Human Lymphoblastoid Cells with Acquired Resistance to C2-Desamino-C2-methyl-N10-propargyl-5,8-dideazafolic Acid: A Novel Folate-based Thymidylate Synthase Inhibitor

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ABSTRACT

We describe the characterization of human lymphoblastoid cell lines with acquired resistance (>20,000-fold) to a novel folate-based thymidylate synthase (TS) inhibitor, C2-desamino-C2-methyl-N10-propargyl-5,8-dideazafolic acid (ICI198583). This acquired resistance was associated with a 64-fold amplification of the TS gene, a similar elevation in the corresponding mRNA, and an ~200-fold increase in both TS activity and TS protein. This amplification was maintained when the cells were grown in the absence of the selective agent, ICI198583, for 340 generations. TS isolated from one of the resistant cell lines, W1-L2:C1, displayed inhibition kinetic parameters similar to those of TS isolated from the parent W1-L2 cell line. It thus appears unlikely that resistance is due to an altered TS enzyme having a lower affinity for ICI198583. The resistant cell line, W1-L2:C1, was cross-resistant to other folate-based TS inhibitors but was as sensitive as the parent cell line, W1-L2, to 5-fluorodeoxyuridine. The W1-L2:C1 cell line was laterally sensitive to the classical dihydrofolate reductase (EC 1.5.1.3) inhibitor methotrexate as well as to the lipophilic dihydrofolate reductase inhibitors metoprine and 2,4-diamino-5-methyl-6-(3,4,5-trimethoxyanilino)ethylquinazoline glucuronic acid salt (also called trimetrexate). When the W1-L2 and W1-L2:C1 cell lines were exposed to 1 μM ICI198583 for 24 h they accumulated the same concentration of total cellular ICI198583 polyglutamates despite the fact that the latter cell line accumulated a 300-fold greater concentration of ICI198583 monoglutamate. As polyglutamates, the tetra- and pentaglutamate forms predominated in the W1-L2 cell line, whereas the diglutamate form predominated in the W1-L2:C1 cell line, with few higher polyglutamates being detected. The lack of tri- and higher polyglutamates of ICI198583 (i.e., the more active species) in the W1-L2:C1 cell line may also contribute to the observed resistance. These findings may have important implications in light of the rapid onset of resistance to antifolates in the clinic.

INTRODUCTION

A major limitation to the use of some chemotherapeutic agents as antimun drugs has been the development of clinical resistance. Acquired resistance to antimetabolites frequently occurs by amplification of the gene encoding the target enzyme, which results in elevated cellular levels of that enzyme (1–5). Acquired resistance to antifolates is exemplified to its good transport via the reduced folate carrier, IC50 = 0.085 and 0.056 μM, respectively). This 40-fold improvement in cytotoxicity with the C2-methyl analogue seems to relate (at least in part) to its good transport via the reduced folate carrier, which CB3717 is thought not to use (24). ICI198583 was found to be only 3-fold less potent than CB3717 as a TS inhibitor but 70-fold less potent as a DHFR inhibitor and is thus a more specific TS inhibitor (25, 26). Moreover, this highly active compound has substrate activity for FPGS similar to that of CB3717 (Km = 40 μM) (25). The resulting polyglutamates of ICI198583 are up to ~100-fold more potent than the mono-

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glutamate as TS inhibitors (25, 27). Intracellular polyglutamation of this compound has recently been demonstrated in the L1210 and W1-L2 cell lines (25, 28) and may thus be an important determinant of cytotoxicity. The similarity in structure of ICI198583 to CB3717 and the difference in transport mechanisms warranted further studies with this analogue.

The present paper is concerned with the development of resistance to ICI198583 in a human lymphoblastoid cell line. The availability of a TS cDNA probe permitted the estimation of TS gene amplification and TS mRNA levels in the resistant cell line. TS was purified from the sensitive and from the resistant cell lines by affinity chromatography for studies on inhibitor binding constants. In vitro cross-resistance patterns were investigated, as were intracellular levels of ICI198583 polyglutamates.

