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DIFFERENTIAL SELECTIVITY OF 5-FLUOROURACIL AND 5′-DEOXY-5-FLUOROURIDINE IN CULTURED HUMAN B LYMPHOCYTES AND MOUSE L1210 LEUKEMIA*

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Abstract—The role of differential metabolic activation of a 5-fluorouracil (FU) prodrug, 5′-deoxy-5-fluorouridine (dFUR), in achieving selective cytotoxocity was investigated in cultured human B lymphocytes and murine leukemia L1210 cells. B cells were cross-sensitive to FU and dFUR. On the other hand, leukemia L1210 cells were sensitive to FU but resistant to dFUR. The difference in the biological activities of FU and dFUR in B and L1210 cells correlated with (a) the metabolism of dFUR to FU by intact B (60% conversion) and L1210 (no conversion) cells, and (b) the phosphorylase activity of B (660 nmoles converted in 2 hr per mg protein) and L1210 (undetectable) cells. The intracellular metabolism of FU and dFUR was studied using a reversed-phase ion-pair high pressure liquid chromatographic assay. FU and dFUR shared similar metabolic pathways in B cells; their anabolites included FU ribose and deoxyribose nucleosides and nucleotides. In L1210 cells, FU was anabolized to 5-fluorouridine triphosphate and 5-fluorodeoxyuridine monophosphate, whereas dFUR was present mainly as the unchanged drug. Further metabolism studies using dFUR with tritium label in either the FU moiety or the altered sugar moiety established that the metabolic pathway of dFUR to cytotoxic FU anabolites in the B cells was via phosphorolysis to FU. These data indicate that, on a cellular level, an FU prodrug such as dFUR, which is activated by cytosolic enzyme, has a different selectivity from that of FU, and that the basis of differential selectivity is the initial phosphorolysis to FU.

5-Fluorouracil (FU) is widely used in the palliative treatment of solid tumors; its antitumor activity stems from the proximal metabolites, including 5-fluorodeoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP) [1]. One of the major difficulties encountered in FU therapy is its severe host toxicities [2]. In the past decade, extensive research efforts have been directed toward the development of FU prodrugs, in search of an antitumor agent with improved tissue selectivity over that of FU. Theoretically, there may be two types of metabolic prodrugs. A prodrug activated by microsomal enzymes such as cytochrome P-450 would be primarily metabolized in the liver, and the FU generated would be distributed to tissues via the general circulation. In this case, the prodrug would behave as a depot of FU and would have no therapeutic advantage over slow FU infusion. Alternatively, a prodrug activated by soluble enzymes would be metabolized in the target tissues, and the intracellularly generated FU would be further utilized. The tissue selectivity of this type of prodrug may, therefore, differ from that of FU. This is supported by the results of recent studies of ftorafur (FT), an FU metabolic prodrug. The antitumor activity of FT was observed in the presence of sub-therapeutic FU plasma concentrations following FT administration [3–5]. This, together with the observation that FT is metabolized by both the cytosol [6] and microsomal enzymes [7–9], suggests that a fraction of the FT dose is activated to FU in the target sites, which is consistent with the observed difference in tissue toxicity between FT and FU, i.e. FT has reduced myelosuppression and increased neurotoxicity [10].

The structures of FU and its analog, 5′-deoxy-5-fluorouridine (dFUR), are compared in Fig. 1. dFUR was synthesized by Cook *et al.* [11]; it has a better therapeutic index than FU in several animal tumors and is metabolized to FU by uridine phosphorylase (EC 2.4.2.3) [12–14], a cytosol enzyme [15, 16]. The selective activation of dFUR by Ehrlich ascites cells has been considered to be the basis for the improved therapeutic index in animals [17].

In this study, the differential selectivity of FU and dFUR was evaluated in cultured human B lymphocytes and leukemia L1210 cells in mice. The relationship of the selectivity of dFUR to its activation to FU by uridine phosphorylase, and to the sequential intracellular metabolism of radiolabeled dFUR to FU and cytotoxic FU anabolites was established.

