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ROLE OF THYMIDINE KINASE IN THE INHIBITORY ACTIVITY OF 5-SUBSTITUTED-2′-DEOXYURIDINES ON THE GROWTH OF HUMAN AND MURINE TUMOR CELL LINES

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Abstract—Twenty-four 5-substituted 2′-deoxyuridines have been evaluated for their inhibitory effects on the growth of three human lymphoblast cell lines (Namalva, Raji and TK^- (thymidine kinase deficient) Raji) and these inhibitory effects were compared to those for two murine leukemia cell lines (L1210/0 and L1210/BdUrd). The latter was selected from the parental L1210/0 cell line by its ability to grow at high concentrations of 5-bromo-dUrd and could also be considered as TK^-. There was a close correlation between the inhibitory effects of the deoxyuridine analogs on Namalva, Raji and L1210 cells: the correlation coefficient (r) for log ID_{50} (median inhibitory dose) for L1210 cell growth, on the one hand, and log ID_{50} for Namalva or Raji cell growth, on the other hand, was 0.902 and 0.929, respectively. There was also a strong correlation ($r = 0.936$) between the log ID_{50} values for the two human lymphoblast cell lines. However, there was no significant correlation ($r < 0.40$) either between the log ID_{50} for the TK^- Raji cells and the parental TK^+ Raji cells, or between the log ID_{50} for the TK^- L1210/BdUrd cells and the parental TK^+ L1210/0 cells. We may conclude therefore, that (i) the murine leukemia L1210 cell system is predictive for the growth-inhibitory effects of 5-substituted 2′-deoxyuridines on human lymphoblast cell lines, and (ii) the antitumor cell activity of the 5-substituted 2′-deoxyuridines is, to a large extent, dependent on the thymidine kinase activity of the tumor cells.

Since rapidly growing cells (i.e., tumor cells, bone marrow cells, etc.) show a high requirement for thymidine (dThd) and its precursors to sustain their DNA synthesis, any antagonist which interferes with the metabolic pathway that leads to the incorporation of dThd into DNA, may be expected to destroy the viability of these fast proliferating cells. Because of their structural analogy with dThd, 5-substituted 2′-deoxyuridines may offer a particular promise as potential antitumor agents. In fact, some 2′-deoxyuridine (dUrd) derivatives, viz. 5-fluoro-dUrd and 5-trifluoromethyl-dUrd, have long been known as antineoplastic agents [1]. 5-Fluoro-dUrd (in its free base form, 5-fluorouracil) is commonly used in the treatment of patients with disseminated breast and colon cancers [2], and 5-trifluoromethyl-dUrd has been shown to induce regression of breast carcinoma and certain pediatric malignancies [3, 4]. Other dUrd derivatives, i.e., 5-ethyl-dUrd [5, 6], 5-ethynyl-dUrd [7], 5-vinyl-dUrd [7], 5-mercapto-dUrd [8], 5-formyl-dUrd [9], 5-nitro-dUrd [10] and 5-hydroxymethyl-dUrd [11] have been reported to inhibit the growth of either L1210, Ehrlich ascites carcinoma, $B_5$59 melanoma or other tumor cells in culture.

In a previous study [12], we have evaluated various 5-substituted dUrd derivatives for their inhibitory effects on the growth of murine leukemia L1210 cells. From these studies the following compounds emerged as the most effective inhibitors (in order of decreasing activity): 5-fluoro-dUrd > 5-trifluoromethyl-dUrd > 5-nitro-dUrd (5′-monophosphate) > 5-ethynyl-dUrd > 5-formyl-dUrd > 5-(1-chlorovinyl)-dUrd. Other dUrd analogs such as 5-methylsulfinylmethyl-dUrd, 5-hydroxy-dUrd, 5-propynyloxy-dUrd and 5-propyl-dUrd were totally ineffective as inhibitors of L1210 cell growth [12]. These studies have now been extended to two human lymphoblast cell lines, Namalva and Raji. The role of phosphorylation by dThd kinase (TK) in the cytotoxic activity of these compounds was assessed by including in our comparative analysis a TK^- mutant Raji cell line and a TK^- mutant L1210 cell line (L1210/BdUrd, selected by its ability to grow in the presence of 5-bromo-dUrd (260 μg/ml)).

