Synergistic effect of 5-fluorodeoxyuridine and quinazoline antifolates on murine leukemia self-cultured in vitro

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Received: 28 August, 1997; accepted: 20 October, 1997

Key words: thymidylate synthase, antifolates, growth inhibition

The effect of thymidylate synthase inhibitors, fluorodeoxyuridine (FdUrd) and its two sulphonamide derivatives was examined in the culture of murine leukemia cells — 5178Y (parental subline) and its fluorodeoxyuridine resistant subline 5178Y/F. A synergistic effect of the antimitobolites on cell survival was observed on exposure of the culture of either line to a slightly inhibitory concentration of FdUrd (1 nM) in combination with 2-desamino-2-methyl-10-propargyl-5,8-dideaza-pteroylsulphoglutamate or 2-desamino-2-methyl-10-propargyl-5,8-dideaza-pteroylsulphoglucose. This effect was accompanied by a marked reduction, in both cell lines of intracellular concentration of 5,10-methyltetrahydropteroyl-polyglutamate, although its concentration in the resistant subline was 3 times as high as in the parental line. The inhibitory effect of combined drugs on the cellular pool of folates in 5178Y line depended also on the sequence of drug addition, whereas in the FdUrd resistant line this sequence was without any effect. The results obtained strongly suggest that under certain conditions inhibition of thymidylate synthesis by antifolates is intensified by a prior use of FdUrd.

Fluoropyrimidine antimitobolites, particularly fluorouracil and FdUrd, are cytotoxic in diverse biological systems and have been extensively used in clinical treatment of carcinomas of ovary, breast and gastrointestinal tracts [1]. Primarily the action of these drugs depends on their conversion to FdUMP, which binds tightly to thymidylate synthase (EC 2.1.1.45) in the presence of methylenetetrahydrofolate (CH2H4PteGlu)n a substrate of the enzyme [2, 3]. Since thymidylate synthase (TS) is the only enzyme providing de novo thymidylate, this enzyme is crucial for DNA biosynthesis and is a target for cancer therapy.

Unfortunately FdUMP is only one of several products derived from FdUrd. The other products can also be incorporated into DNA,

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*This work was supported by the State Committee for Scientific Research, a grant No. 6P20301807.
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Abbreviations: TS, thymidylate synthase; 5,10CH2H4PteGlu, 5,10-methyltetrahydropteroyl-polyglutamate; FdUrd, fluorodeoxyuridine; FdUMP, fluorouridinemonomonucleotide; DMPDDS(Glu or Gly), 2-desamino-2-methyl-10-propargyl-5,8-dideaza-pteroyl-sulphoglucose or γ-glutamate; PDDF, 10-propargyl-5,8-dideazafolic acid; pABAGlu, p-aminobenzoylglutamate; MTX, methotrexate; TMTX, trimetrexate; H2PteGlu, dihydro-pteroyl-polyglutamate; H4PteGlu, tetrahydro-pteroyl-polyglutamate.
RNA and sugars [4–6]. Multiplicity of FdUrd metabolites inspired studies directed toward
development of folate analogs of TS inhibitors. 10-N-propargyl-5,8-dideazaflolate
developed by Jones and coworkers in 1981 [7] appeared to be an extremely potent inhibitor
of TS. Since that time a whole family of dideazafolates inhibiting TS has been syn-
thetized in different laboratories including ours [8, 9].

It seemed also of interest to examine the effect of two TS inhibitors on cell survival.
Studies on the effect of continuous exposure of murine leukemia cells to either FdUrd or
the newly synthesized antifolates — sulphonamidase derivatives of 10-propargyl-5,8-die-
dazaflolates, a poly-γ-glutamable (DMPD-

DSFGlu), and a nonpolyglutamable (DMPD-

DSFGly) (Fig. 1) could provide some infor-
mation on the mechanism of TS inhibition via
interference with intracellular pools of fol-

lates and DNA synthesis.

MATERIALS AND METHODS

Materials

Fisher’s medium, Dulbecco medium, bovine
fetal serum, bovine new born serum and
trypsin were purchased from Grand Island
Biochemical Company, Life Technologies
Ltd., Paisley, Scotland.

10-Propargyl-5,8-dideazafloric acid (PDDF)
and its p-aminosulphonyl derivative
(DMPDDSDF) were purchased or synthesized
as described previously [8, 9].