METHODS

Chemicals and reagents. All chemicals and reagents used were of analytical grade or spectroquality. Scintillation counting mixture was obtained from the Amersham Corp. (Arlington Heights, IL). dFUR, [2-^{14}C]dFUR (sp. act. 105 μCi/mmole), and

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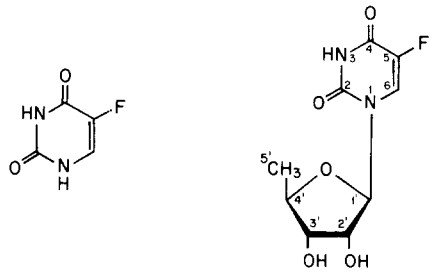

Fig. 1. Structures of FU and dFUR.

[5′-^{3}H]dFUR (sp. act. 14 μCi/μmole) were gifts from Hoffman-LaRoche (Nutley, NJ). Their purities were analyzed by high pressure liquid chromatography (HPLC). Unlabeled dFUR was >99% pure; 0.1% was present as FU and 0.3% as 5-fluorouridine (FUR). Five percent of the [2-^{14}C]dFUR was represented by [2-^{14}C]FU. [5′-^{3}H]dFUR was >99% pure. [2-^{14}C]FU (sp. act. 50 μCi/μmole), [6-^{3}H]FU (sp. act. 18 Ci/mmole), [6-^{3}H]dFUR (sp. act. 750 mCi/mmole), and [6-^{3}H]deoxyuridine (UdR) (sp. act. 20 μCi/mmole) were purchased from Moravek Biochemicals (City of Industry, CA). RPMI-1640 culture medium was obtained from Gibco (Grand Island, NY). 5-Fluorouridine diphosphate (FUDP) and triphosphate, and 5-fluorodeoxyuridine diphosphate (FdUDP) and triphosphate (FdUTP), were obtained from Sierra Bioresearch (Tucson, AZ).

Instruments. Drug analysis was done on an HPLC unit consisting of a M6000A solvent pump equipped with a three-way solvent draw valve, a 440 dual wavelength u.v. detector at 254 and 280 nm, and a U6K injector (Waters Associates, Milford, MA). HPLC was run at ambient temperature at constant flow rate. Radioactivity was determined using a Packard 3330 scintillation counter (Packard Instrument, Downers Grove, IL). Quenching was corrected by an external standard method. The counting efficiencies were 40 and 85% for ^{3}H and ^{14}C respectively. Spectrophotometric assays were run on a Cary 118 (Varian Instruments, Palo Alto, CA).

Cell procurement. Cultured human B (RPMI-1788) cells harvested during the mid-log growth phase were used in this investigation. This cell line has been characterized previously [18] and is free of mycoplasma organism. The doubling time for these cells is 24 hr. Leukemia L1210 cells were obtained on day 4 after transplantation of 10^6 cells i.p. into female DBA/2 mice (Charles River, MA).

Inhibition of cell growth. B cells ($3–5 \times 10^5$/ml) were incubated with various concentrations of dFUR and FU in a culture medium consisting of 3.5% heat-inactivated fetal calf serum (Gibco) and 220 mg/100 ml sodium bicarbonate in RPMI-1640 for 24, 48 or 72 hr. The number of trypan-blue-excluding cells, considered as viable cells, remaining at the end of the incubation period was determined.

