[CANCER RESEARCH 53, 3687–3693, August 15, 1993]

Differences in Activities and Substrate Specificity of Human and Murine Pyrimidine Nucleoside Phosphorylases: Implications for Chemotherapy with 5-Fluoropyrimidines[1]

Mahmoud H. el Kouni,[2] Mustapha M. el Kouni, and Fardos N. M. Naguib

Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912 [M. H. K., M. M. K., F. N. M. N.], and Department of Pharmacology and Comprehensive Cancer Center, University of Alabama at Birmingham, Birmingham, Alabama 35294 [M. H. K., F. N. M. N.]

ABSTRACT

Enzyme inhibition studies on extracts from human liver, mouse liver, and human placenta indicate that there are considerable differences between human and murine hepatic uridine phosphorylases (UrdPase, EC 2.4.2.3) and thymidine phosphorylases (dThdPase, EC 2.4.2.4.) with regard to their specificities and roles in the phosphorolysis of natural and 5-fluoropyrimidine nucleosides. To confirm further these differences between human and murine pyrimidine nucleoside phosphorylases, UrdPase and dThdPase were isolated from human liver, mouse liver, and human placenta using diethylaminoethyl-cellulose ion exchange chromatography. The pattern of elution from the column suggests that the hydrophobicity or charges on the human enzymes at pH 8 are different from those on their murine counterparts. The amount of each enzyme present differed between tissues and species. The apparent K_m, V_{max}, and efficiency of catalysis (V_{max}/K_m) values were determined for each enzyme using uridine, thymidine, deoxyuridine, 5-fluorouridine (FUrd), 5-fluoro-2'-deoxyuridine (FdUrd), and 5'-deoxy-5-fluorouridine (5'-dFUrd) as substrates. Kinetic parameters and inhibition studies were used to ascertain the binding affinity, substrate specificity, and contributions of UrdPase and dThdPase to the phosphorolysis of the various nucleosides in the 3 tissues.

The roles of UrdPase and dThdPase in human liver were quite distinct from those of their counterparts from human placenta and mouse liver. In human liver, UrdPase appears to be highly specific to uridine. Human hepatic UrdPase contributes only 15% to the cleavage of FUrd and does not contribute to the cleavage of the deoxyribosides (thymidine, deoxyuridine, FdUrd, and 5'-dFUrd). In mouse liver, UrdPase has a broader specificity as it cleaves over 85% of FUrd, 15% of FdUrd, and 25% of 5'-dFUrd. On the other hand, human hepatic dThdPase has a broader specificity than murine hepatic dThdPase. Human hepatic dThdPase cleaves all nucleosides tested including the ribosides, uridine, and FUrd. Approximately 15% of uridine and 85% of FUrd phosphorolysis in human liver is carried out by dThdPase. This contrasts with the murine hepatic dThdPase, which is more specific to deoxyribosides, as it does not contribute to the phosphorolysis of uridine, and contributes only 15% toward the cleavage of FUrd. dThdPase is the principal enzyme responsible for the phosphorolysis of 5'-dFUrd in both human and murine livers. The specificities of UrdPase and dThdPase from human placenta resembled the enzymes from the murine liver more than those from human liver. Thus, it appears that the specificities of human hepatic pyrimidine nucleoside phosphorylases are distinct from those from extrahepatic tissues. This suggests the existence of tissue-specific isozymes of pyrimidine nucleoside phosphorylases in humans.

The inter- and intraspecies differences in substrate specificities and activities between human and murine pyrimidine nucleoside phosphorylases may have an important impact on the validity of attempts to introduce inhibitors of these enzymes into the clinic or on drawing conclusions about the metabolism and the chemotherapeutic use of pyrimidine analogues in humans based on studies in mice.

INTRODUCTION

Pyrimidine nucleoside phosphorylases play an important role in the metabolism of pyrimidines, as they have the capacity to modulate the salvage pathway by anabolizing or catabolizing pyrimidine (except cytosine) nucleosides and their analogues. In most mammalian cells, there are 2 different pyrimidine nucleoside phosphorylases: UrdPase[3] (EC 2.4.2.3) and dThdPase (EC 2.4.2.4), which catalyze the following reversible reaction:

Pyrimidine (deoxy)riboside + ↔ pyrimidine base + (deoxy)ribose-1-P_i

Previous studies have indicated that UrdPase from various sources cleaves primarily pyrimidine ribosides, but is relatively nonspecific as it also cleaves pyrimidine 2'- and 5'-deoxyribosides (1–13). dThdPase, on the other hand, is reported to be specific for pyrimidine 2'- and 5'-deoxyribosides (3, 4, 9–12, 14–19). UrdPase is present in almost all tissues and tumors studied, whereas dThdPase activity is evidently reduced or absent from many tumors (1, 3, 11, 14, 20–28).

The importance of pyrimidine nucleoside phosphorylases in cancer chemotherapy stems from the fact that these enzymes are responsible for the deactivation or activation of several chemotherapeutic pyrimidine analogues, most notably the 5-fluoropyrimidines (3, 7, 9–12, 15–19, 25, 29–31). Inhibitors of UrdPase have been shown to increase the levels of FdUrd in liver perfusates (32) and potentiate its efficacy and selective toxicity against human tumors *in vitro* and *in vivo*, especially those tumors that have little or no dThdPase activity (25, 33, 34). In addition, UrdPase is also involved in the regulation of uridine concentration in plasma. UrdPase inhibitors were shown to increase the concentration and half-life of uridine in plasma (35–38), as well as the salvage of uridine by various tissues (36–39), and to protect against FUra (36, 38, 39) and FdUrd (40) host toxicities. dThdPase is the primary enzyme responsible for degradation of FdUrd in tissues that contain both enzymes (3, 4, 25). Few inhibitors of this enzyme are currently known (10, 41–43). These inhibitors, however, are not sufficiently potent; hence, assessment of their use in cancer chemotherapy has yet to be determined.