Trimethotrexate (TMTX) was a gift from
Glaxo-Wellocome Company. Methotrexate
(MTX), folic acid (both purified, prior to use
by ion-exchange — DEAE cellulose chromat-
ography), tetrahydrofolate, ATP, thymidy-
late synthase (from bovine liver) and dihy-
drofolate reductase (from bovine liver) were
purchased from Sigma Chemical Co. (St.
Louis, MO, U.S.A.). pABAGlu₉ was a gift
from Prof. Rzeszotarska and Dr. Krzyża-
nowski from the Opole University.

All radioactive compounds were purchased
from Moravek Biochemicals, Inc., Brea, CA,
U.S.A. or Amersham, International plc,
Amersham, U.K.

All other chemicals were reagent grade.

Methods

Cell culture. Murine leukemia 5178Y cells
free of Mycoplasma were grown in suspen-
sion in 60 mm Falcon dishes in Fisher’s
medium supplemented with 8% of bovine
new born serum under a 5% CO₂ atmosphere
as described previously [8–10]. Murine
leukemia 5178Y/F cells containing 6 times more
of TS protein than the parental line [10] were
obtained in our laboratory by passing 5178Y
cells on the media containing the in-
creasing concentration of FdUrd, starting
from 0.1 nM to reach 80 μM [10] after 18
months.

Cell survival. The 5178Y cells were plated
at a density of 1 × 10⁴ cells/well (Bibby
Sterilin, Ltd., England). After 2 h the drugs
were added for 48 h at the concentration
indicated and the number of viable cells was
counted in Neubauer camera using the try-
pan-blue staining.

Enzyme assays. For the enzyme assay

cells were collected (by centrifugation),
washed twice in ice cold phosphate buffer-
saline, sonicated in Branson Sonifier 250

Figure 1. Chemical structures of thymidylate syn-
thase inhibitors.
sonicator in 100 mM phosphate buffer (pH 7.4) containing 10 mM 2-mercaptoethanol, and centrifuged for 20 min at 20,000 × g. The supernatant was used for the enzyme assay.

Folypolyglutamate synthetase (EC 6.3.2.-17) was assayed according to McGuire et al. [11] using tetrahydrofolate (250 μM) as a substrate.

γ-Glutamyl hydrolase (EC 3.4.19.9) was assayed in cell extracts according to Sikora et al. [12], or determined by high performance liquid chromatography (Beckman Ultrasil AX 10 µ 0.4 cm × 25 cm column) using pABA-Glu₅ as a substrate.

Cell protein was estimated according to Bradford [13].

**Thymidylate synthesis.** Deoxyuridylate incorporation was a measure of de novo thymidylate biosynthesis by the intact cultured cells. The cells exposed to the drugs were exposed in turn to 1 μCi of [5-³H]dUrd for 40 min and the reaction was terminated by addition of activated charcoal suspended in 0.15 M trichloroacetic acid. Recovery of ³H₂O radioactivity was a measure of the rate of thymidylate synthesis in situ [9]. The results were expressed in pmoles/well.

**[6-³H]dUrd incorporation to DNA.** Following cell treatment by the drug cultures were exposed to 10 nM (1 μCi) of [6-³H]dUrd for 40 min. Incorporation of the radiolabel into DNA in intact cells was measured following alkaline hydrolysis of RNA in 0.5 M KOH at 37°C according to Hryniuk et al. [14].

**Determinations of 5,10CH₂H₄PteGlu₅ and H₂PteGlu₅.** Cells exposed to the drugs were extracted by boiling in a solution containing 50 mM Tris, 1 mM EDTA and 50 mM ascorbate (pH 7.4) and 5,10CH₂H₄PteGlu₅ was measured in the supernatant according to Banni et al. [15]. Then the samples were incubated for 30 min with 10 pmol of [³H]PdUMP and 10 pmol of bovine TS in a total volume of 100 μl at 37°C. The reaction was stopped by adding 1% SDS and boiling for 10 min. The [³H]PdUMP-thymidylate synthase-5,10CH₂H₄PteGlu₅ complex was quantitated following separation of unreacted [³H]PdUMP by G-25 Sephadex filtration. H₂PteGlu₅ was measured after reduction to H₄PteGlu₅ by dihydrofolate reductase in the presence of NADPH and assayed as 5,10CH₂H₄PteGlu₅ in the presence of 6 mM formaldehyde in the thymidylate synthase reaction. The amount of dihydrofolate was calculated by subtracting the amount of 5,10CH₂H₄PteGlu₅ present in the initial sample from the amount formed by dihydrofolate reductase.