MATERIALS AND METHODS

Cells. Murine leukemia L1210 cells (referred to as L1210/0) were grown in 75 cm^2 tissue culture flasks (Falcon 3024F; Becton Dickinson France S.A., Grenoble, France) in Eagle's minimal essential medium, supplemented with 10% (v/v) inactivated fetal calf serum (Gibco Bio-Cult, Glasgow, Scotland,

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U.K.) and 2 mM L-glutamine (Flow Laboratories, Irvine, Scotland, U.K.).

TK^+ and TK^- Raji cells were provided by Dr. H. Wolf (München, F.D.R.) whereas Namalva cells were a kind gift of Prof. D. C. Burke (Warwick, U.K.). The cells were grown as described for the murine leukemia L1210 cells, but the growth medium was supplemented with 1% THM (5 mM Tes, 7.5 mM Hepes and 5 mM MOPS) buffer (pH 5.3) and 25 U/ml of nystatine (S.A. Labaz N.V., Brussels, Belgium).

Test compounds. The source of the compounds was as follows: 5-fluoro-dUrd (Aldrich Chemical Co., Milwaukee, WI), 5-bromo-dUrd (Sigma Chemical Co., St Louis, MO), 5-iodo-dUrd (Sigma Chemical Co.), 5-nitro-dUrd (5'-monophosphate) (see [13, 14]), 5-thiocyano-dUrd (see [15, 16]), 5-hydroxy-dUrd (Sefochem Fine Chemicals, Emek Hayarden, Israel), 5-propynyloxy-dUrd (see [17]), 5-cyano-dUrd (see [18]), 5-ethynyl-dUrd (see [19, 20], 5-formyl-dUrd (see [21, 22], 5-oxime of 5-formyl-dUrd (see [23]), 5-trifluoromethyl-dUrd (P-L Biochemicals, Milwaukee, WI), 5-ethyl-dUrd (see [24, 25]), 5-propyl-dUrd (see [26]), *E*-5-(2-chlorovinyl)-dUrd (see [27, 28]), *E*-5-(2-bromovinyl)-dUrd (see [20, 28, 29]), *E*-5-(2-iodovinyl)-dUrd (see [20, 29], 5-hydroxymethyl-dUrd (Calbiochem–Behring Corp., Lucerne, Switzerland), 5-azidomethyl-dUrd (see [21, 22]), 5-methylthiomethyl-dUrd (see [21, 30]), 5-methylsulfinylmethyl-dUrd (see [30]), 5-methylsulfonylmethyl-dUrd (see [30]), 5-iodoacetamidomethyl-dUrd (see [21, 22]) and 5-(1,3-dithiolan-2-yl)-dUrd (see [23]).

Radiochemicals. The radiolabelled nucleoside [methyl-^{3}H]-dThd (sp. act., 47 Ci/mmole) was obtained from the Institute of Radio-Elements (IRE, Fleurus, Belgium).

Thymidine (dThd) kinase assay. One-ml cell pellets were first washed with 0.9% NaCl–0.01 M Tris–HCl buffer, pH 8.0; then 1 ml of 0.05 M Tris–HCl, pH 8.0 containing 0.02 M β-mercaptoethanol was added. The suspension was sonicated two times for 10 sec, cleared by centrifugation at 100,000 *g* for 45 min and stored in aliquots at −70°.