Because of the importance of pyrimidine nucleoside phosphorylases in the regulation of pyrimidine metabolism and their roles in cancer chemotherapy with pyrimidine analogues, we are interested in developing inhibitors for these enzymes. In the course of our investigation, we noticed that the activities of pyrimidine nucleoside phosphorylases in extracts from human liver differed from those from murine liver and other animal tissues previously studied (1–7, 9–14, 25, 44, 45), with regard to their specificity and role in cleaving the glycosidic bond of pyrimidine nucleosides, particularly those of the 5-fluoropyrimidine analogues.

Therefore, in the present study, we separated, characterized, and compared UrdPase and dThdPase from human liver, mouse liver, and human placenta to assess their roles in the phosphorolysis of natural

Received 3/19/93; accepted 6/5/93.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This investigation was supported in part by Grant CA-13943 awarded by the National Cancer Institute, Department of Health and Human Services.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: UrdPase, uridine phosphorylase; BAU, 5-benzylacyclouridine or 5-benzyl-1-[(2-hydroxyethoxy)methyl]uracil; 5'-dFUrd, 5'-deoxy-5-fluorouridine; dThdPase, thymidine phosphorylase; FdUrd, 5-fluoro-2'-deoxyuridine; FUra, 5-fluorouracil; FUrd, 5-fluorouridine.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/53/16/3687/2451328/cr0530163687.pdf by guest on 24 August 2022

and 5-fluoropyrimidines. Human placenta was used to ascertain whether or not the differences observed between human hepatic pyrimidine nucleoside phosphorylases are general characteristics of the enzymes from human tissues or particular to the human liver enzymes. The results indicate significant inter- and intraspecies differences in substrate specificities and phosphorolytic efficiencies of these 2 enzymes. These findings could have an important impact on the validity of attempts to introduce inhibitors of UrdPase and dThdPase into the clinic on the basis of studies conducted with rodents. In addition, the present results also delineate the role of these 2 enzymes in the metabolism of 5-fluoropyrimidine nucleosides in human and murine tissues. This information may aid in the rational design of successful chemotherapeutic regimens utilizing 5-fluoropyrimidine nucleosides in combination with inhibitors of pyrimidine nucleoside phosphorylases. A preliminary report has been presented (46).

MATERIALS AND METHODS

Chemicals. [2-^{14}C]Thymidine (56 Ci/mol), [2-^{14}C]deoxyuridine (56 Ci/mol), [2-^{14}C]uridine (56 Ci/mol), [2-^{14}C]FdUrd (56 Ci/mol), [2-^{14}C]FUrd (56 Ci/mol), and [6-^{3}H]5'-dFUrd (1.6 Ci/mmol) were obtained from Moravek Biochemicals, Inc., Brea, CA; DEAE-cellulose (DE-23) from Whatman, Inc., Clifton, NJ; silica gel G/UV$_{254}$ thin layer chromatography plates from Brinkmann, Westbury, NJ; and Bio-Rad protein assay kits from Bio-Rad Laboratories, Richmond, CA. BAU was synthesized by Dr. Shih Hsi Chu, Brown University, Providence, RI, by methods described elsewhere (4). 5'-dFUrd was a gift from Hoffman-LaRoche, Nutley, NJ. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

Source of Tissues. Human liver specimens were obtained from donors through the Alabama Regional Organ Bank. Fresh human placenta specimens were obtained from the Woman and Infant Hospital, Providence, RI. Mouse livers were obtained from Swiss Albino (CD1) mice (Charles River Laboratories, Boston, MA). To avoid the matinal circadian nadir in UrdPase activity (47, 48), mice were killed in the afternoon by cervical dislocation and their livers removed.

Preparation of Extracts. Organs were weighed and then homogenized (3:1, v:w) in ice-cold 20 mM potassium phosphate buffer (pH 8) containing 1 mM EDTA and 1 mM dithiothreitol with a polytron homogenizer (Brinkmann). Immediately after homogenization, phenylmethylsulfonylfluoride was added to the homogenates to achieve a final concentration of 1 mM. The homogenates were then centrifuged at 105,000 × g for 1 h at 4°C. The supernatant fluids (cytosol) were collected and used as the enzyme source.

Enzyme Assays. Nucleoside cleavage was measured radioisotopically by following the formation of nucleobases from their respective nucleosides as described previously (49). The standard assay mixture contained 20 mM potassium phosphate, pH 8.0; 1 mM dithiothreitol, 1 mM EDTA, 125 μM 2-^{14}C- or 3-^{3}H-labeled nucleoside at 1.8 Ci/mol, and 25 μl of enzyme in a final volume of 50 μl. The incubation was carried out for 30 min at 37°C. Under these conditions, the activity was linear with time and enzyme concentration. The reactions were initiated by addition of enzyme and terminated by boiling in a water bath for 2 min followed by freezing for at least 20 min. Proteins were removed by centrifugation, and 10 μl of the supernatant fluid were spotted on silica gel thin layer chromatography plates. The plates were then developed in a mixture of chloroform:methanol:acetic acid (90:5:5, v:v:v). The R_f values were: uridine, 0.07; deoxyuridine, 0.1; thymidine, 0.14; FUrd, 0.05; FdUrd, 0.07; 5'-dFUrd, 0.16; uracil, 0.43; thymine, 0.62; and FUra, 0.41. The amounts of radioactivity in the substrate (nucleoside) and product (nucleobase) were calculated on a percentage basis using a Berthold LB-2821 automatic thin-layer chromatography linear analyzer.