Standard curves were generated using 5,10CH₂H₄PteGlu₇ and H₂PteGlu₇. The results were expressed as nmol/g cell protein.

**Functional interactions between drugs.** The combined drug effect was evaluated using the Chou & Talalay [16] analysis based on the median effect principle. The data were analyzed using the concentration-effect statistical program.

**RESULTS AND DISCUSSION**

**Effect on cell survival**

The activities of three antimetabolites —FdUrd and DMPDPSFGlu or DMPDPSFGly — inhibitors of thymidylate synthase are shown in Table 1. The decrease in survival of 5178Y cells caused by FdUrd was the most pronounced with I₅₀ approximately at 0.002 μM on 48 h exposure to the antimetabolite. In the case of the two antifolates: DMPDSFGlu and DMPDPSFGly I₅₀ values were 3 μM and 20 μM, respectively.

Continuous exposure of 5178Y/F cells to either antifolate caused only a slight increase in I₅₀ value in comparison with parental 5178Y cells (Table 1). DMPDPSFGlu appears to be a much potent (about 7 times) inhibitor than DMPDPSFGly in both cell lines due to its ability to undergo polyglutamylation [17, 18].

A possible effect on interaction of the antimetabolites at low inhibitory concentration on cell survival was also tested (Table 2). A 48 h exposure of 5178Y cells successively to DMPDPSFGlu and FdUrd at concentrations corresponding to I₅₀ resulted in a more significant lowering of cell survival than expected from the sum of their separate effects. The addition of DMPDPSFGlu for 48 h at the same concentration after 2 h exposure of the cells to FdUrd resulted in even higher syner-
Table 1. Effect of FdUrd, DMPDSSF\textsubscript{Glu} and DMPDSSF\textsubscript{Gly} on survival of 5178Y and 5178Y/F cells.

The results are the means ± S.D. from 4–6 independent experiments.

<table>
<thead>
<tr>
<th>Antimetabolite</th>
<th>$I_{50}$ (μM)</th>
<th>5178Y cells</th>
<th>5178Y/F cells</th>
<th>5178Y cells</th>
<th>5178Y/F cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>FdUrd</td>
<td>0.002</td>
<td>0.010</td>
<td>0.001</td>
<td>0.05 ± 0.008</td>
<td></td>
</tr>
<tr>
<td>DMPDSSF\textsubscript{Gly}</td>
<td>20.00 ± 1.00</td>
<td>30.00 ± 7.00</td>
<td>1.00 ± 0.10</td>
<td>3.00 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>DMPDSSF\textsubscript{Glu}</td>
<td>3.00 ± 0.70</td>
<td>5.00 ± 1.00</td>
<td>0.20 ± 0.03</td>
<td>0.50 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

The logistic effect than when the drugs were added at the same time. A slightly lower synergistic inhibitory effect was observed with 5178Y/F cells irrespective of the succession of drug addition (Table 2). We assumed that these results might be related to the differences in the intracellular folate pool between 5178Y and 5178Y/F cells, since the intracellular concentration of folates i.e. $5,10\text{CH}_2\text{H}_4\text{PteGlu}_n$ and $\text{H}_2\text{PteGlu}_n$ in 5178Y/F cells is about 3 times higher than that in the parental cell line 5178Y [18] (Table 3). This supposition proved to be correct as we have observed that leucovorin (5-CHO-H$_2$PteGlu), a reduced folate, added at 5 μM concentration together with FdUrd and one of the antimitabolites decreased the number of survived cells by 30% in both cell lines (Table 4).

[19]; being without any effect on synergistic action of antifolates (data not shown). One has to take into account that the enzyme, a folate substrate ($5,10\text{CH}_2\text{H}_4\text{PteGlu}_n$) and the antimitabolite (FdUMP) form tertiary complex [20]. Stability of this complex depends on folate concentration [21–23]. Moreover the complex formed in the presence of dideazafolates was found to be more stable than the one formed without an inhibitor [21, 24] which is consistent with our observation (Table 4).

De novo thymidylate biosynthesis

DMPDSSF\textsubscript{Glu} and DMPDSSF\textsubscript{Gly} exert a moderate inhibition of thymidylate synthesis de novo (below 35%) (Table 5). FdUrd showed

Table 2. Effect of low concentration ($I_{10}$) of FdUrd, DMPDSSF\textsubscript{Glu} and DMPDSSF\textsubscript{Gly} on survival of 5178Y and 5178Y/F cells.

