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Thymidine Kinase as a Determinant of the Response to 5-Fluoro-2′-deoxyuridine in Transplantable Murine Leukemias

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SUMMARY

The responsiveness to 5-fluoro-2′-deoxyuridine of different transplantable mouse leukemias *in vivo* was inversely correlated with tumor cell levels of thymidine kinase (EC 2.7.1.21). This enzyme transforms the drug to the pharmacologically active nucleotide 5-fluoro-2′-deoxyuridylic acid. However, it also provides an alternative pathway for circumventing the drug-induced blockade of thymidylate biosynthesis.

INTRODUCTION

The antitumor agent 5-fluoro-2′-deoxyuridine is a direct precursor of 5-fluoro-2′-deoxyuridylic acid, which is a potent inhibitor of thymidylate synthetase (1, 2). Thymidine kinase (EC 2.7.1.21) has a dual role in the pharmacology of FUdR,[2] serving both to convert the drug to dFUMP (3) and to provide a pathway for circumventing the dFUMP blockade of thymidylate biosynthesis (4). The first step in FUdR catabolism is degradation of the compound to 5-fluorouracil by uridine phosphorylase (EC 2.4.2.3) (5). The FU can be converted to FUMP by sequential action of uridine phosphorylase and uridine kinase (EC 2.7.1.48) (6) or by a phosphoribosyltransferase (7). Pathways for conversions of FUMP to dFUMP have not yet been established. A summary of transformations of FU is shown in Fig. 1.

The present study was designed to delineate determinants of responsiveness to FUdR in the transplantable murine leukemias, and to determine which role of thymidine kinase would be most relevant to FUdR responsiveness.

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[2] The abbreviations used are: FUdR, 5-fluoro-2′-deoxyuridine; dFUMP, 5-fluoro-2′-deoxyuridylic acid; FU, 5-fluorouracil; FUMP, 5-fluorouridylic acid.

MATERIALS AND METHODS

Chemicals. FUdR-2-^{14}C (12 mCi/mmole) and thymidine-2-^{14}C (20 mCi/mmole) were purchased from Calbiochem. Unlabeled FU and FUdR were provided by Hoffmann-La Roche, and dFUMP by Dr. Charles Heidelberger. Other chemicals were purchased from commercial sources.

Animal tumors. Sources of ascitic tumor

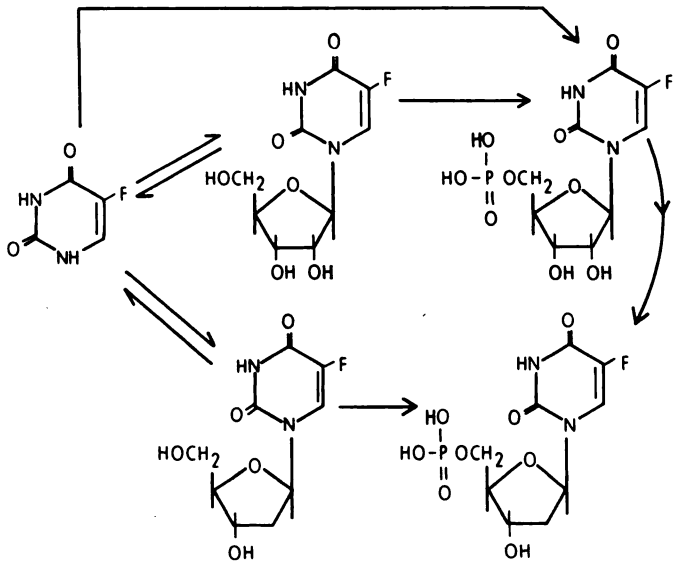

FIG. 1. *Pathways of FU and FUdR metabolism*

cell lines have been described (8). All tumors were carried intraperitoneally in murine hosts. Survival data were obtained by treatment of tumor-bearing animals, from day 1 to day 10 after transplantation, with FUdR (80 mg/kg/day). Survival times were calculated in terms of percentage increase in the life span of treated vs. control animals. Groups of at least 10 animals were used to establish survival data for each tumor line (Fig. 2).