The cell extracts were assayed for dThd kinase activity in a standard reaction mixture containing 5 mM ATP, 5 mM $MgCl_2 \cdot 6\ H_2O$, 9 mM KF, 5 mM phosphoenolpyruvate, 5 μg pyruvate kinase (Sigma Chemical Co.), 10 mM β-mercaptoethanol, 0.2 mM (0.1 μCi) [methyl-^{3}H]-dThd and 10 μl cell extract in a total volume of 40 μl Tris–HCl 0.05 M, pH 8.0. The reaction mixture was incubated at 37° for 15 min and the reaction terminated by addition of 75 μl of ice-cold 0.05 M Tris–HCl buffer, pH 8.0. After boiling for 2 min, the mixture was applied onto DE81 discs and washed with NH_4OOCH 1 mM, pH 8.2, ethanol and ether. The filters were then assayed for radioactivity in a toluene-based scintillant.

[Methyl-^{3}H]-dThd incorporation into cell DNA. The incorporation of [methyl-^{3}H]-dThd into cellular DNA was measured in Linbro microplates (model FB-48-TC, Linbro Chemical Co., New Haven, CT). To each well were added 10^5 L1210 cells and 5.31 pmoles (0.25 μCi) of [methyl-^{3}H]-dThd. The cells were allowed to proliferate for 20 hr at 37° in a humidified, CO_2-controlled atmosphere. At the end of this incubation period, the contents of the wells (200 μl) were brought onto 25-mm glass fiber filters (type A/E, Gelman Instrument Company, Ann Arbor, MI), mounted on a Millipore 3025 sampling manifold apparatus. The filters were washed twice with cold NaCl/P_i (phosphate-buffered saline), twice with cold 10% trichloroacetic acid, twice with cold 5% trichloroacetic acid, once with cold ethanol, and once with cold ether. The filters were then allowed to dry for 10 min at 60° and assayed for radioactivity in a toluene-based scintillant.

In preliminary experiments we established that [methyl-^{3}H]-dThd was linearly incorporated for a period of 20 hr into DNA of both TK^+ cells (i.e., L1210/0) and TK^- cells (i.e., L1210/BdUrd).

Selection of L1210/BdUrd cells. L1210 cells were seeded into tissue culture dishes (Falcon 3002F, Becton Dickinson France S.A., Grenoble, France) at 2×10^5 cells/dish in the presence of 6 ml growth medium supplemented with 25 μg/ml 5-bromo-dUrd. After 2 or 3 days, the cells were distributed to 3 new tissue culture dishes, again in the presence of 6 ml growth medium plus 25 μg/ml 5-bromo-dUrd. Then the dose of 5-bromo-dUrd was gradually increased at each cell passage until it reached 260 μg/ml. We were able to select this L1210 subline after about ten to fifteen passages. Following a similar procedure, we were also able to select L1210 sublines that were resistant to dThd (140 μg/ml) and 5-nitro-dUMP (10μg/ml).

Inhibition of tumor cell growth. All assays were also performed in Linbro microplates. The cells were suspended in growth medium and added to the microplate wells at 5×10^4 cells/well (L1210/0 and L1210/BdUrd) or 6.5×10^4 cells/well (Raji and Namalva) in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 42–48 hr (L1210 cells) or 72–84 hr (Raji and Namalva cells) at 37° in a humidified, CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter Counter (Coulter Electronics Ltd., Harpenden, U.K.). The ID_{50} (median inhibitory dose) was defined as the concentration of compound that reduced the number of living cells by 50%.

RESULTS

As reflected by their respective ID_{50} values (Table 1), the inhibitory effects of 5-substituted 2'-deoxyuridines on tumor cell growth varied markedly from one compound to the other. The extreme values were obtained for 5-fluoro-dUrd, which inhibited L1210 cell growth at an ID_{50} of 0.001 μg/ml, and for 5-methylsulfinylmethyl-dUrd, 5-hydroxy-dUrd, 5-propynyloxy-dUrd and 5-propyl-dUrd, which did not inhibit L1210 cell growth even at 1 mg/ml. The compounds with the highest activity against L1210 cells, viz. 5-fluoro-dUrd, 5-trifluoromethyl-dUrd, 5-nitro-dUMP and 5-ethynyl-dUrd, were also the most inhibitory to Namalva and TK^+ Raji cells; and those compounds that were inactive as inhibitors of L1210/0 cell growth, viz. 5-hydroxy-dUrd, 5-propynyloxy-dUrd and 5-propyl-dUrd, were also inactive against Namalva and TK^+ Raji cells. In