Isolation of Uridine Phosphorylase and Thymidine Phosphorylase from Cytosol Extracts by DEAE-Cellulose Column Chromatography. DEAE-cellulose was equilibrated with Buffer A (20 mM potassium phosphate buffer, pH 8, containing 1 mM EDTA and 1 mM dithiothreitol) and packed into a column (6.0 × 2.6 cm). Approximately 3 ml (~120–150 mg protein) of cytosol were applied to the column. The column was then eluted with 100 ml of Buffer A followed by a 100-ml linear gradient of NaCl (0 to 1.0 M) in Buffer A. Fractions of 10 ml were collected at 4°C at a flow rate of 60 ml/h. Each fraction was assayed for uridine and thymidine (0.125 mM, 6 Ci/mol) cleavage using the standard assay, except that the reaction was allowed to proceed for 1 h. Fractions that contained UrdPase and dThdPase were pooled, desalted, and concentrated in 20 mM potassium phosphate buffer (pH 8) containing 1 mM EDTA and 1 mM dithiothreitol using Centriprep-30 concentrators (Amicon, Danvers, MA). The enzymes (4–6 mg/ml) were then stored at −70°C until further use. Under these conditions, the enzymes were stable with no loss of activity for over 6 months.

Kinetic Studies. These experiments were conducted with enzymes partially purified by DEAE-cellulose column chromatography. The assay method is that described above, except that different concentrations of each substrate were used. The ranges of substrate concentrations used were dependent on the substrate, enzyme, and enzyme source and are shown in Table 3. Apparent K_m and V_{max} values were calculated using a computer program that uses the Wilkinson-Cleland procedure (50, 51). The program was written by Dr. S. Cha and modified to fit IBM BASIC by Dr. F. N. M. Naguib.

Protein Determination. Protein concentrations were determined by the method of Bradford (52) using bovine γ-globulin as a standard.

RESULTS

Table 1 shows the phosphorolytic activities of UrdPase and dThdPase toward different nucleosides in cytosolic extracts from human liver, mouse liver, and human placenta. The activity of UrdPase and dThdPase in the mixture was deduced from the inhibition by BAU, a specific inhibitor of UrdPase (4). The activity inhibited by BAU is due to UrdPase. The remaining activity is attributed to dThdPase. Table 1 shows that although uridine is cleaved mainly (83%) by UrdPase in human liver, it can also serve as a substrate for human hepatic dThdPase, as 17% of uridine phosphorolysis is due to dThdPase activity. In contrast, in mouse liver and human placenta uridine are cleaved almost exclusively (96–97%) by UrdPase, as little uridine (3–4%) is cleaved by dThdPase. This indicates that dThdPase in human liver is less specific than its counterpart in the other 2 tissues. BAU did not have an inhibitory effect on thymidine cleavage in human liver but had some effect on thymidine cleavage in human placenta (2%) and mouse liver (5%). Therefore, in mouse liver, and to a lesser extent in human placenta, but not human liver, UrdPase can utilize thymidine as a substrate. The differences between the enzymes from the various tissues were further accentuated when the 5-fluoropyrimidines were tested as substrates. BAU inhibited FUrd cleavage by no more than 15% in human liver. In contrast, BAU inhibited the phosphorolysis of 5-FUrd in mouse liver and human placenta by 80% and 70%, respectively. Phosphorolysis of FdUrd, and 5'-dFUrd was less inhibited by BAU in the human tissues (0–2% and 0–10%, respectively) than in mouse liver (12% and 31%, respectively). Thus, the phosphorolysis of the 5-fluoropyrimidine deoxyribosides, FdUrd and 5'-dFUrd, proceeds principally by dThdPase in human and mouse tissues. Never-

Table 1 *Phosphorolysis of various pyrimidine nucleosides by the cytosol extracts from human liver, mouse liver, and human placenta and percent inhibition by BAU*

Substrate	Tissue		
	Human liver	Mouse liver	Human placenta
Uridine	82.6 ± 10.2 [a]	73.0 ± 1.4	185.8 ± 4.8
% inhibition [b]	83	96	97
Thymidine	148.8 ± 2.7	68.9 ± 1.0	201.9 ± 1.1
% inhibition	0	5	2
FUrd	96.9 ± 3.1	51.1 ± 0.7	97.9 ± 5.0
% inhibition	15	80	70
FdUrd	146.9 ± 1.2	63.5 ± 1.5	198.6 ± 4.5
% inhibition	0	12	2
5'-dFUrd	124.3 ± 2.6	42.4 ± 1.9	97.4 ± 1.8
% inhibition	0	31	10

[a] Specific activity (pmol/min/mg protein ± SE of estimation) calculated from at least 3 estimates using 0.125 mM substrate.
[b] Percent inhibition by 100 μM BAU.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/53/16/3687/2451328/cr0530163687.pdf by guest on 24 August 2022

theless, UrdPase plays a significant role in the phosphorolysis of these deoxyribosides in mouse liver and, to a lesser extent, in human placenta, but not human liver.