Inhibition of incorporation of [6-^{3}H]UdR into DNA. B and mouse L1210 cells at 5×10^6 cells/ml of complete medium were incubated with 10 μm concentrations of the drugs for 2 hr at 37°. The complete medium consisted of 10% mycoplasma-tested horse serum in RPMI-1640, and a buffer system of 8 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethane sulfonic acid and 16 mM morpholinopropane sulfonic acid (Sigma Chemical Co., St. Louis, MO) in 0.7 N NaOH, pH adjusted to 7 with hydrochloric acid. At the end of the incubation period, cells were removed and centrifuged at 800 *g* for 3 min. The relative concentrations of dFUR and its metabolite, FU, in the cell-free supernatant fractions were analyzed by HPLC. Cells were washed three times with ice-cold culture medium and resuspended in drug-free medium and incubated with [6-^{3}H]UdR (1 μCi/ml) for 30 min at 37°. Small aliquots of cell suspensions were removed at the end of incubation period and the number of trypan-blue-excluding cells was determined to ensure no substantial loss of viable cells. Cell pellets obtained after incubation with [6-^{3}H]UdR were precipitated with 100 μl of 6% perchloric acid in 0.1 M ammonium formate buffer. The acid-insoluble pellets were washed twice with 6% perchloric acid, and the DNA was digested by boiling the pellets in 6% perchloric acid for 15 min. DNA content was determined using a spectrophotometric assay [19], and the radioactivity in the DNA fraction was determined.

Antitumor activity in mice bearing L1210 leukemia cells. L1210 cells (10^6) were transplanted i.p. in 8- to 9-week-old female DBA/2 mice. Chemotherapy was started 24 hr after transplantation with i.p. injections of dFUR at 250–500 $mg \cdot kg^{-1} \cdot day^{-1}$ for 1–5 days, or of FU at 20–100 $mg \cdot kg^{-1} \cdot day^{-1}$ for 1–5 days. Concentrations of dFUR in the peritoneal fluid immediately, and 30 and 60 min after injection of 500 mg/kg were determined.

Phosphorylase activity of 105,000 g cellular fraction. The activities of uridine phosphorylase in B and L1210 cells harvested during the mid-log growth phase were determined. Cells were suspended in 1.15% potassium chloride in 10 mM phosphate buffer, pH 6.6, and lyzed by sonication at 60% power for 60 sec in a Sonic 300 dismembrator (Artek Systems Corp., Farmingdale, NY). After centrifugation at 105,000 *g* at 4° for 1 hr, the supernatant cellular fractions were obtained. dFUR at 167 μM concentrations was incubated separately with extracts of about 10^7 cells at 25° for 2–24 hr. FU was incubated with a similar mixture of enzyme solution, as a control measure of its stability under these conditions. The disappearance of dFUR and the formation of FU were analyzed by HPLC. Protein contents in the supernatant fraction were determined by the method of Lowry *et al.* [20].

Intracellular metabolism of radiolabeled FU and dFUR. B or mouse L1210 cells at about 5×10^6 cells/ml were incubated with 1 μCi of drugs at 37° for up to 2 hr. [2-^{14}C]FU, [6-^{3}H]FU, [2-^{14}C]dFUR, and [6-^{3}H]dFUR were used at a final radioactivity concentration of 1 μCi/ml of incubation mixture, and [5′-^{3}H]dFUR at 2 μCi/ml. The corresponding drug concentrations of the incubation mixtures were 40 μM for [2-^{14}C]FU and [2-^{14}C]dFUR, 1.3 to 10.0 μM for [6-^{3}H]dFUR, 7.5 to 10.0 μM for [6-^{3}H]FU, and 164.0 μM for [5′-^{3}H]dFUR. dFUR molecular species, with tritium label in different portions of the molecule, i.e. [6-^{3}H]dFUR with ^{3}H

in the FU moiety and [5'-^{3}H]dFUR with ^{3}H in the altered sugar moiety, were used to establish the metabolic pathways of dFUR to FU nucleosides and nucleotides. At the end of the incubation period, cells were removed and washed free of the drugs with ice-cold saline. The cellular macromolecules were precipitated with 200 μl acetonitrile, and the soluble cellular constituents were extracted with 300 μl distilled water. The macromolecule-free fractions were transferred and evaporated under a gentle stream of nitrogen to about 50 μl, and analyzed for intracellular metabolites of FU and dFUR by a reversed-phase ion-pair high pressure liquid chromatographic (IP-HPLC) assay.