**MATERIALS AND METHODS**

RPMI 1640 [containing 20 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer and lacking sodium bicarbonate and L-glutamine] was from Flow Laboratories (Irvine, Scotland). Donor horse and fetal bovine sera were obtained from Imperial Laboratories (Salisbury, England). Folic acid (calcium leucovorin, 3 mg/ml) was from David Bull laboratories (Warwick, England). [±]-l-Tetrahydrofolic acid (HCl) (97% pure) was purchased from Fluka (Neu-Ulm, Germany). [5-3H]-dUMP and [benzoyl-3H]ICI198583 were supplied by the Radiochemical Centre (Amersham, Buckinghamshire, England). [6-3H]-dUMP was obtained from Moravek Biochemicals, Inc. (Brea, CA). The folate-based TS inhibitor ICI198583 was synthesized and supplied by ICI Pharmaceuticals PLC (Macclesfield, Cheshire, England). This compound was dissolved in sodium bicarbonate (0.15 m NaHCO₃) to yield a 10 mm solution. The ICI198583 polyglutamate standards were synthesized at the Institute of Cancer Research (27). Methotrexate was obtained from Nils Klass Klausen (Denmark). TMQ glucuronic acid salt (trimetrexate) and DDMP were gifts from the National Cancer Institute (Bethesda, MD). DDATHF was a gift from Dr. Grindey (Eli Lilly, Indianapolis, IN). All other reagents were purchased from either Fisons (Loughborough, England) or Sigma (London).

Development of Resistant Cell Lines. Human lymphoblastoid W1-L2 cells were originally cultured from the spleen of a patient suffering from hereditary spherocytosis anemia (29). The cells were grown in suspension using RPMI 1640 supplemented with 20 mm L-glutamine, 10% fetal bovine serum, 0.2% amphotericin B solution, and 0.24% gentamicin using RPMI 1640 supplemented with 2 mM L-glutamine, 10% fetal bovine sera were obtained from Imperial Laboratories (Salisbury, England). Folic acid (calcium leucovorin, 3 mg/ml) was from David Bull laboratories (Warwick, England). [±]-l-Tetrahydrofolic acid (HCl) (97% pure) was purchased from Fluka (Neu-Ulm, Germany). [5-3H]-dUMP and [benzoyl-3H]ICI198583 were supplied by the Radiochemical Centre (Amersham, Buckinghamshire, England). [6-3H]-dUMP was obtained from Moravek Biochemicals, Inc. (Brea, CA). The folate-based TS inhibitor ICI198583 was synthesized and supplied by ICI Pharmaceuticals PLC (Macclesfield, Cheshire, England). This compound was dissolved in sodium bicarbonate (0.15 m NaHCO₃) to yield a 10 mm solution. The ICI198583 polyglutamate standards were synthesized at the Institute of Cancer Research (27). Methotrexate was obtained from Nils Klass Klausen (Denmark). TMQ glucuronic acid salt (trimetrexate) and DDMP were gifts from the National Cancer Institute (Bethesda, MD). DDATHF was a gift from Dr. Grindey (Eli Lilly, Indianapolis, IN). All other reagents were purchased from either Fisons (Loughborough, England) or Sigma (London).

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The present paper is concerned with the development of resistance to ICI198583 in a human lymphoblastoid cell line. The availability of a TS cDNA probe permitted the estimation of TS gene amplification and TS mRNA levels in the resistant cell line. TS was purified from the sensitive and from the resistant cell lines by affinity chromatography for studies on inhibitor binding constants. In vitro cross-resistance patterns were investigated, as were intracellular levels of ICI198583 polyglutamates.
Table 1 Characterization of the ICI198583-resistant cell line

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IC50 for ICI198583</th>
<th>-Fold increase in TS specific activity</th>
<th>-Fold increase in DHFR specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1-L2</td>
<td>0.056</td>
<td>13.4 ± 1.92</td>
<td>3.59 ± 0.13</td>
</tr>
<tr>
<td>W1-L2:C1</td>
<td>1.540</td>
<td>27.500</td>
<td>200</td>
</tr>
<tr>
<td>W1-L2:C4</td>
<td>1.139</td>
<td>3.59 ± 0.32</td>
<td>0.13 ± 0.22</td>
</tr>
<tr>
<td>W1-L2:C5</td>
<td>3.139</td>
<td>3.59 ± 0.32</td>
<td>0.13 ± 0.22</td>
</tr>
<tr>
<td>W1-L2:C6</td>
<td>1.08</td>
<td>3.59 ± 0.32</td>
<td>0.13 ± 0.22</td>
</tr>
</tbody>
</table>

RESULTS

A number of cloned cell lines were isolated from the W1-L2 cells resistant to 50 and 500 μM ICI198583. Two were obtained with acquired resistance to 50 μM ICI198583 (W1-L2:C1 and W1-L2:C2), and four with acquired resistance to 500 μM ICI198583 were also obtained (W1-L2:C3, C4, C5, and C6). Mean generation times were 17 h for the W1-L2 cell line, 18 h for the W1-L2:C1, C2, C3, C5, and C6 cell lines, and 24 h for the W1-L2:C4 and C6 cell lines. The six cell lines W1-L2:C1-C6 were all actually more than 20,000-fold resistant to ICI198583 as accessed by IC50 values. The six resistant cell lines showed greatly increased TS activities (100- to 200-fold) when compared with the parental cell line, W1-L2.