To confirm further the differences in substrate specificity between human and mouse UrdPase and dThdPase, UrdPase and dThdPase were isolated from the 105,000 × *g* supernatant of human liver, mouse liver, and human placenta by DEAE-cellulose ion exchange chromatography. Fig. 1 shows that the elution pattern of the human enzymes is different from that obtained with the enzymes from mouse liver. Human UrdPase eluted during the wash, whereas dThdPase eluted after applying the salt gradient. The reverse is observed when mouse liver extract is chromatographed.

Table 2 shows that the amounts of UrdPase and dThdPase per g tissue differ greatly between the 3 tissues. Human liver has the highest dThdPase activity (110 units/g), followed by human placenta (66 units/g), with mouse liver having the lowest (26 units/g). UrdPase activity was highest in human placenta (53 units/g), followed by mouse liver (15 units), with the lowest activity in human liver (12 units/g).

The kinetic parameters of glycosidic bond cleavage by the partially purified UrdPase and dThdPase were determined using various nucleosides, including the 5-fluoropyrimidines, as substrates. Table 3 shows the apparent K_m, V_{max}, and efficiency of catalysis (V_{max}/K_m) values for UrdPase and dThdPase from the 2 species. In general, the binding affinity of ligands to the enzymes, as determined by the apparent K_m values, was similar for the enzymes from the 3 tissues. However, there were some noticeable exceptions. There were differences between the binding affinities of human and murine hepatic UrdPases for FdUrd and 5′-dFUrd, and between the binding of human and murine dThdPases for uridine, FdUrd, and FUrd. The V_{max} values also differed between enzymes from the 2 human tissues as well as between enzymes from human and murine tissues. The V_{max} values obtained for dThdPase from human liver with the deoxyribosides (thymidine, deoxyuridine, FdUrd, and 5′-dFUrd) and ribosides (uridine and FUrd) were higher by at least 11- and 4-fold, respectively, than those obtained with dThdPase from human placenta. These values were also at least 6- and 3-fold higher than those observed with the murine hepatic enzyme. With regard to UrdPase, the murine enzyme showed higher V_{max} values than the human UrdPase with all nucleosides tested, with the exception of uridine. The efficiency of catalysis (V_{max}/K_m) values, which reflect the specificity of UrdPase and dThdPase, also showed marked variations within and between species. The data in Table 3 show that UrdPases are generally more specific to ribosides, whereas dThdPases are specific to deoxyribosides with one striking exception. In human liver, the phosphorolysis of FUrd, a riboside, is catalyzed 5-fold more efficiently by dThdPase than UrdPase. The results in Table 3 also illustrate the diversity in the specificities of UrdPases and dThdPases, which appear to be species-, tissue-, and substrate-dependent.

Table 4 shows the percentage of relative efficiency of UrdPase and dThdPase from the 3 tissues in catalyzing the phosphorolysis of different nucleosides as compared to their natural substrates, uridine and thymidine, respectively. In general, the relative efficiencies of catalysis of human and murine UrdPase and dThdPase were similar, with some noticeable exceptions. One apparent difference is the higher relative efficiency of catalysis of FUrd cleavage by murine hepatic UrdPase (19%) compared to the enzyme from human tissues (5%). Human dThdPase, on the other hand, has at least a 2.8-fold better relative efficiency of catalysis with FdUrd (37–72%) and 5′-dFUrd (23% and 28%) than the murine dThdPase (13% and 7%, respectively).

Table 5 shows the percent contribution by UrdPase and dThdPase, within each tissue, to the phosphorolysis of the various nucleosides, including the 5-fluoropyrimidines as calculated from the data in Table 3. The results in Table 5 confirm the data obtained using cytosol and BAU at a fixed substrate concentration and emphasize the importance of using inhibitors to differentiate between the contribution of UrdPase and dThdPase in the phosphorolysis of the different nucleosides (Table 1). Uridine is cleaved mainly by UrdPase in all 3 tissues. However, dThdPase in human liver is responsible for approximately 15% of the phosphorolysis of uridine, whereas in mouse liver and human placenta, phosphorolysis by dThdPase is negligible. Thymidine and deoxyuridine cleavage is carried out exclusively by dThdPase in human liver, whereas in mouse liver and human placenta dThdPase is responsible for 94–98% of this reaction. Most (83%) of

Table 2 *Units of activity of UrdPase and dThdPase in human liver, mouse liver, and human placenta*

Activity	Tissue		
	Human liver	Mouse liver	Human placenta
UrdPase	11.8 ± 3.2[a]	14.9 ± 5.2	52.6 ± 8.0
dThdPase	110.3 ± 19.9	26.1 ± 8.4	65.7 ± 3.3
UrdPase/dThdPase	0.11	0.57	0.80

[a] Units (nmol/min in 1 g tissue ± SD) calculated from at least 3 estimates using 0.125 mM substrate.