HPLC analysis. Quantitation of dFUR in the peritoneal fluid and of dFUR and FU in culture medium during metabolism studies by either intact cells or 105,000 *g* cellular fractions was done using an HPLC assay. These compounds were eluted from a μBondapak C_{18} reverse phase column (Waters Associates) with 1.5% acetonitrile in 2.5 mM ammonium acetate buffer, pH 4.0. The elution volumes of FU and dFUR were 5.0 and 28.6 ml respectively. The identities of the u.v. absorbing peaks were further determined by the characteristic u.v. absorbance ratios at 254:280 nm of 1.5 and 1.0 for FU and dFUR respectively.

The intracellular metabolism of FU and dFUR to FU nucleosides and nucleotides was studied using a previously described IP–HPLC assay by two-step isocratic elution from μBondback C_{18} column with two solvents [21]. The solvent composition was slightly modified to prolong the column life. Solvent A consisted of 2.5 mM tetraethylammonium hydroxide, 0.2 mM tetrabutylammonium hydrogen sulfate, 2.5 mM sodium acetate, 2.0 mM sodium/potassium phosphate, and 2% methanol, pH adjusted to 6.0 with acetic acid. Solvent B contained an additional 30 mM phosphate. Under the conditions used, the chromatographic behaviours of uracil derivatives were similar to those of their fluorinated analogs. Therefore, uridine diphosphoglucose (UDPG) was used as a marker for 5-fluorouridine diphosphoglucose (FUDPG). After FU, dFUR, FU nucleosides, FU monophosphate nucleotides, and UDPG had been eluted with solvent A, solvent B was passed through the column and the di- and triphosphate nucleotides were eluted subsequently. The elution volumes of FU, FUR, 5-fluorodeoxyuridine (FUdR), 5-fluorouridine monophosphate (FUMP), dFUR, FdUMP, UDPG, FUDP, FdUDP, and FUTP were 5.5, 12.2, 17.1, 26.4, 38.4, 48.0, 63.8, 83.6, 89.0, and 95.4 ml respectively. The baseline separation of these ten components allowed clear distinction and identification of each radioactivity peak eluting from the HPLC column. The radioactivities in 1-min fractions of eluents were determined. Quenching by HPLC solvents was 25% for ^{3}H and 15% for ^{14}C.

RESULTS

Inhibition of growth of B lymphocytes. The growth inhibitory effects of FU and dFUR on B cells were concentration and time dependent. After 24 hr, the growth inhibition ranged from 0 to 30%, with cell viability of greater than 90%. The most pronounced effects were observed after 72 hr, and the results are illustrated in Fig. 2. The viability of the control cells was 95%. The viability of drug-treated cells varied inversely with drug concentration and ranged from 1 to 99% for FU and from 38 to 90% for dFUR. At 4 μM concentrations, FU and dFUR inhibited the growth of B cells at 72 hr by 80–90%. The corresponding concentrations for 50% growth inhibition of B cells were 0.5 and 1.3 μM for FU and dFUR respectively.

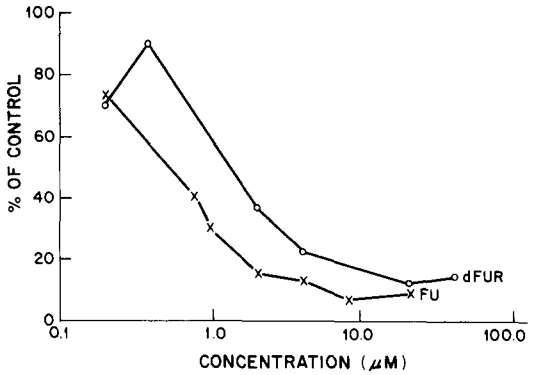

Fig. 2. Growth inhibitory effects of FU and dFUR on cultured human B lymphocytes. Results represent four separate experiments where B cells at 3–5 × 10^5/ml were grown for 72 hr. The final concentrations of trypan-blue-excluding cells in the controls ranged from 8 to 20 × 10^5/ml. Numbers of trypan-blue-excluding cells after incubation with various drug concentrations are expressed as percents of control.