The W1-L2:C1 cell line was selected for further characterization. This cell line was 27,000-fold resistant to ICI198583, and the TS activity was 200-fold increased compared with the parental cell line (Table 1). The degree of resistance was maintained, and only a 40% decrease in TS activity was noted when the cell line was grown in the absence of ICI198583 for ~340 generations (Table 1).

Studies using a TS cDNA probe demonstrated that the TS gene was ~64-fold amplified in the W1-L2:C1 cell line (Fig. 2). A similar enhancement in the level of TS mRNA was also observed (Fig. 3). The elevated level of TS gene copy number was maintained when the W1-L2:C1 cell line was grown in the absence of ICI198583 for ~340 generations (Fig. 4).

There was no significant difference between the W1-L2 and W1-L2:C1 cell lines in DHFR enzyme activity (Table 1).

Enzyme Kinetic Data. The values of Ks for IC198583 and CB3717 using partially purified TS from the W1-L2:C1 cell line were similar to those obtained using TS from the parent W1-L2 cell line (Table 2). The Ks values for the substrate (±)5,10-CH2FH4 using TS isolated from the W1-L2 and W1-L2:C1 cell lines were also similar (Table 2). The formation and dissociation of the ternary complex of W1-L2 TS-[6-3H]dUMP and ICI198583 were monitored and compared with those of W1-L2:C1 TS-[6-3H]dUMP and ICI198583. The same procedure was repeated using CB3717 instead of ICI198583. Thus the values of K1/2 for complex formation at saturating levels of ICI198583 or CB3717 were obtained for TS from W1-L2 and W1-L2:C1 cell lines (Table 3). The values of K1/2 for complex dissociation were also estimated. There was little difference between the values of K1/2 obtained using W1-L2 TS and those obtained using W1-L2:C1 TS. However, complex formation was about 3-fold slower for IC198583 relative to that of CB3717. The rate of complex dissociation was slightly faster for IC198583 relative to CB3717 (~1.4-fold).

Cytotoxicity Data. The values of IC50 for a number of folate-based TS inhibitors against the W1-L2 and W1-L2:C1 cell lines are shown in Table 4. The W1-L2:C1 cell line was highly cross-resistant to IC198583-related analogues which also have TS as their cytotoxic locus of action (CB3717, C2-desamino-CB3717, and ICID1694) (39-41). The W1-L2:C1 cell line was not very cross-resistant to C2-desamino-N0-hydrogen-CB3717, a compound suspected of having both TS- and DHFR-inhibitory activities in cultured cell lines (40).

The W1-L2 and W1-L2:C1 cell lines were equally sensitive to the pyrimidine nucleoside 5-FdUrd. The W1-L2:C1 cell line showed collateral sensitivity to MTX (3.5-fold more sensitive) (Table 4). Collateral sensitivity was also observed with the lipophilic DHFR inhibitors TMQ glucuronic acid salt and DDMP. The W1-L2 and W1-L2:C1 cell lines were equally sensitive to the glucosamimid ribonucleotide transformylase inhibitor DDAHTH (Table 4).

Table 5 contains the IC50 values for the pyrimidine-based TS inhibitor 5-FdUrd in the sensitive and resistant W1-L2 cell lines in the presence of folinic acid (5 μM) and/or dThd (10 μM) and/or dThd (10 μM).
DISCUSSION

Acquired and stable resistance to the TS inhibitor, ICI198583, in the W1-L2:C1 cell line (made resistant to 50 μM) was associated with a 64-fold amplification of the TS gene, a similar elevation in the corresponding mRNA level, and a 200-fold increase in TS activity (Figs. 2 and 3 and Table 1). Furthermore, Western blot analysis using polyclonal antibodies (raised against human recombinant TS) indicated a 100–200-fold increase in TS enzyme levels in W1-L2:C1 cells (42). It is therefore probable that most if not all of the amplified genes are transcriptionally active in this cell line. There was no evidence of any major rearrangements in gene structure during the amplification process (Figs. 2 and 4). There was some discrepancy between the elevated levels of TS mRNA (64-fold elevated) and the increase in TS enzyme activity (200-fold elevated). This may be due to an increase in the rate of translation or to the stabilization of the TS enzyme in the W1-L2:C1 cell line.