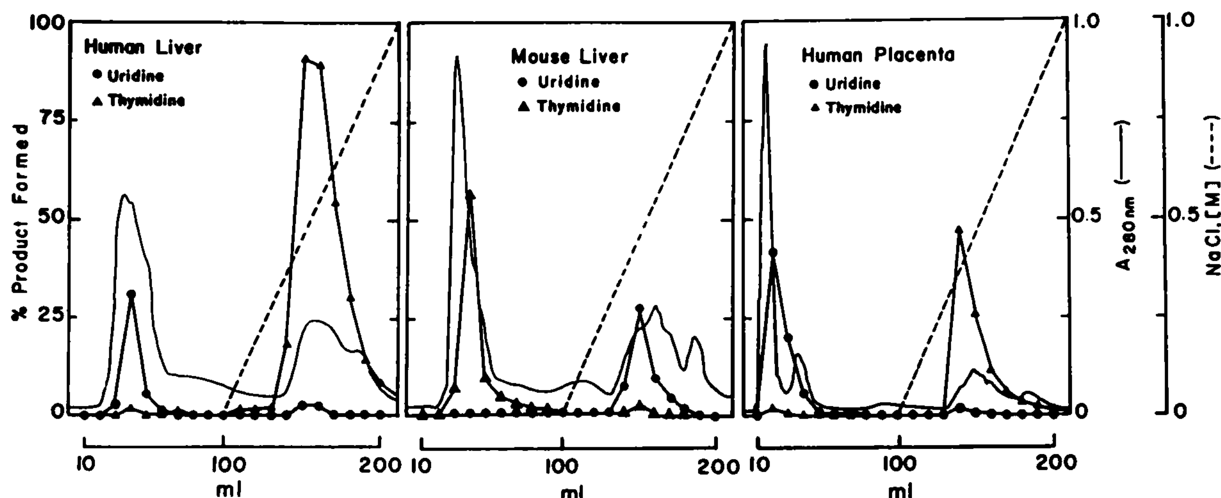

Fig. 1. Isolation of uridine phosphorylase (●) and thymidine phosphorylase (▲) from cytosol extracts of human liver, mouse liver, and human placenta by DEAE-cellulose ion exchange chromatography.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/53/16/3687/2451328/cr0530163687.pdf by guest on 24 August 2022

Table 3 *Kinetic parameters of uridine phosphorylase and thymidine phosphorylase partially purified from human liver, human placenta, and mouse liver with various pyrimidine nucleosides*[a]

Substrate	Parameters	Uridine phosphorylase			Thymidine phosphorylase		
		Human liver	Mouse liver	Human placenta	Human liver	Mouse liver	Human placenta
Uridine	K_m[b]	115 ± 24	66 ± 7.4	98 ± 12	274 ± 77	802 ± 153	298 ± 93
	V_{max}[c]	64.0 ± 3.82	65.3 ± 2.58	106 ± 3.6	16.2 ± 1.45	4.59 ± 0.56	2.81 ± 0.35
	Concentration[d]	0.006–0.5	0.03–1.0	0.03–1.4	0.03–0.5	0.006–1.0	0.06–1.4
	Efficiency[e]	0.717	0.935	1.242	0.124	0.006	0.010
Thymidine	K_m	57 ± 14	105 ± 11	94 ± 26	152 ± 13	195 ± 24	96 ± 18
	V_{max}	1.30 ± 0.08	4.49 ± 0.21	1.34 ± 0.10	1,468 ± 46	241 ± 18.0	85.7 ± 3.79
	Concentration	0.03–0.5	0.01–1.0	0.03–1.4	0.03–1.5	0.1–0.5	0.03–1.4
	Efficiency	0.024	0.039	0.019	9.745	1.268	1.00
Deoxyuridine	K_m	958 ± 232	1,515 ± 274	828 ± 150	735 ± 75	1,024 ± 137	887 ± 157
	V_{max}	11.2 ± 2.05	59.0 ± 9.68	13.9 ± 1.73	3,837 ± 198	596 ± 48.1	335 ± 41.5
	Concentration	0.06–2.0	0.01–1.0	0.03–0.5	0.06–2.0	0.01–0.5	0.01–0.5
	Efficiency	0.011	0.039	0.017	5.247	0.581	0.353
FUrd	K_m	213 ± 33	206 ± 21	155 ± 34	1,294 ± 67	632 ± 84	1,849 ± 263
	V_{max}	6.63 ± 0.34	31.7 ± 0.92	11.4 ± 0.65	193 ± 5.85	16.6 ± 1.43	48.2 ± 4.45
	Concentration	0.18–3.0	0.01–1.0	0.18–1.5	0.18–1.5	0.03–0.5	0.18–1.5
	Efficiency	0.031	0.174	0.076	0.149	0.026	0.026
FdUrd	K_m	392 ± 49	509 ± 60	409 ± 47	325 ± 27	757 ± 64	433 ± 63
	V_{max}	7.11 ± 0.41	16.5 ± 0.91	11.4 ± 0.65	2,282 ± 80	127 ± 6.16	173 ± 15.6
	Concentration	0.01–1.0	0.01–3.0	0.03–1.0	0.03–1.0	0.01–1.0	0.06–1.0
	Efficiency	0.021	0.032	0.029	7.001	0.160	0.366
5′-dFUrd	K_m	830 ± 163	1,749 ± 424	939 ± 96	198 ± 19	206 ± 20	200 ± 21
	V_{max}	24.0 ± 2.63	59.9 ± 3.88	37.0 ± 2.37	533 ± 11.4	19.6 ± 1.07	46.7 ± 2.29
	Concentration	0.06–2.0	0.18–3.0	0.18 1.5	0.06–0.5	0.01–0.5	0.03–0.5
	Efficiency	0.034	0.034	0.039	2.692	0.095	0.234

[a] Kinetic parameters are weighted means from at least 2 experiments.
[b] Apparent ± K_m, μM SE of estimation.
[c] V_{max}, nmol/min/g tissue ± SE of estimation.
[d] Range of concentration of substrate used in mM to determine the kinetic parameters.
[e] Efficiency of catalysis (V_{max}/K_m).

the FUrd is cleaved by dThdPase in human liver. In contrast, 87% and 75% of FUrd is cleaved by UrdPase from mouse liver and human placenta, respectively. FdUrd is cleaved exclusively by dThdPase, in human liver (100%), and mainly by dThdPase in human placenta (93%) and mouse liver (83%), whereas the remaining phosphorolysis is due to UrdPase activity. The cleavage of 5′-dFUrd also proceeds mainly by dThdPase in the 3 tissues, albeit to different degrees. dThdPase is responsible for 73, 86, and 99% of the phosphorolysis of 5′-dFUrd in mouse liver, human placenta, and human liver, respectively.

