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Minireview

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 γ-polyglutamate forms [4, 6], which besides dihydrofolate reductase inhibit also thymidylate synthase (EC 2.1.1.45), glycinamide ribonucleotide and aminoimidazolecarboxyamide 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. metoprin 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 nitrogens 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 glycinamide 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 glycinamide ribonucleotide formyltransferase – has been identified (Fig. 2).

The initial observations of combined drug synergism exhibited by the dihydrofolate reduc-

[1] Abbreviations used: 5,10-$CH_2H_4PteGlu_n$, 5,10-methylenetetrahydropteroylpolyglutamate; 10-HCO-$H_4PteGlu_n$, 10-formyltetrahydropteroylpolyglutamate; 5-$CH_3H_4PteGlu$, 5-methyltetrahydropteroylglutamate; DDATHF, 5,10-dideazatetrahydrofolate; MTX, methotrexate; PDDF, 10-propargyl-5,8-dideazafolic acid; TMTX, trimetrexate; dUMP, deoxyuridine monophosphate

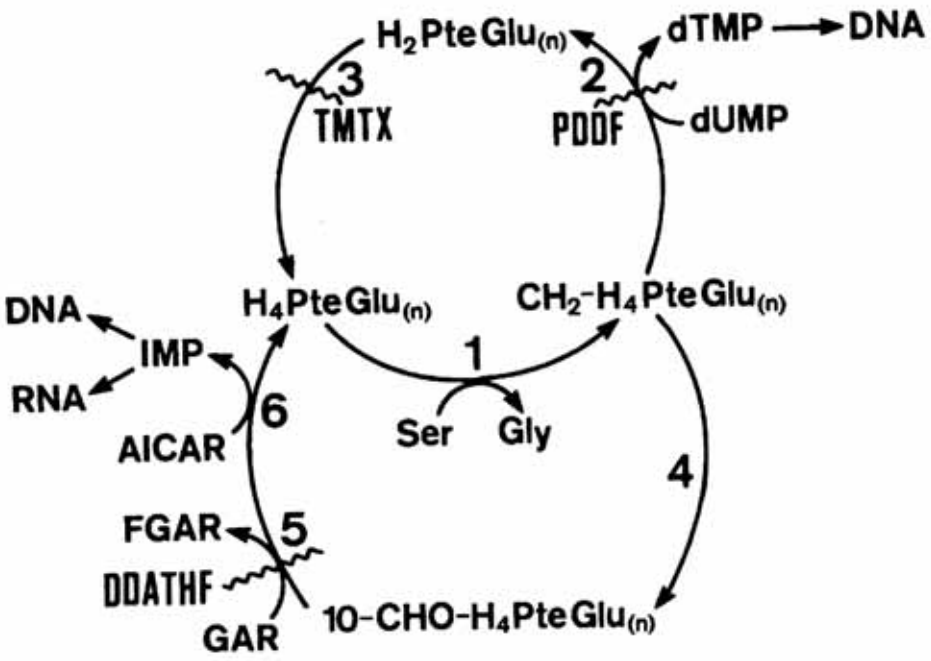

Fig. 1. Inhibition by antifolates of metabolic cycles involved in purine and pyrimidine synthesis.
Enzymes: 1, serine hydroxymethyltransferase; 2, thymidylate synthase; 3, dihydrofolate reductase; 4, multifunctional protein possessing the activities of 5,10-methylenetetrahydrofolate dehydrogenase, 5,10-methenyltetrahydrofolate cyclohydrolase and 10-formyltetrahydrofolate synthetase; 5, glycinamide ribonucleotide formyltransferase; 6, aminoimidazolecarboxamide ribonucleotide formyltransferases.

tase (TMTX) and thymidylate synthase (PDDF) inhibitors come from experiments with *Lactobacillus casei* [20]. Recent studies with the same bacteria were extended by including the glycinamide ribonucleotide formyltransferase inhibitor (DDATHF). When DDTHF was used in combination with TMTX their overall effect on enzyme activity was greater than the action of each agent alone, thus suggesting synergy [21].

These studies on growth inhibition prompted the assessment of the effect of drug interaction on mammalian cells such as rat hepatoma or human lymphoma. In general, for the drug combination both TMTX and PDDF were used at concentrations that caused no or only modest growth inhibition. Under these conditions with either TMTX or PDDF, the growth inhibition (I_{50}) was decreased, about 10-fold as compared to that exerted by PDDF alone (from 5.6 M to 0.46 M) [22, 23]. Similar effects were obtained with DDATHF. Growth inhibition data were analysed by median effect analysis and the results were consistent with drug synergy [22, 24].

The results of these studies suggested that the dihydrofolate reductase inhibitors raised sensitivity of the cells to PDDF and DDATHF action.