Antitumor activity against mice bearing leukemia L1210. The average survival time (AST) in the untreated group was 7.5 ± 0.5 days (mean ± S.D., N = 5). The AST of animals treated with a single 100 mg/kg dose of FU was 8.1 ± 0.3 days (N = 5), while treatment with a divided dose of 20 mg·kg^{-1}·day^{-1} for 5 days increased the AST by 40% to 10.5 ± 1.2 days (N = 5). In contrast, dFUR at dosages up to 500 mg·kg^{-1}·day^{-1} for 5 days had no antitumor activity against this tumor, and the AST of the dFUR-treated group was 7.0 ± 0.5 days (N = 5).

Concentration of dFUR in peritoneal fluid. The averaged concentrations of dFUR in peritoneal fluid,

Table 1. Inhibition of [6-^{3}H]UdR incorporation into DNA*

Drug	% of control	
	B	L1210
FU	73.4 ± 7.9† (5)	11.0 ± 3.4† (8)
dFUR	82.7 ± 14.0† (6)	91.6 ± 6.4‡ (4)

* Cells were incubated with a 10 μM concentration of drugs at 37° for 2 hr, washed, and incubated with [6-^{3}H]UdR for 30 min. Results are expressed as means ± S.D. of observations obtained from an individual experiment. Numbers of observations are in parentheses. Statistical analysis was done using an unpaired, two-tailed *t*-test.

† Significantly different from control, $P < 0.001$.

‡ Not significantly different from control.

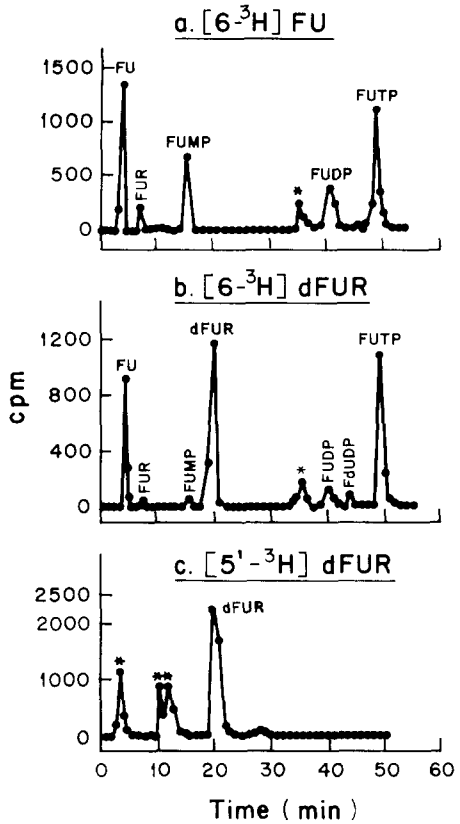

Fig. 3. HPLC-radioactivity profiles of intracellular metabolites in B cells of [6-^{3}H]FU (a), [6-^{3}H]dFUR (b), and [5'-^{3}H]dFUR (c). The radioactivity peak eluted between FUDP and FUTP is tentatively identified as FdUDP. The HPLC flow rate was 2 ml/min. Elution times were not corrected for the void volume from the u.v. detector to the fraction collector (3 ml). Key: (*) unknowns.