DISCUSSION

The present studies reveal that there are distinct differences between the pyrimidine nucleoside phosphorylases from human and mouse liver. Such differences are not limited to enzymes from the 2 species, but are also observed between enzymes from different tissues within the same species.

The differences in the elution profiles of human UrdPase and dThdPase resulting from DEAE-cellulose column chromatography and that obtained from mouse liver (Fig. 1), as well as those reported by others for enzymes from other animal sources (1–4, 45), suggest that the charge or possibly the hydrophobicity on the surface of the human enzymes is different from those from other sources (1–4, 45). At pH 8, human UrdPase eluted during the wash, whereas dThdPase eluted after applying the salt gradient (Fig. 1). Thus, at pH 8, human UrdPase appears to carry a positive charge or has a significant hydrophobic surface since it does not bind to the ion exchanger, whereas dThdPase appears to carry a negative charge since it binds. This is the opposite of what was observed for UrdPase and dThdPase from mouse tissue (Fig. 1) and other animal sources (1–4, 45). The patterns of elution for UrdPase and dThdPase from human liver and placenta were similar. This indicates that the reverse elution profile of human hepatic enzymes is a general characteristic of human pyrimidine nucleoside phosphorylases and not due to peculiarities of the hepatic tissue.

The disparity in the apparent K_m values reflects differences in substrate binding affinities between the enzymes from the 2 species (Table 3) and suggests structural differences between the active sites

Table 4 *Percent relative efficiency of catalysis (V_{max}/K_m) of uridine phosphorylase and thymidine phosphorylase from human liver, mouse liver, and human placenta with various pyrimidine nucleosides*

Percent relative efficiency of catalysis is estimated as percent efficiency of catalysis of uridine or thymidine phosphorylase with a nucleoside, relative to that of the natural substrate, uridine or thymidine, respectively.

Substrate	Uridine phosphorylase			Thymidine phosphorylase		
	Human liver	Mouse liver	Human placenta	Human liver	Mouse liver	Human placenta
Uridine	100 (0.72)[a]	100 (0.94)	100 (1.24)	1	0.4	1
Thymidine	3	4	2	100 (9.75)	100 (1.27)	100 (1.00)
Deoxyuridine	2	4	2	54	46	35
FUrd	4	19	6	2	2	3
FdUrd	3	3	2	72	13	37
5′-dFUrd	4	3	4	28	7	23

[a] Numbers in parentheses, efficiency of catalysis (V_{max}/K_m) obtained from Table 3.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/53/16/3687/2451328/cr0530163687.pdf by guest on 24 August 2022

Table 5 *Percent contribution by uridine (UrdPase) and thymidine (dThdPase) phosphorylases from human liver, mouse liver, and human placenta toward the phosphorolysis of various pyrimidine nucleosides*

Percent contribution of UrdPase or dThdPase toward a specific substrate is calculated, using data from Table 3, as percent efficiency of catalysis (V_{max}/K_m) of that substrate by UrdPase or dThdPase/(sum of efficiencies of catalysis of the same substrate by the two phosphorylases).

Substrate	Human liver		Mouse liver		Human placenta	
	UrdPase	dThdPase	UrdPase	dThdPase	UrdPase	dThdPase
Uridine	85	15	100	0	99	1
Thymidine	0	100	3	97	2	98
Deoxyuridine	0	100	6	94	4	96
FUrd	17	83	87	13	75	25
FdUrd	0	100	17	83	7	93
5′-dFUrd	1	99	27	73	14	86

of the enzymes from the 2 sources. Distinctions between the enzymes from human and murine tissue were also evident when the maximum velocity (V_{max}) of the enzymes with various substrates were compared (Table 3). However, measurements of ligand binding (K_m values) or V_{max} alone may overshadow the actual contribution of an enzyme in the catalysis of a specific nucleoside within a given tissue. A more accurate measurement of substrate specificity or the relative roles of UrdPase and dThdPase in the phosphorolysis of various nucleosides within a given tissue can be obtained by using potent and specific enzyme inhibitors (Table 1) or inferred from the catalytic efficiency (V_{max}/K_m) of the enzymes (Tables 3–5).

Differences in the relative catalytic efficiency of cleavage of the glycosidic bonds of the various nucleosides (Table 4) illustrate the differences in substrate specificity between human and murine UrdPases and dThdPases. In general, the data show that the addition of a fluorine atom to *C5* of uridine (*i.e.*, FUrd) reduces the efficiency of UrdPase in cleaving the riboside. However, this reduction in the efficiency of UrdPase was more pronounced in human tissues than in mouse liver. Addition of a fluorine atom to *C5* of deoxyuridine (*i.e.*, FdUrd), on the other hand, enhances the phosphorolytic efficiency of human, but not mouse, dThdPase (Table 4).