The drug concentration was the same as in Table 1. Results are the means ± S.D. from 4–6 independent experiments.

<table>
<thead>
<tr>
<th>Addition</th>
<th>FdUrd</th>
<th>DMPD-DSF\textsubscript{Glu}</th>
<th>DMPD-DSF\textsubscript{Gly}</th>
<th>5178Y cells</th>
<th>5178Y/F cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>observed</td>
<td>expected\textsuperscript{c}</td>
<td>observed</td>
<td>expected\textsuperscript{c}</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>100 ± 5</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>92 ± 1</td>
<td>98 ± 7</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>90 ± 1</td>
<td>90 ± 6</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>91 ± 3</td>
<td>90 ± 1</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>21 ± 3</td>
<td>68.2</td>
</tr>
<tr>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>29 ± 4</td>
<td>69.8</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>7 ± 1</td>
<td>68.3</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>15 ± 6</td>
<td>64.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}FdUrd was added 2 h after the cells were plated and DMPDSSF\textsubscript{Glu} was added after another 4 h for 48 h; \textsuperscript{b}DMPDSSF\textsubscript{Gly} was added 2 h after the cells were plated and FdUrd was added after another 4 h for 49 h; \textsuperscript{c}The expected values were calculated after [16].
Table 3. Effect of FdUrd combined with DMPDSSF Glu or DMPDSSF Gly on cellular concentration of 5,10CH₃H₄PteGlu₄ and H₂PteGlu₄.

The cells were cultured in the presence of drugs at I₀ concentrations. For details see Materials and Methods. The results are means ± S.D. from 3–4 independent experiments.

<table>
<thead>
<tr>
<th>FdUrd</th>
<th>DMPDSSF Glu</th>
<th>DMPDSSF Gly</th>
<th>Intracellular concentration (pmoles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5,10CH₃H₄PteGlu₄</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5178Y cells</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>3.6 ± 0.47</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>−</td>
<td>1.2 ± 0.20</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>−</td>
<td>4.3 ± 0.20</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>+</td>
<td>4.6 ± 0.17</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>+</td>
<td>0.9 ± 0.20</td>
</tr>
<tr>
<td>+ b</td>
<td>+ b</td>
<td>−</td>
<td>0.2 ± 0.07</td>
</tr>
</tbody>
</table>

*DMPDSSF Gly was added 2 h after the cells were plated and FdUrd was added after another 4 h; +FdUrd was added 2 h after the cells were plated and DMPDSSF Glu was added after another 4 h.

a much lower inhibition (below 20%) than could be expected from the cell survival. This lack of relation between the effects on cell survival and inhibition of de novo thymidylate synthesis suggested that a simple blocking of thymidylate biosynthesis can not be responsible for cytotastic action of FdUrd but it might indicate dissociation of thymidylate from DNA [25].

The combined effect of FdUrd with DMPDSSF Glu or DMPDSSF Gly on thymidylate synthesis showed slight synergy in intact cells of both lines (Table 5). However, the assay gives no information on the mechanism of this effect.

Effect of FdUrd combined with DMPDSSF Glu or DMPDSSF Gly on [6-³H]dUrd incorporation into DNA. Incorporation of [6-³H]dUrd into DNA was measured to compare FdUrd interference into DNA and thymidylate synthases. More intensive incorporation of [6-³H]dUMP into DNA in the presence of FdUrd than in the presence of either antifolate indicates that the inhibitory effect of FdUrd was mainly directed to DNA synthesis rather than effects on thymidylate synthesis (Table 5). Although antifolates inhibit DNA synthesis, this effect is not as great as their inhibition of thymidylate synthesis and it is probably indirect, due to changes in concentration of deoxyribonucleoside-5'-phosphates [24, 25].

The effect of FdUrd combined with DMPDSSF Glu and DMPDSSF Gly on the activity of γ-folylpolyglutamate synthase and γ-glutamyl hydrolase. The activities of these two enzymes crucial for cellular polyglutamylation were measured in the extracts of the cells treated with the drugs (Table 6). Only a moderate inhibitory

Table 4. Effect of 5 μM leucovorin (5-CHO-H₂PteGlu) on survival of 5178Y and 5178Y/F cells treated with FdUrd combined with DMPDSSF Glu or DMPDSSF Gly at low concentrations (I₁₀).