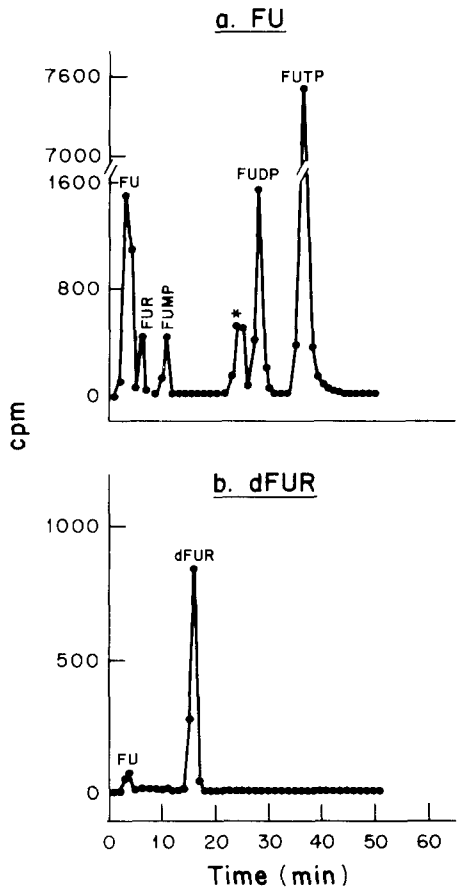

Fig. 4. HPLC-radioactivity profiles of intracellular metabolites of [6-^{3}H]FU (a) and [6-^{3}H]dFUR (b) in L1210 cells. The HPLC flow rate was 2.5 ml/min. Elution times were not corrected for the void volume from the u.v. detector to the fraction collector (3 ml). Key: (*) unknowns.

determined in three groups of two mice bearing L1210 leukemia, were 14.5, 1.3 and 0.3 mM at 0, 30 and 60 min after the i.p. injection of 500 mg/kg.

Inhibition of [6-^{3}H]UdR incorporation into DNA. The effects of dFUR and FU on the incorporation of [6-^{3}H]UdR into the DNA fractions of the B and L1210 cells are summarized in Table 1. In B cells, FU and dFUR at 10 μM concentrations caused significant inhibition of [6-^{3}H]UdR incorporation. In L1210 cells, FU inhibited the [6-^{3}H]UdR incorporation into DNA to about 10% of control values, but no inhibition was observed with dFUR.

Metabolism of dFUR to FU by intact cells. Since FU and dFUR have similar molar extinction coefficients [11], the relative concentrations of these compounds in culture medium were estimated by the area ratios of their corresponding u.v.-absorbing peaks eluted from HPLC. The extent of metabolic conversion of 10 μM dFUR to FU after 2 hr of incubation at 37° without cells (control), or with intact B or L1210 cells was calculated from the ratios of FU concentration to the sum of FU and the parent drug concentrations. No spontaneous degradation of dFUR was observed in the control. There was a significant difference in the metabolism of dFUR by the B and L1210 cells ($P < 0.001$). Metabolism of dFUR (65.7 ± 24.0%, N = 5) by B cells was evident. However, L1210 cells were incapable of converting dFUR to FU on up to eight separate determinations.

Phosphorolysis by the 105,000 g cellular fractions. The extents of conversion of dFUR to FU by the 105,000 *g* cellular fractions after 2- and 24-hr incubations were identical, indicating that metabolism was essentially completed in 2 hr. There was no spontaneous degradation of dFUR under the conditions used. dFUR was metabolized to FU by B cell extracts at a rate of 660 nmoles converted in 2 hr per mg protein. In contrast, the activity of uridine phosphorylase in L1210 cell extracts was undetectable.

Intracellular metabolism of FU and dFUR. HPLC-radioactivity profiles of the intracellular metabolites of FU and dFUR in B and L1210 cells after 1 hr of incubation are shown in Figs. 3 and 4. Table 2 summarizes the pool sizes of intracellular macromolecule-free metabolites of FU and dFUR in B and L1210 cells. There was a 2- to 4-fold variation in the pool sizes between experiments even when the same drug concentration and the same incubation period were used. The cause of this fluctuation is unknown; one possible explanation is the variation in enzyme activity in different cell batches, as suggested previously [22].