We have used both specific inhibition of UrdPase (Table 1) and kinetic studies (Table 5) to delineate the role of UrdPase and dThdPase in the catalysis of the phosphorolysis of natural pyrimidine nucleosides, as well as their 5-fluorinated analogues. Both methods gave similar results. They indicate that the degree of participation by UrdPase and dThdPase in the phosphorolysis of pyrimidine nucleosides varies between different tissues. The roles of UrdPase and dThdPase in human liver were quite distinct from those of their counterparts in human placenta and mouse liver. Although UrdPase activity in the 3 tissues seems directed mainly toward the phosphorolysis of pyrimidine ribosides (*i.e.*, uridine and FUrd) rather than deoxyribosides (thymidine, deoxyuridine, FdUrd, and 5′-dFUrd), UrdPase from human liver seems highly specific to the phosphorolysis of uridine, as it contributes only 17% to the cleavage of FUrd, compared to 75% and 83% by UrdPase from human placenta and mouse liver, respectively (Table 5). Human liver UrdPase also has little or no activity towards the deoxyribosides, in contrast to the enzymes from mouse liver, human placenta (Table 5), and other human and animal tissues and tumors (1–13), in which UrdPases showed considerable activities with the deoxyribosides. Thus, it appears that in humans, unlike other animals studied, cytoplasmic UrdPase in the liver is distinct from the enzyme from extrahepatic tissues and tumors as indicated by its narrow specificity. This suggests the existence of tissue-specific isozymes of UrdPase in humans.

The role of dThdPase from all 3 tissues appears to be preferentially directed toward the phosphorolysis of deoxyribosides. Nevertheless, the role of dThdPase in human liver is distinct from its role in the other 2 tissues. Approximately 15–17% of the total phosphorolysis of uridine in human liver is due to dThdPase (Tables 1 and 5). dThdPase in human placenta and mouse liver contributes little, if any, toward the cleavage of uridine. This is the first reported instance of a dThdPase playing a significant role in the phosphorolysis of uridine and sharply contrasts with the generally held belief that dThdPase is specific for deoxyribosides. The enzyme from various sources is reported to cleave deoxyuridine, FdUrd, and 5′-dFUrd, but not uridine (3, 4, 9–12, 16–18, 44), as indeed is indicated by our results with dThdPase from mouse liver and human placenta (Table 5). The present results also show that dThdPase in human liver is the principal enzyme responsible for cleavage of FUrd, a riboside. The efficiency of FUrd cleavage by human dThdPase was 5-fold better than that by UrdPase. This is the reverse of what we observed in mouse liver or human placenta, where FUrd is cleaved mainly by UrdPase (Tables 1 and 5). The significant role of dThdPase in the phosphorolysis of uridine and FUrd in human liver sharply contrasts with the role of dThdPase in extrahepatic tissues, and suggests the presence of dThdPase isozymes in humans. The present results also indicate that dThdPase is the principal enzyme responsible for the phosphorolysis of 5′-dFUrd in both human and murine tissues. This finding is in disagreement with the reports that 5′-dFUrd is cleaved mainly by UrdPase in murine tissues and by dThdPase in human tissues (7–9).

The significant contribution of dThdPase (15–17%) to the degradation of uridine in human liver (Tables 1 and 5) could have important implications for chemotherapy. The liver is considered to be the main organ involved in the regulation of uridine metabolism (35, 53–58). It has been demonstrated in rats that more than 90% of plasma uridine entering the liver by the portal vein is degraded in a single pass, whereas a constant amount of uridine from *de novo* biosynthesis is released into the blood of the hepatic vein (53, 54). Activity of hepatic UrdPase is the first step in elimination of plasma uridine delivered to the liver (35, 38, 39, 56). Inhibitors of UrdPase, which protect against host toxicity of FUra (36, 38, 39) in murine systems, were shown to increase the concentration and salvage of uridine (35, 36–39). The finding that dThdPase could be involved in the catabolism of uridine in human but not mouse livers may render UrdPase inhibitors less effective in the prevention of uridine degradation in humans and consequently less useful in protecting humans against FUra toxicity.

On the other hand, UrdPase inhibitors would still be useful in treating some tumors in humans, especially in regimens using FdUrd. Although dThdPase is the principal enzyme responsible for degradation of FdUrd in tissues that contain both enzymes (3, 4, 25), its activity is low or absent in many human and animal neoplasms (1, 3, 11, 14, 20–28). It has been shown that the cleavage of thymidine and FdUrd detected in such tumors is due to UrdPase (3). Therefore, coadministration of a UrdPase inhibitor with FdUrd should enhance the selective toxicity of FdUrd against the tumor, because host tissues still contain high dThdPase, and hence can efficiently cleave FdUrd and overcome its toxicity, even when UrdPase activity is inhibited. Additionally, since the level of thymidine kinase, the enzyme that phosphorylates FdUrd into its active metabolite, fluorodeoxyuridine 5′-monophosphate (FdUMP) (59), is elevated in most tumors, as opposed to nondividing tissues, which virtually lack this enzyme (60),

Downloaded from http://aacrjournals.org/cancerres/article-pdf/53/16/3687/2451328/cr0530163687.pdf by guest on 24 August 2022

selective toxicity of FdUrd may be further enhanced by coadministration of a UrdPase inhibitor. Thymidine kinase is not inhibited by UrdPase inhibitors (3, 4, 61). Indeed, this is what we have found in mice bearing human tumors (25, 33, 34). It is reassuring that the present results demonstrate that dThdPase in human liver and placenta is the principal enzyme degrading FdUrd. Therefore, the use of UrdPase inhibitors in cancer chemotherapy in humans, particularly in conjunction with FdUrd, remains promising.