Table 1. Inhibitory effects of 5-substituted 2'-deoxyuridines on the proliferation of L1210/0, L1210/BdUrd, Namalva, TK$^+$ Raji and TK$^-$ Raji cells

Compound	ID_{50} (μg/ml) L1210/0*	L1210/BdUrd	Namalva	TK$^+$ Raji	TK$^-$ Raji
5-Fluoro-dUrd	0.001	1.75	0.017	0.126	1.03
5-Trifluoromethyl-dUrd	0.007	36.8	0.220	0.097	168.0
5-Nitro-dUMP	0.035	40.5	0.132	0.138	85.0
5-Ethynyl-dUrd	0.091	268	0.128	0.078	78
5-Formyl-dUrd	0.275	240.6	0.046	0.310	327
5-Hydroxymethyl-dUrd	3.61	190	17.9	—	—
5-Oxime of 5-formyl-dUrd	4.2	>1000	3.69	2.39	>1000
5-Ethyl-dUrd	8.5	>1000	9.77	19.9	864
5-Thiocyano-dUrd	22.5	165	2.79	4.58	93
(*E*)-5-(2-Iodovinyl)-dUrd	24.3	1.40	17.5	24.6	31.5
5-Bromo-dUrd	26.0	>1000	21.3	12.4	>1000
(*E*)-5-(2-Bromovinyl)-dUrd	26.9	1.25	95	36.4	44
5-Azidomethyl-dUrd	35.0	120.6	3.60	23.1	13.4
5-Iodoacetamidomethyl-dUrd	45.7	—	10.5	21.0	35.0
5-Iodo-dUrd	61.2	>1000	4.63	3.26	>1000
5-Cyano-dUrd	210	>1000	11.6	109	>1000
5-Methylsulfonylmethyl-dUrd	265	335	96	315	640
(*E*)-5-(2-Chlorovinyl)-dUrd	327	305	45.8	44.3	248
5-(1,3-Dithiolan-2-yl)-dUrd	625	>1000	460	283	>1000
5-Methylthiomethyl-dUrd	640	≥1000	174	278	>1000
5-Methylsulfinylmethyl-dUrd	>1000	>1000	520	>1000	>1000
5-Hydroxy-dUrd	>1000	>1000	>1000	>1000	>1000
5-Propynyloxy-dUrd	>1000	>1000	>1000	>1000	>1000
5-Propyl-dUrd	>1000	—	>1000	>1000	>1000

* Data for L1210/0 are taken from ref. 12.

Table 2. Ratio of the ID_{50} for TK^- Raji and L1210/BdUrd cells to the ID_{50} for their parent cell lines

Compound	$\frac{ID_{50}\ Raji\ TK^-}{ID_{50}\ Raji\ TK^+}$	$\frac{ID_{50}\ L1210/BdUrd}{ID_{50}\ L1210/0}$
5-Fluoro-dUrd	7.9	1750
5-Trifluoromethyl-dUrd	1732	5257
5-Nitro-dUMP	616	1157
5-Ethynyl-dUrd	1000	2945
5-Formyl-dUrd	1055	875
5-Hydroxymethyl-dUrd	—	52.6
5-Oxime of 5-formyl-dUrd	>418	>238
5-Ethyl-dUrd	43	>118
5-Thiocyano-dUrd	20	7.33
(*E*)-5-(2-Iodovinyl)-dUrd	1.2	0.06
5-Bromo-dUrd	>81	>38.5
(*E*)-5-(2-Bromovinyl)-dUrd	1.2	0.05
5-Azidomethyl-dUrd	0.6	2.93
5-Iodoacetamidomethyl-dUrd	1.7	—
5-Iodo-dUrd	>306	>16.3
5-Cyano-dUrd	>9.2	>4.76
5-Methylsulfonylmethyl-dUrd	2.0	1.26
(*E*)-5-(2-Chlorovinyl)-dUrd	5.6	0.93
5-(1,3-Dithiolan-2-yl)-dUrd	>3.5	>1.60
5-Methylthiomethyl-dUrd	>3.6	≥1.56
5-Methylsulfinylmethyl-dUrd	—	—
5-Hydroxy-dUrd	—	—
5-Propynyloxy-dUrd	—	—
5-Propyl-dUrd	—	—