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-$CH_2H_4PteGlu_n$ – 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

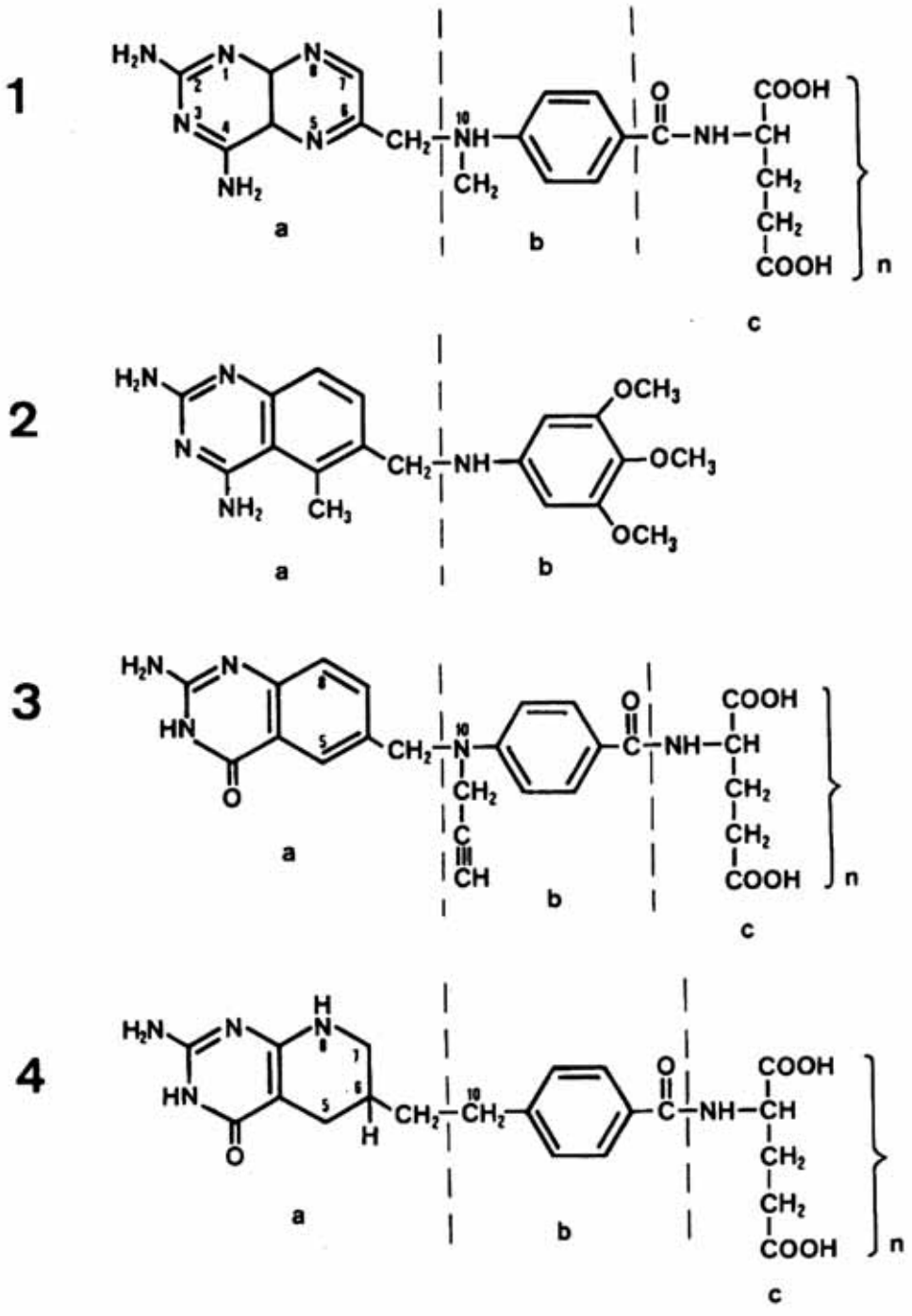

Fig. 2. Structures of antifolates.
1, Methotrexate (MTX); 2, Trimetrexate (TMTX); 3, 10-propargyl-5,8-dideazafolic acid (PDDF): 4, 5,10-dideazatetrahydrofolic acid (DDATHF). a, Pteridine ring; b, *p*-aminobezoyl ring; c, glutamic acid; n, number of γ-linked glutamate residues.

[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 polyglutamates-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 glycinamide ribonucleotide formyltransferase by DDATHF. However, in some cellular systems, dihydrofolate reductase inhibitors can cause reduction in the intracellular level of 10-HCO-$H_4PteGlu_n$ (substrate for glycinamide ribonucleotide formyltransferase) [9, 28]. There are no kinetic data on the interaction between DDATHF and 10-HCO-$H_4PteGlu_n$ with regard to glycinamide ribonucleotide formyltransferase. A strong synergism between the dihydrofolate reductase and glycinamide ribonucleotide formyltransferase 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-$CH_3H_4PteGlu$, 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 nullifing the therapeutic benefits. Futher investigations are required for better understanding of the mode of action of antifolate combinations and of their terapeutic potential.

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