Preparation of cell extracts. Freshly isolated tumor cells (9) were suspended in 1.5 volumes of 0.25 M sucrose containing 0.05 M Tris-HCl buffer (pH 7.6), 5 mM mercaptoethanol, and 1 mM EDTA. The suspensions were rapidly frozen, thawed in a 10° bath, treated briefly with a sonic oscillator to disperse clumps, and then centrifuged at 100,000 × *g* for 30 min. The supernatant fluid was collected and used for studies of enzyme levels. Protein levels ranged from 7.5 to 15 mg/ml. Dialysis of the fluid at 4° against 0.05 M Tris buffer (pH 7.5) containing 5 mM mercaptoethanol and 1 mM EDTA did not change any result reported here. The cell extracts could be stored at −20° for several weeks without loss of activity, although repeated freezing and thawing led to some inactivation of uridine phosphorylase.

Measurement of enzyme levels. To measure levels of thymidine kinase and uridine phosphorylase, a modification (10) of Sköld's procedure (11) was used. Portions of 25 μl of the cell extracts were diluted with an equal volume of a mixture containing 2 mM labeled nucleoside (thymidine-2-^{14}C or 5-fluoro-2′-deoxyuridine-2-^{14}C), 12 mM ATP, 30 mM potassium phosphate, and 120 mM Tris-HCl, the two buffers being adjusted to pH 7.5. After incubation for 2, 5, 10, or 20 min at 37°, 5 μl of 0.1 M acetic acid containing 100 mM carrier nucleoside, pyrimidine base, and nucleotide were added, and the mixture was heated to 100° for 10 sec. The precipitated protein was removed by centrifugation, and a 10-μl aliquot of the supernatant fluid was analyzed by thin-layer chromatography (10) to determine the rate of conversion of the labeled nucleoside to the pyrimidine base (via uridine phosphorylase) and to the nucleotide (via thymidine kinase). The observed rates were linear for at least 10 min. The results obtained by this procedure were confirmed by an optical

assay for phosphorylase activity (12), and by the DEAE-cellulose disc method for detection of thymidine kinase (13). Protein levels were measured by the method of Lowry *et al.* (14).

RESULTS AND DISCUSSION

Survival studies. The drug-promoted increase in survival time of tumor-bearing animals is shown in Table 1.

Enzymes involved in FUdR metabolism. A summary of the data obtained is shown in Fig. 2 and Table 1. Levels of thymidine kinase were measured with labeled thymidine as the substrate. Substitution of FUdR did not alter the relationship between enzyme activity and responsiveness to FUdR shown in Fig. 2; in these cell lines FUdR was phosphorylated about as rapidly as thymidine. Phosphorolysis of FUdR by all

TABLE 1

Pyrimidine phosphorylase levels in cell extracts

Cell extracts were incubated with labeled FUdR or thymidine (TdR), $MgCl_2$, ATP, inorganic phosphate, and Tris-HCl buffer as described in the text. Initial rates of product formation were calculated from chromatographic analysis of reaction mixtures.

Cell line	Increased life span	Uridine phosphorylase level[b] measured with	
		TdR	FUdR
	%	*μmoles/g protein/hr*	
P288	10	5	9
Lymphoma 4	15	—[c]	4
RCS	25	—	8
Mecca	50	—	8
P388/VCR	55	130	230
P388/38280	60	0.5	1.5
L1210	60	3	5
Lymphoma 2	70	—	11
P388	80	22	45
P815/VLB	116	—	3.5
P1534Ja	200	8	12

[a] Increase in life span of tumor-bearing animals produced by administration of FUdR. See the legend to Fig. 2 for derivation of these numbers.

[b] Quantities refer to production of 5-fluorouracil or thymine.

[c] Not done.