general, the ID_{50} values for the two human lymphoblast cell lines corresponded well to the ID_{50} values for L1210/0 cells, except for (*E*)-5-(2-chlorovinyl)-dUrd and 5-iodo-dUrd, which were about 10–20 times more inhibitory to Namalva and TK^+ Raji cells than to L1210/0 cells, and for 5-azidomethyl-dUrd and 5-cyano-dUrd, which were about 10–20 times more inhibitory to Namalva than to L1210 and TK^+ Raji cells (Table 1).

While the ID_{50} values for TK^+ Raji cells were roughly similar to those recorded for Namalva and L1210/0 cells (with the few exceptions noted above), no such correspondence was observed either between the ID_{50} values for TK^+ Raji cells and for TK^- Raji cells, or between the ID_{50} values for L1210/0 and for L1210/BdUrd cells (Table 1). For most compounds, the ID_{50} for TK^- Raji and for L1210/BdUrd cells was significantly higher than the ID_{50} for the TK^+ Raji and L1210/0 cells, and for the more active compounds, namely 5-trifluoromethyl-dUrd, 5-formyl-dUrd and 5-ethynyl-dUrd the difference in ID_{50} for TK^- and TK^+ Raji cells, as well

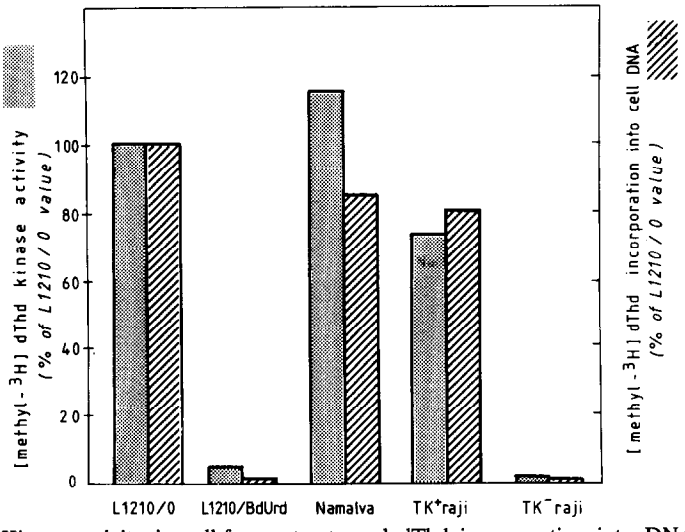

Fig. 1. dThd Kinase activity in cell-free extracts and dThd incorporation into DNA of L1210/0, L1210/BdUrd, Namalva, TK^+ Raji and TK^- Raji cells.

