosynthesis [17 - 19] (cf. Fig. 1). This derivative, 5,10-dideazatetrahydrofolate (DDATHF), has the 5 and 10 nitrogens replaced by carbon and a fully reduced pyrazine ring (Fig. 2).

Effects of combinations of antifolates

The first effective combinations of inhibitors of two enzymes in the folate pathway were directed at thymidylate synthase and dihydrofolate reductase as targets. Recently, an additional target of these drug combinations – the glycaminamide ribonucleotide formyltransferase – has been identified (Fig. 2).

The initial observations of combined drug synergism exhibited by the dihydrofolate reductase...
Fig. 1. Inhibition by antifolates of metabolic cycles involved in purine and pyrimidine synthesis.

Enzymes: 1, serine hydroxymethyltransferase; 2, thymidylate synthase; 3, dihydrofolate reductase; 4, multifunctional protein possessing the activities of 5,10-methylenetetrahydrofolate dehydrogenase, 5,10-methylene tetrahydrofolate cyclohydrolase and 10-formyltetrahydrofolate synthetase; 5, glycaminamide ribonucleotide formyltransferase; 6, aminolimidazolecarboxamide ribonucleotide formyltransferases.

Possible mechanism of action of antifolate combinations

The studies of the mechanism of action of antifolate combinations on cell growth inhibition are in their early stage. The dihydrofolate reductase inhibitors were used at non-cytotoxic concentrations which have little (< 25%) inhibitory effect on purine and pyrimidine biosynthesis [24, 25], but inhibited significantly dihydrofolate reductase (but not thymidylate synthase). Dihydrofolate reductase inhibitors led to a depletion of the intracellular pool of reduced folates, accompanied by an accumulation of dihydrofolate [24, 26]. Analysis of the folate pool in the TMTX-treated cells shows a 75% reduction of total folates, among them 5,10-CH$_2$H$_4$PteGlu$_n$ – substrate of thymidylate synthase [22, 24]. In addition, the depletion of endogenous reduced folates facilitates glutamylation of folate analogues with a glutamyl residue (Fig. 2), which then are accumulated in the cell. In TMTX-treated cells the accumulation of PDDF polyglutamates was nearly doubled [22, 25], resulting in a 6 - 8-fold increase in the growth inhibition of the cells treated with PDDF in the presence of TMTX.
Fig. 2. Structures of antifolates.
1. Methotrexate (MTX); 2. Trimetrexate (TMTX); 3. 10-propargyl-5,8-dideoxafoolic acid (PDDF); 4. 5,10-dideoaza-5-hydrofoolic acid (DDATHF). a. Pteridine ring; b. p-aminoazacycl ring; c. glutamic acid; n. number of γ-linked glutamate residues.

[26]. This increase is quantitatively nearly equivalent to the extent of enhancement of PDDF activity as evaluated by growth inhibition or cytotoxicity [27]. In addition, the cellular level of dUMP (second substrate for thymidylate synthase) was markedly elevated (up to 30 fold) in PDDF-treated cells in the presence of dihydrofolate reductase inhibitors. This elevation and formation of more effective inhibitors (PDDF-polyglutamates) indicate that these conditions favor the interaction of PDDF polyglutamate with thymidylate synthase and result in formation of a stable PDDF polyglutamate-thymidylate synthase-dUMP complex.
This complex may lead to a more effective blockage of thymidylate synthase by PDDF in TMTX-treated cells [25].

The data accumulated so far do not allow to draw a firm conclusion that intracellular substrate depletion facilitates inhibition of glycynamide ribonucleotide formyl transferase by DDATHF. However, in some cellular systems, dihydrofolate reductase inhibitors can cause reduction in the intracellular level of 10-HCO-H4PteGlu11 (substrate for glycynamide ribonucleotide formyl transferase) [9, 28]. There are no kinetic data on the interaction between DDATHF and 10-HCO-H4PteGlu11 with regard to glycynamide ribonucleotide formyl transferase. A strong synergism between the dihydrofolate reductase and glycynamide ribonucleotide formyl transferase inhibitors is probably due to the incorporation of two single carbon units per purine base (C-2 and C-8). In the situation where the intracellular level of reduced folates is limited by dihydrofolate reductase inhibitors [29], partial inhibition of purine biosynthesis by DDATHF would cause an increase in potency of growth inhibition [30, 31].

Thus far limit information is available concerning the possible response in vivo (in mice bearing L1210 tumor) to the combinations of TMTX and PDDF or TMTX and DDATHF [23, 32]. Since the cells in cultures are for the most part grown on folic acid, and the circulating reduced folate in mammals is 5-CH3H4PteGlu, it should be kept in mind that the use of combinations of dihydrofolate reductase inhibitors with PDDF or DDATHF could raise the sensitivity of the host to these agents, thus nullifying the therapeutic benefits. Further investigations are required for better understanding of the mode of action of antifolate combinations and of their therapeutic potential.

REFERENCES