Table 3 Summary of binding studies with TS purified from the W1-L2 and W1-L2:C1 cell lines

<table>
<thead>
<tr>
<th>TS inhibitor</th>
<th>W1-L2 TS</th>
<th>W1-L2:C1 TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICI198583</td>
<td>50, 40</td>
<td>53, 50, 43</td>
</tr>
<tr>
<td>CB3717</td>
<td>17, 10</td>
<td>13, 16, 37</td>
</tr>
<tr>
<td>ICI198583</td>
<td>136, 136</td>
<td>233, 133, 167</td>
</tr>
<tr>
<td>CB3717</td>
<td>100, 103</td>
<td>137, 113, 104</td>
</tr>
</tbody>
</table>

* t_{1/2} time required for reaction to reach 50% completion.  
b K_{app}, association/dissociation rate constant (0.693/t_{1/2}).

d Table 4 Cross-resistance studies

<table>
<thead>
<tr>
<th>Compound</th>
<th>W1-L2 I_{50} (μM)</th>
<th>W1-L2:C1 I_{50} (μM)</th>
<th>-Fold resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICI198583</td>
<td>0.056</td>
<td>1540</td>
<td>27,500</td>
</tr>
<tr>
<td>CB3717</td>
<td>2.6</td>
<td>&gt;500</td>
<td>&gt;192</td>
</tr>
<tr>
<td>C'-desamino-CB3717</td>
<td>0.42</td>
<td>2300</td>
<td>5,500</td>
</tr>
<tr>
<td>C'-desamino-N^6-hydrogen-CB3717</td>
<td>0.255</td>
<td>7.6</td>
<td>30</td>
</tr>
<tr>
<td>ICID1694ᵇ</td>
<td>0.0058</td>
<td>&gt;100</td>
<td>&gt;17,241</td>
</tr>
<tr>
<td>5-FdUrd</td>
<td>0.0056</td>
<td>0.007</td>
<td>1.25</td>
</tr>
<tr>
<td>MTX</td>
<td>0.012</td>
<td>0.0034</td>
<td>0.28</td>
</tr>
<tr>
<td>TMQ</td>
<td>0.0178</td>
<td>0.0068</td>
<td>0.38</td>
</tr>
<tr>
<td>DDMF</td>
<td>0.028, 0.03</td>
<td>0.0054, 0.0057</td>
<td>0.19</td>
</tr>
<tr>
<td>DDAHThF</td>
<td>0.025, 0.027</td>
<td>0.034, 0.038</td>
<td>1.38</td>
</tr>
</tbody>
</table>

* Insoluble at concentrations >500 μM.  
b C'-desamino-C'-methyl-N^6-methylthiophene ring analogue of CB3717.

d Table 5 Modulation of 5-fluorodeoxyuridine cytotoxicity in the W1-L2 and W1-L2:C1 cell lines

<table>
<thead>
<tr>
<th>Additions</th>
<th>W1-L2 I_{50} (μM)</th>
<th>W1-L2:C1 I_{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FdUrd</td>
<td>0.0056</td>
<td>0.007</td>
</tr>
<tr>
<td>5-FdUrd + folinic acid</td>
<td>0.0005, 0.0008</td>
<td>0.27, 0.27</td>
</tr>
<tr>
<td>5-FdUrd + dThd</td>
<td>50, 80</td>
<td>0.4</td>
</tr>
<tr>
<td>5-FdUrd + dThd + 10 μM folinic acid</td>
<td>46, 93</td>
<td>87</td>
</tr>
</tbody>
</table>

μM). The presence of folinic acid potentiated the cytotoxicity of 5-FdUrd (~10-fold) in the W1-L2 cell line and antagonized the cytotoxicity in the W1-L2:C1 cell line (~40-fold). dThd antagonized the cytotoxic effects of 5-FdUrd in both the W1-L2 (~12,000-fold) and W1-L2:C1 (~60-fold) cell lines. The coadministration of folinic acid and dThd further antagonized the cytotoxic effect of 5-FdUrd in the W1-L2:C1 cell line.