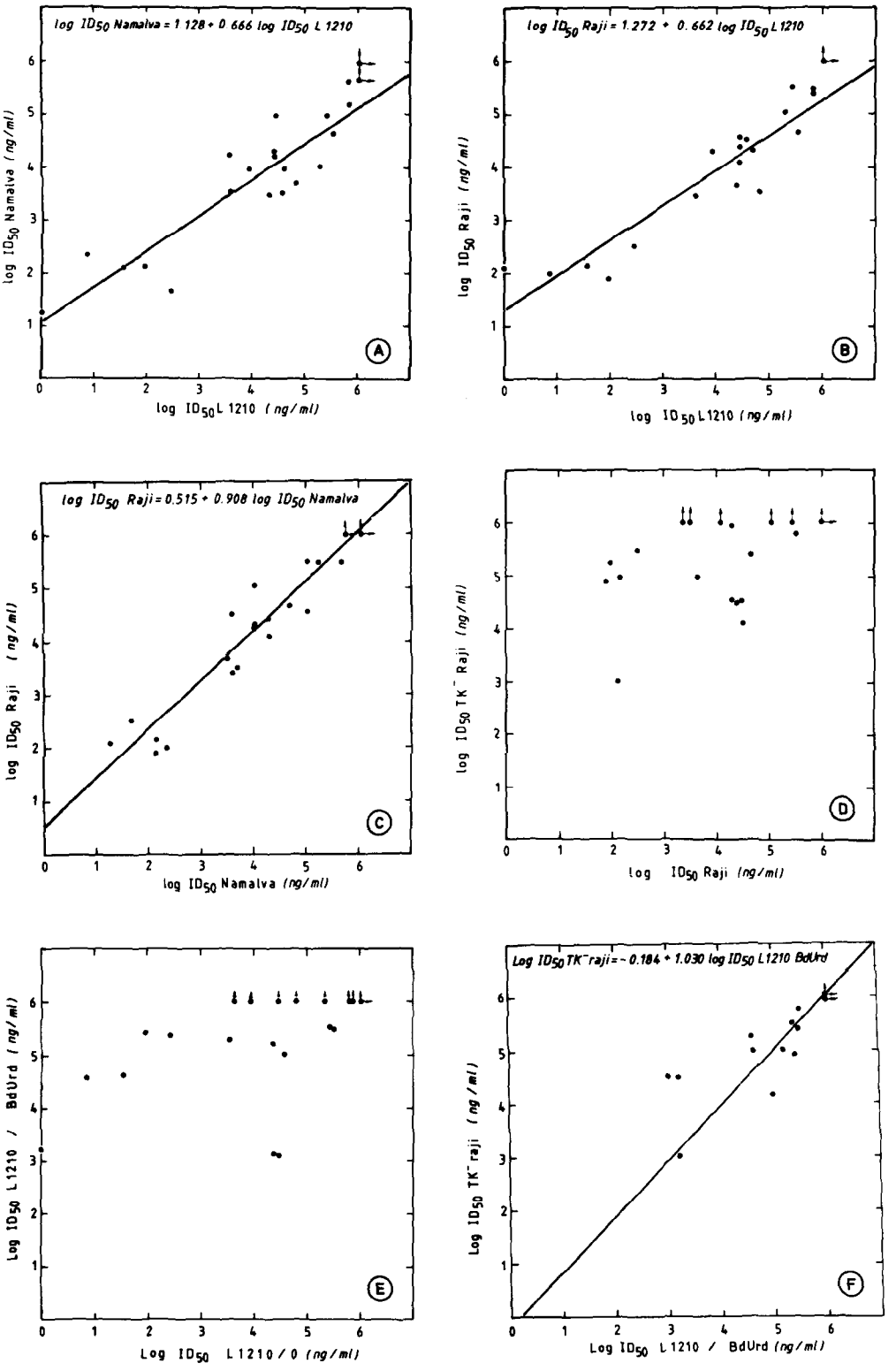

Fig. 2. Correlations between the log ID_{50} values for several cell lines. (A) Linear regression line for log ID_{50} for Namalva cell growth as a function of log ID_{50} for L1210 cell growth. (B) Linear regression line for log ID_{50} for TK^+ Raji cell growth as a function of log ID_{50} for L1210 cell growth. (C) Linear regression line for log ID_{50} for TK^+ Raji cell growth as a function of log ID_{50} for Namalva cell growth. (D) Log ID_{50} for TK^- Raji cell growth as a function of log ID_{50} for TK^+ Raji cell growth. (E) Log ID_{50} for L1210/BdUrd cell growth as a function of log ID_{50} for L1210/0 cell growth. (F) Linear regression line for log ID_{50} for TK^- Raji cell growth as a function of log ID_{50} for L1210/BdUrd cell growth.