Table 2. Metabolism of dFUR and FU in B and L1210 cells*

Drug	Concn (μM)	Time (min)	dFUR	FU (pmoles/10^7 cells)	FU anabolites	Ratio of FU anabolites to parent drug
B cells						
dFUR	1.3	30	9.8	7.8	11.6	1.2
	1.3	60	3.2	1.7	20.1	6.3
	1.3	60	25.7	17.2	66.7	2.6
	40.0	30	95.0	48.2	148.6	1.6
FU	7.5	60	NA†	40.5	106.0	2.6
	40.0	120	NA	25.4	55.4	2.2
L1210 cells						
dFUR	1.3	60	1.4	0.1	0.1	0.1
	10.0	60	8.5	1.0	1.7	0.2
	10.0	60	8.9	1.1	2.5	0.3
	40.0	30	4.2	1.9	0	0
FU	1.3	60	NA	17.0	86.3	5.1
	10.0	60	NA	11.4	43.0	3.8
	10.0	60	NA	27.3	122.9	4.5
	40.0	30	NA	15.8	82.5	6.2

* Pool sizes of intracellular macromolecule-free metabolites and parent drugs were determined by collecting the radioactivity peaks following HPLC separation.
† Not applicable.

In B cells incubated with dFUR, a radioactivity peak that eluted between FUDP and FUTP had a retention time identical to FdUDP and has been tentatively identified as FdUDP. More vigorous analysis is needed to confirm its identity. Similar patterns of intracellular metabolites were observed for FU and dFUR in B cells; their anabolites consisted of FUR, FUdR, FUMP, FUDP, FdUDP, and FUTP. The ratio of intracellular metabolites to unchanged FU or dFUR ranged from 1.2 to 6.3. Unchanged FU and dFUR accounted for 13–35% of the total radioactivity. FdUMP was absent in B cell extracts. In view of the inhibitory effects of FU and dFUR on [6-^{3}H]UdR incorporation into DNA of B cells, the intracellularly formed FdUMP might have been bound to thymidylate synthetase and, therefore, not been measurable in the macromolecule-free fraction. The difference between the metabolite profiles of FU and dFUR in L1210 cells is illustrated in Fig. 4. FU was extensively metabolized; its anabolites included FUR, FUdR, FUMP, FUDP, FUTP, as well as FdUMP. These FU anabolites represented over 80% of the total radioactivity recovered. In the case of dFUR, over 70% of the radioactivity was accounted for by the unchanged drug. The presence of small amounts of FU and FU anabolites may have resulted from exposing cells to the 5% impurity present as FU in the radiolabled dFUR. The ratio of FU metabolites to unchanged FU was 20- to 60-fold greater than that to dFUR.

In addition to the aforementioned FU nucleosides and nucleotides, three unknown radioactivity peaks were observed in B and L1210 cells. The one which eluted prior to FU is also present in the commercially available [6-^{3}H]FU and is believed to be tritiated water (communicated to us by J. Moravek of Moravek Biochemicals). The other two peaks correspond to FU-related metabolites. Their elution pattern from the IP-HPLC suggests that they correspond to species which are highly ionized at pH 6 such as nucleotides or their derivatives.

Metabolic pathway of dFUR to FU nucleosides and nucleotides. The HPLC-radioactivity profile of macromolecule-free metabolites of [5′-^{3}H]dFUR in B cells after 1 hr of incubation is shown in Fig. 3c. The metabolites of [5′-^{3}H]dFUR differed from those of [6-^{3}H]dFUR (Fig. 3b) in that the radioactivity peaks derived from [5′-^{3}H]dFUR did not correspond to FU-related anabolites. Since the tritium label in [5′-^{3}H]dFUR was in the altered sugar moiety, these radioactivity peaks represented derivatives of either the sugar moiety or of the intact dFUR which was unrelated to the cytotoxic FdUMP or FUTP.