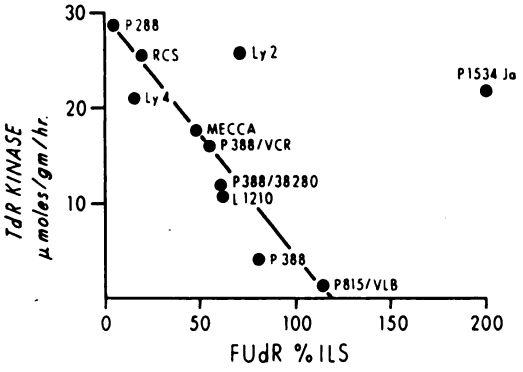

FIG. 2. *Correlation between thymidine (TdR) kinase levels and responsiveness to FUdR as measured by increase in life span (ILS)*

Enzyme activity was determined with labeled thymidine as the substrate, as described in the text. Survival data are reported in terms of percentage increase in the life span of drug-treated vs. untreated tumor-bearing animals.

extracts proceeded more rapidly than the analogous conversion of thymidine to thymine (Table 1). In related studies, Roberts (15) examined levels of thymidylate synthetase in four tumor lines: L1210, P288, P388, and P1534Ja. The data show no correlation between levels of this dFUMP-inhibited enzyme and responsiveness of the different cell lines to FUdR.

We found that inherent or "natural" resistance of nine of these murine leukemias to FUdR was inversely correlated with their capacity to form dTMP from thymidine and dFUMP from FUdR. The capacity of the cell lines to degrade FUdR to FU was not an important determinant of responsiveness to FUdR (compare P388, P388/VCR, and P388/38280, Table 1). Data derived from studies *in vitro* do not necessarily reflect the situation *in vivo*. The present study suggests that the cell lines least responsive to FUdR contain the highest levels of thymidine kinase.

An explanation for this apparent paradox might be that the action of thymidine kinase, i.e., formation of dTMP from thymidine, serves to circumvent the blockade by dFUMP of thymidylate synthesis *de novo*. The validity of this explanation would de-

pend on the availability of an exogenous supply of thymidine in FUdR-treated animals. Alternatively, the presence of high levels of thymidine kinase in certain FUdR-resistant lines, e.g., P288, might be associated with other abnormalities in levels or characteristics of enzymes involved in DNA synthesis which could be responsible for inherent resistance to FUdR.

The behavior of the Lymphoma 2 and P1534Ja cell lines remains unexplained. These lines showed greater responsiveness to FUdR than expected from rates of TdR phosphorylation (Fig. 2). Perhaps such lines have low exogenous supplies of thymidine. Alternatively, the "abnormality" leading to the insensitivity of P288 to FUdR, associated with high thymidine kinase activity, could be lacking in P1534Ja. Further discussion must await the delineation of the role of thymidine kinase in cell division.

In contrast to the results shown here, selection for resistance to FUdR by exposure to the drug led to a *fall* in thymidine kinase activity (16–18). In the particular cell lines employed, conversion of the drug to dFUMP by thymidine kinase may have been the only route of dFUMP formation. Since two FUdR-resistant cell lines so derived were responsive to 5-fluorouracil (18), the uridine phosphorylase-catalyzed interconversion of FUdR and FU was apparently absent, with thymidine kinase the only route of dFUMP formation from FUdR. The presence of a phosphoribosyltransferase (7) could account for the conversion of FU to pharmacologically active nucleotide forms in a cell line lacking uridine phosphorylase. An example of this phenomenon has been reported elsewhere (10). We emphasize that the data reported here apply only to cell lines exhibiting various degrees of natural or inherent, as opposed to drug-induced, resistance to FUdR.

The ineffectiveness of FUdR against human leukemias might be explained, in part, by the high levels of thymidine kinase that have been found in human leukemic cells (19–21). Thymidine kinase levels were also generally elevated in malignant as compared with normal human tissue (22).

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