as the difference in ID_{50} for L1210/BdUrd and L1210/0 cells, were about 1000-fold (Table 2). However, for a limited number of dUrd derivatives, i.e., 5-azidomethyl-dUrd, 5-methylsulfonylmethyl-dUrd and 5-iodoacetamidomethyl-dUrd, the differences in ID_{50} for the mutant and parent cell lines were rather small. Furthermore, (*E*)-5-(2-bromovinyl)-dUrd and (*E*)-5-(2-iodovinyl)-dUrd were equally active against TK^- and TK^+ Raji cells, yet 20 times more inhibitory to L1210/BdUrd than to L1210/0 cells (Table 2).

That TK^- Raji and L1210/BdUrd cells were deficient in dThd kinase activity, was assessed by measuring (i) the incorporation of [methyl-^{3}H]-dThd into cellular DNA, and (ii) the dThd kinase activity in extracts prepared from the cells (Fig. 1). For L1210/BdUrd and TK^- Raji cells [methyl-^{3}H]-dThd incorporation into DNA was decreased to 0.86% and 0.36% of the L1210/0 cell value (35.95 ± 3.25 pg per 10^5 cells per hr). Concomitantly, the dThd kinase activity decreased to 4.77% (L1210/BdUrd) and 1.61% (TK^- Raji), relative to 100% for L1210/0 (18.22 ± 9.86 ng per mg protein per hr) (Fig. 1).

DISCUSSION

With the 24 dUrd analogs that were evaluated for inhibition of tumor cell growth there appeared to be a linear correlation between the log ID_{50} for L1210 cell growth and the log ID_{50} for Namalva cell growth (Fig. 2A) and TK^+ Raji cell growth (Fig. 2B). The correlation coefficients* (r) were 0.901 and 0.929, respectively. There was also a strong linear correlation ($r = 0.936$) between the log ID_{50} for Namalva and TK^+ Raji cells (Fig. 2C). Thus, from the results obtained with a given 5-substituted 2′-deoxyuridine in the mouse L1210 tumor cell system, one might readily predict its inhibitory activity for human lymphoblast cell lines such as Namalva and Raji.

However, this extrapolation only holds for TK^+ cell lines. Indeed, when the ID_{50} values for TK^- Raji cells were compared with the ID_{50} values for TK^+ Raji cells (Fig. 2D), Namalva or L1210/0 cells (data not shown), the correlation was not significant: $r =$ 0.270, 0.352 and 0.385, respectively. In addition, when the ID_{50} values for L1210/BdUrd cells were compared with the ID_{50} values for L1210/0 cells (Fig. 2E), Namalva or TK^+ Raji cells (data not shown), the correlation coefficients were again very low: $r = 0.251$, 0.020 and 0.024, respectively. In contrast, the correlation coefficient between the log ID_{50} for TK^- Raji and the log ID_{50} for L1210/BdUrd was 0.654 (Fig. 2F).

Most compounds inhibited TK^- Raji and L1210/BdUrd cell growth at a dose that was higher by several orders of magnitude than the dose required to inhibit TK^+ Raji and L1210/0 cells. 5-Trifluoromethyl-, 5-nitro-, 5-ethynyl-, 5-formyl-dUrd and the 5-oxime of 5-formyl-dUrd did not inhibit TK^- Raji and L1210/BdUrd cell growth unless they were applied at 100–5000 times the ID_{50} for TK^+ Raji and L1210/0 cell growth. For these compounds the cell-growth inhibitory effects would seem highly dependent on the presence of thymidine kinase activity in the host cell. It is likely that these compounds must first be phosphorylated to their 5′-monophosphate form before they could exert their antitumor action.