Polyglutamation Experiments. Table 6 compares the cellular uptake of [³H]ICI198583 and its intracellular metabolism to polyglutamate forms in W1-L2 and W1-L2:C1 cell lines. The uptake of ICI198583 was 30-fold higher in the resistant cell line (W1-L2:C1) as estimated by the total cellular tritium after 24 h of incubation. Although the total level of polyglutamates found in the two cell lines exposed to 1 μM [³H]ICI198583 was similar (1-2 μM), this represents 91% of cellular tritium in the sensitive cell line (W1-L2) and only 2% in the resistant cell line (W1-L2:C1). Furthermore, as polyglutamates the tetra- and pentaglutamates were the predominant forms in the W1-L2:C1 cell line, while the diglutamate predominated in the W1-L2:C1 cell line. Incubation with a 10-fold higher extracellular concentration of [³H]ICI198583 (10 μM) did not alter the total level of cellular tritium in the W1-L2:C1 cell line but did increase the level of polyglutamates (3-fold), with the diglutamate again predominating.

Table 2 Kinetic studies on partially purified TS from W1-L2 and W1-L2:C1 cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>K₅₀ 5,10-CH₂FH₄ (μM)</th>
<th>K₅₀ CB3717 (nm)</th>
<th>K₅₀ ICI198583 (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1-L2</td>
<td>18.36 ± 1.24, 19.24 ± 1.1</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>W1-L2:C1</td>
<td>16.05 ± 1.84, 20.32 ± 2.2</td>
<td>1.05</td>
<td>5.75</td>
</tr>
</tbody>
</table>

* See "Materials and Methods" for K₅₀ determinations.
Preliminary cytogenetic studies suggested the presence of some double minute chromosomes in the W1-L2:C1 cell line. The W1-L2 and W1-L2:C1 cell lines are aneuploid, and it was therefore difficult to identify homogeneous staining regions or abnormal banding regions. The stability of the resistant phenotype and TS gene amplification (Table 1 and Fig. 4) would suggest that the amplified genes are also integrated into the cellular chromosome as homogeneous staining regions or abnormal banding regions (1, 43).

Resistance in this W1-L2:C1 cell line did not appear to be due to an altered TS enzyme with reduced affinity for the selective agent ICI198583 as determined by inhibition kinetic and binding studies (Tables 2 and 3). Such a mutation in TS has been described for 5-FdUrd-resistant cell lines whereby the mutant TS has a lower affinity for 5,10-CH$_2$FH$_4$ and/or 5-FdUMP (44-46). CB3717 was found to bind to TS for a slightly longer period of time compared with ICI198583, and this is reflected in its lower $K_i$ value (Tables 2 and 3).

The high degree of resistance to ICI198583 (27,000-fold) in the W1-L2:C1 cell line was not reflected by a particularly large increase in TS activity (200-fold), and hence other factors, in addition to TS overproduction, may be involved. It is interesting to note that the W1-L2 cell lines with acquired resistance to only 5 and 20 $\mu$M ICI198583 were both actually 20,000-fold resistant as assessed by IC$_{50}$ values. There is evidence that ICI198583 enters L1210 cells by the same mechanism as reduced folates and MTX (24, 47). It is unlikely that a transport defect exists in the W1-L2:C1 cell line, since it shows collateral sensitivity to MTX and is sensitive to DDAHF, a compound also known to use this transport mechanism (Table 4) (48). It is thus interesting that impaired transport, which plays a central role in resistance to MTX (49), does not appear to be a factor in resistance to ICI198583. This is despite the fact that these antifolates share the same transport mechanism. The discrepancy between the degree of resistance to TS enzyme level may relate to the saturable uptake mechanism known to exist for ICI198583 (24), thereby limiting the intracellular level of drug. High levels of resistance often result from multiple-step selections and are known to be associated with multiple mechanisms of resistance within the same cells, as exemplified by MTX resistance (50, 51).