The compounds are added together 2 h after cells were plated. Results are means ± S.D. from 3–4 independent experiments.

<table>
<thead>
<tr>
<th>FdUrd</th>
<th>DMPDSSF Glu</th>
<th>DMPDSSF Gly</th>
<th>Cell survival (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>5178Y cells</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>+</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>41 ± 3</td>
</tr>
</tbody>
</table>
Table 5. Effect of FdUrd combined with DMPDDSFGlu or DMPDDSFGly at low concentration (I₀) on de novo thymidylate biosynthesis in 5178Y and 5178Y/F cells.

The cells were cultured in the presence of the drug as described in Table 2. De novo thymidylate synthesis was measured by incorporation of [5-³H]FdUrd or [6-³H]FdUrd into DNA as described in Materials and Methods. The results are the means ± S.D. from 3 independent experiments.

<table>
<thead>
<tr>
<th>Addition</th>
<th>FdUrd</th>
<th>DMPD-DSFGLu</th>
<th>DMPD-DSFGly</th>
<th>Incorporation (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[5-³H]FdUrd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5178Y cells</td>
</tr>
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<td></td>
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<td>5178Y cells</td>
</tr>
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<td>−</td>
<td>−</td>
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<td>−</td>
<td>100 ± 1</td>
</tr>
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<td>72 ± 2</td>
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<td>56 ± 4</td>
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<td>69 ± 3</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>30 ± 1</td>
</tr>
</tbody>
</table>

*effect on folyl-γ-polyglutamate synthetase activity was observed in the cells exposed to DMPDDSFGlu, probably because of drug polyglutamylation (Table 6).*

**Effect of FdUrd combined with DMPDDSFGlu and DMPDDSFGly on cellular concentration of H₂PteGlu₉ and 5,10CH₂H₂PteGlu₉.** During continuous exposure of 5178Y and 5178Y/F cells to 1 nM and 50 nM FdUrd, respectively, the cellular pool of 5,10CH₂H₂PteGlu₉ was reduced approximately by 50% with only 20% of reduction in thymidylate synthesis as measured by [5-³H]deoxyuridylic incorporation (Tables 3, 5). The cellular pool of H₂PteGlu₉ under the same conditions was slightly decreased. When the cells of either line were exposed to DMPDDSFGly or DMPDDSFGlu at I₀ con-

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Table 6. Effect of FdUrd, DMPDDSFGlu and DMPDDSFGly at I₀ concentration on the activity of folylpolyglutamate synthetase (FPGS) and γ-glutamyl hydrolase in 5178Y and 5178Y/F cells.

The drugs were added to the cell culture at the same time. The results are means ± S.D. from 3–6 independent experiments.

<table>
<thead>
<tr>
<th>Addition</th>
<th>FdUrd</th>
<th>DMPD-DSFGLu</th>
<th>DMPD-DSFGly</th>
<th>Enzyme activity (nmoles/h per mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FPGS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5178Y cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5178Y cells</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.31 ± 0.01</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.28 ± 0.04</td>
</tr>
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<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
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<td>−</td>
<td>0.17 ± 0.01</td>
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<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>+</td>
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<td>−</td>
<td>−</td>
<td>0.31 ± 0.01</td>
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<td>0.18 ± 0.04</td>
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<tr>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>0.28 ± 0.04</td>
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centrations the 5,10CH₂H₂PteGlu₄ pool increased by about 25%, similarly as deoxyuridylate incorporation. The inhibitory effect of combined drugs on cellular pools of the two folates depended on the sequence of their addition. When the cells were exposed first to 0.001 μM FdUrd and then to 0.02 μM or 0.003 μM DMPDDSFGly or DMPDDDSFGlu, respectively, the inhibitory effect on cellular pool of 5,10CH₂H₂PteGlu₄ was as high as 80% (Table 3). In comparison, a 2 h exposure of the cells to antifolates followed by incubation with FdUrd (at I₅₀ concentration) resulted in a 65% decrease of the 5,10CH₂H₂PteGlu₄ intracellular pool.

These results suggest that TS inhibitors, FdUrd and dideazafolates (DMPDDSFGlu or DMPDDDSFGly), can act synergistically and effect of synergy does not depend on TS level in the cells either in 5178Y or 5178YF.

It is hoped that application of combined drugs-TS inhibitors may lead to better understanding of the interactions between intracellular folates, DNA and thymidylate synthesis and to a successful use of these drugs in cancer therapy.

REFERENCES