According to our previous studies, the target for the antitumor action of 5-substituted dUrd derivatives such as 5-trifluoromethyl-dUrd, 5-formyl-dUrd, 5-ethynyl-dUrd, 5-nitro-dUrd and the 5-oxime of 5-formyl-dUrd may well be thymidylate synthetase [12]. Other compounds such as 5-iodo-dUrd and 5-bromo-dUrd may owe their cytotoxic action primarily to incorporation into host cell DNA [31]. This incorporation is also dependent on prior phosphorylation by the thymidine kinase.

It is noteworthy that the phosphorylated form of 5-nitro-dUrd, 5-nitro-dUMP, was only weakly active against TK^- Raji cells (Table 1). This may indicate that the compound was first dephosphorylated to its nucleoside form before or immediately after it had been taken up by the cells.

5-Fluoro-dUrd was only 8 times less active against TK^- than against TK^+ Raji cells (Table 1). If the antitumor action of 5-fluoro-dUrd was solely based on an inhibition of thymidylate synthetase [2, 12], one may have expected a greater difference in its activity towards TK^- and TK^+ cells and higher ID_{50} values for TK^- Raji and L1210/BdUrd cells. The inhibitory activity of 5-fluoro-dUrd in TK^- Raji and L1210/BdUrd cells should be mediated by one or another mechanism which does not require previous phosphorylation. For example, 5-fluoro-dUrd could be cleaved to deoxyribose and 5-fluorouracil [32–34], and the latter may be incorporated into cellular RNA [1, 35], thereby producing a fraudulent RNA.

Unlike the other dUrd analogs, (*E*)-5-(2-iodovinyl)-dUrd and (*E*)-5-(2-bromovinyl)-dUrd inhibited the growth of L1210/BdUrd cells to a greater extent than the growth of the parent L1210/0 cells. These findings argue against the role of the normal cellular TK activity in the antitumor activity of (*E*)-5-(2-bromovinyl)-dUrd and (*E*)-5-(2-iodovinyl)-dUrd. In fact, (*E*)-5-(2-bromovinyl)-dUrd showed little, if any, affinity for the dThd kinase of L1210/0 ($K_i/K_m = 223$) and TK^+ Raji cells ($K_i/K_m > 100$).

The relatively strong inhibitory effects of (*E*)-5-(2-bromovinyl)-dUrd on the growth of TK^- mutant cell lines (TK^- Raji and L1210/BdUrd) may be achieved by a mechanism that is independent of the cell's dThd kinase activity. However, (*E*)-5-(2-bromovinyl)-dUrd might still act through the phosphorylation pathway if it were specifically phosphorylated in the TK^- mutant cell lines (as a consequence of the altered substrate affinity of the dThd kinase).

In preliminary attempts to elucidate the mechanism of cytotoxicity of (*E*)-5-(2-bromovinyl)-dUrd, we have determined its affinity for the dThd kinase of the different cell lines tested. Its K_i/K_m for L1210/BdUrd dThd kinase was 2.65, as compared to 223 for L1210/0 dThd kinase (average of 4 experiments). Thus, in parallel with an increased cytotoxic response, (*E*)-5-(2-bromovinyl)-dUrd showed a lower K_i/K_m value for the dThd kinase of the TK^- mutant cell line. This lower K_i/K_m ratio may be

* When determining the correlation coefficients, the ID_{50} values > 1000 μg/ml were not taken into account.

interpreted to mean that (i) (*E*)-5-(2-bromovinyl)-dUrd serves as a better inhibitor of the mutant cell dThd kinase than of the parent cell dThd kinase, or (ii) if it is phosphorylated by these dThd kinases, it may be phosphorylated to a greater extent by the mutant than by the parental cell line. Additional experiments (with radiolabeled compound) will be required to further examine these (and other) possibilities.

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