The intracellular metabolism of ICI198583 to polyglutamated forms has been shown to be an important determinant of its cytotoxicity in the L1210 cell line (25, 28, 52). Thus defective ICI198583 polyglutamation in this W1-L2:C1 cell line was investigated. Recently human leukemic cell lines have been described which acquire resistance to MTX by virtue of defective MTX polyglutamation (22, 23). The data on ICI198583 metabolism to polyglutamates are difficult to interpret. The 30-fold greater amount of total cellular tritium found in the W1-L2:C1 cell line (compared with the W1-L2 cell line) can be explained by the binding of ICI198583 to the increased level of TS (Table 6). This may also explain the low level of ICI198583 polyglutamates (relative to monoglutamates) found in this cell line. The tetra- and pentaglutamate forms of ICI198583 predominated in the W1-L2 cell line, whereas the diglutamate was the major polyglutamate in the W1-L2:C1 cell line. ICI198583-diglutamate has a 20-fold lower $K_i$ for TS when compared with ICI198583 itself (27, 28) and may be bound to the excess TS in the W1-L2:C1 cell line, thus making it less available for further polyglutamation by FPGS. Therefore the lower percentage of total polyglutamates found in the W1-L2:C1 cell line may not be due to an alteration in FPGS per se. Indeed, further evidence supporting these data comes from the sensitivity of W1-L2:C1 cells to DDAHF (Table 4), a compound known to form polyglutamates which are important for its cytotoxicity (48). The near absence of the more active tri- and higher polyglutamates of ICI198583 in the W1-L2:C1 cell line may also explain the difference between the degree of resistance and the level of TS activity found in this cell line.

Folate-based TS inhibitors are also known to inhibit DHFR; hence DHFR activity (Table 1) and gene copy number (data not shown) were examined in the W1-L2:C1 cell line and found not to be significantly different from those found in the W1-L2 cell line. This is in contrast to the CB3717-resistant L1210 cell line, where a 2.6-fold increase in DHFR activity and a 30-40-fold increase in gene copy number were noted (3, 18). This is probably due to the greater TS specificity of ICI198583 compared with CB3717 (25, 26).

The W1-L2:C1 cell line was cross-resistant to CB3717 and other folate-based TS inhibitors but showed collateral sensitivity to DHFR inhibitors (Table 4). This cell line may have a greater demand for reduced folates, which may explain the increased sensitivity to DHFR inhibition. The correlation between TS activity and MTX sensitivity has been demonstrated previously in a variety of cell lines (53-57).

The equal sensitivity of the W1-L2 and W1-L2:C1 cell lines to the pyrimidine-based TS inhibitor 5-FdUrd was in accord with the findings of Jackman et al. (3) with the L1210 and L1210:C15 cell lines. It was hypothesized that the stable ternary complex of TS, 5-FdUMP, and 5,10-CH$_2$FH$_4$ in the presence of sufficiently high TS levels (W1-L2:C1 and L1210:C15 cell lines) would cause the depletion of intracellular reduced folates as they become sequestered in the formation of the ternary complex. This would result in the inhibition of both thymidylate and purine synthesis by substrate depletion. The differential effects of dThd alone in preventing 5-FdUrd cytotoxicity in the W1-L2 and W1-L2:C1 cell lines can thus be explained by the additional requirement for 5,10-CH$_2$FH$_4$ replenishment in the W1-L2:C1 cell line (Table 5). The cytotoxicity of 5-FdUrd in the parent W1-L2 cell line was potentiated by the coadministration of folic acid. It had previously been demonstrated that the optimal concentration of folic acid or of folic acid for cell growth was not sufficient for optimal cytotoxicity by 5-FdUrd
(58–60). Conversely, the presence of folic acid antagonized the cytotoxicity of 5-FdUrd in the TS-overproducing cell line W1-L2:C1. In accordance with the previously published hypothesis (3) we conclude that the addition of folic acid to W1-L2:C1 cells enables de novo purine and de novo thymidylate synthesis to proceed in the presence of 5-FdUrd by increasing the reduced folate pool. In contrast to the above findings, Danenberg and Danenberg (19) have reported substantial cross-resistance to 5-FdUrd but not to 5-fluorouracil in a mouse mammary carcinoma cell line (FM3A) with acquired resistance to CB3717. Resistance in this cell line was due to a 200-fold amplification of the TS gene and a corresponding increase in TS activity. The FM3A cell line may contain higher endogenous levels of reduced folates compared with the L1210 and W1-L2 cell lines, which would explain the cross-resistance to 5-FdUrd.

The clinical utility of folate-based TS inhibitors may thus be limited by the development of resistance. The high level of TS gene amplification reported here as a result of continuous cell lines, which would explain the cross-resistance to 5-FdUrd.

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REFERENCES

RESISTANCE TO THE THYMIDYLATE SYNTHASE INHIBITOR IC1198583


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