DISCUSSION

In this investigation, the biological effects and metabolism of FU and its precusor, dFUR, were examined in cultured human B lymphocytes and leukemia L1210 cells in mice. B cells were cross-sensitive to FU and dFUR, which suggests that these two analogs share a similar mode of action. The difference between the 90% growth inhibition and the 20% inhibition of precursor incorporation by these compounds was due to the different exposure times to drugs, i.e. 72 hr in the growth inhibition study and 2 hr in the [6-^{3}H]UdR incorporation study. The 20% reduction in [6-^{3}H]UdR incorporation correlated better with the up to 30% growth inhibition observed after 24 hr of incubation. These data indicate that the observed cytotoxicity to B cells may have been partly due to the inhibition of DNA synthesis. In mice bearing L1210 leukemia, FU produced a 40% AST; however, dFUR at doses 25-fold that of FU did not produce any antitumor activity. In addition, FU nearly completely abolished the

incorporation of [6-^{3}H]UdR into DNA of L1210 cells while dFUR had no effect.

The metabolic activation of FU and dFUR was compared in B and L1210 cells on two levels. Using the 105,000 *g* cellular fractions and intact cells, the phosphorolysis of dFUR to FU was significantly greater in B cells than in L1210 cells. The activity of uridine phosphorylase in B cells was 660 nmoles converted in 2 hr per mg protein while that in L1210 cells was undetectable. This difference in phosphorylase activity between B and L1210 cells was also reflected in their metabolic activation of dFUR, as indicated by the ratios of metabolites to parent drug (Table 2). Results of the *in vitro* studies of radiolabeled FU and dFUR using a newly developed IP-HPLC assay indicate that FU and dFUR were metabolized to nucleosides and nucleotides in B cells. The metabolic pathway of dFUR to FU nucleosides and nucleotides was established by comparing the intracellular metabolites of [6-^{3}H]dFUR and [5′-^{3}H]dFUR in B cells. The FU-related anabolites were observed after incubating cells with [6-^{3}H]dFUR and not with [5′-^{3}H]dFUR, thus indicating that the formation of FU anabolites proceeds via FU as the intermediate. In L1210 cells, FU was incorporated to form nucleosides and nucleotides. The pool sizes of total FU anabolites were comparable to those in B cells. In contrast, dFUR was not utilized to any significant extent. The ratios of FU and anabolites to unchanged dFUR in L1210 cells were about one-tenth of those in B cells, indicating a drastic difference in the activation of dFUR by the two cell lines. The contribution of the difference in dFUR uptake in B and L1210 cells to its selective cytotoxicity is not known. The accumulation of dFUR was significantly greater in B cells than in L1210 cells at equal extracellular concentrations, i.e. 1.3 and 40.0 μM (Table 2). However, it is more appropriate to compare the uptake of dFUR in experiments where B cells were exposed to 1.3 μM dFUR, which produced 50% growth inhibition, to those where L1210 cells were exposed to 10.0 μM dFUR, which is attainable during chemotherapy. Under these conditions, the average intracellular dFUR pool size in B cells (14 pmoles/10^7 cells) was only 50% greater than that in L1210 cells (9 pmoles/10^7 cells), while the difference in their FU anabolite pools was more than 20-fold. These data strongly suggest that it is the metabolic activation rather than the drug transport which determines the differential cytotoxicity of dFUR in B and L1210 cells.

In summary, results of comparative biological activities, phosphorolysis by intact cells and 105,000 *g* cellular fractions, and intracellular metabolism of radiolabeled compounds show that dFUR has selectivity that is different than that of FU, and that its cytotoxicity is determined by its initial activation to FU and subsequently to the cytotoxic FdUMP and FUTP. dFUR represents a type of prodrug which is activated to FU by soluble enzymes; their antitumor activity and host tissue toxicities would depend on the quantitative difference in their intracellular activation in the respective tissues. This type of prodrug may have improved selectivity over that of FU and deserves further investigation.

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