In conclusion, the present results demonstrate that distinct differences clearly exist between the pyrimidine nucleoside phosphorylases from human and mouse tissues with regard to amounts (units/g tissue), charge, or hydrophobicity on the enzymes surface, kinetic parameters, substrate specificities, and their roles in the metabolism of natural pyrimidine nucleosides, as well as their 5-fluorinated analogues. The results also indicate that substrate specificity varies between enzymes from different human tissues, suggesting the presence of isozymes. These findings may have important implications for attempts to introduce inhibitors of these enzymes into the clinic or draw conclusions about the metabolism and the chemotherapeutic usefulness of pyrimidine analogues in humans based on studies in mice.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Sungman Cha for many helpful suggestions and discussions and for his continuous support throughout the course of this study. Thanks are also due to Kevin Merrell and Norma J. Messier for their excellent technical assistance.

REFERENCES

1. Krenitsky, T. A., Barclay, M., and Jacquez, J. A. Specificity of mouse uridine phosphorylase. Chromatography, purification and properties. J. Biol. Chem., *239:* 805–812, 1964.
2. Krenitsky, T. A., Mellors, J. W., and Barclay, R. K. Pyrimidine nucleosidases, their classification and relationship to uric acid ribonucleoside phosphorylase. J. Biol. Chem., *240:* 1281–1286, 1965.
3. Niedzwicki, J. G., el Kouni, M. H., Chu, S. H., and Cha, S. Pyrimidine acyclonucleosides, inhibitors of uridine phosphorylase. Biochem. Pharmacol., *30:* 2097–2101, 1981.
4. Niedzwicki, J. G., Chu, S. H., el Kouni, M. H., Rowe, E. C., and Cha, S. 5-Benzylacyclouridine and 5-benzyloxybenzylacyclouridine, potent inhibitors of uridine phosphorylase. Biochem. Pharmacol., *31:* 1857–1861, 1982.
5. Pontis, H., Degerstedt, G., and Reichard, P. Uridine and deoxyuridine phosphorylase from Ehrlich ascites tumor. Biochim. Biophys. Acta, *51:* 138–147, 1961.
6. Kraut, A., and Yamada, E. W. Cytoplasmic uridine phosphorylase of rat liver: characterization and kinetics. J. Biol. Chem., *246:* 2021–2030, 1971.
7. Ishitsuka, H., Miwa, M., Takemoto, K., Fukuoka, K., Itoga, A., and Maruyama, H. B. Role of uridine phosphorylase for antitumor activity of 5′-deoxy-5-fluorouridine. Jpn. J. Cancer Res., *71:* 112–123, 1980.
8. Miwa, M., Nakamura, J., and Ishitsuka, H. A simple and convenient assay method for the phosphorolysis of 5′-deoxy-5-fluorouridine. Jpn. J. Cancer Res., *72:* 965–967, 1981.
9. Kono, A., Hara, Y., Sugata, S., Karube, Y., Matsushima, Y., and Ishitsuka, H. Activation of 5′-deoxy-5-fluorouridine by thymidine phosphorylase in human tumors. Chem. Pharm. Bull., *31:* 175–178, 1983.
10. Niedzwicki, J. G., el Kouni, M. H., Chu, S. H., and Cha, S. Structure-activity relationship of ligands of the pyrimidine nucleoside phosphorylase. Biochem. Pharmacol., *32:* 399–415, 1983.
11. Woodman, P. W., A. M. Sarrif, and C. Heidelberger. Specificity of pyrimidine nucleoside phosphorylases and the phosphorolysis of 5-fluoro-2′-deoxyuridine. Cancer Res., *40:* 507–511, 1980.
12. Veres, Z., Szabolcs, A., Szinai, I., Denes, G., Kajtar-Peredy, M., and Jeney, A. Enzymatic cleavage of 5-substituted-2′-deoxyuridines by pyrimidine nucleoside phosphorylases. Biochem. Pharmacol., *35:* 1057–1059, 1986.
13. el Kouni, M. H., Naguib F. N. M., Chu, S. H., Cha, S., Ueda, T., Gosselin, G., Imbach, J-L., Shealy, F., and Otter, B. A. Effect of the *N*-glycosidic bond conformation and modifications in the pentose moiety on the binding of nucleoside ligands to uridine phosphorylase. Mol. Pharmacol., *34:* 104–110, 1988.
14. Zimmerman, M., and Seidenberg, J. I. Thymidine phosphorylase and nucleoside deoxyribosyltransferase in normal and malignant tissues. J. Biol. Chem., *239:* 2618–2621, 1964.
15. Nakayama, C., Wataya, Y., Meyer, R. B. Santi, D. V., Saneyoshi, M., and Ueda, T. Thymidine phosphorylase. Substrate specificity for 5-substituted 2′-deoxyuridines. J. Med. Chem., *23:* 962–964, 1980.
16. Desgranges, C., Razaka, G., Rabaud, M., and Bricaud, H. Catabolism of thymidine in human blood platelet: purification and characterization of thymidine phosphorylase. Biochim. Biophys. Acta, *654:* 211–213, 1981.
17. Kono, A., Hara, Y., Sugata, S., Matsushima, Y., and Ueda, T. Substrate specificity of a thymidine phosphorylase in human liver tumor. Chem. Pharm. Bull., *32:* 1919–1921, 1984.
18. Iltzsch, M. H., el Kouni, M. H., and Cha, S. Kinetic studies of thymidine phosphorylase from mouse liver (Abstract). Proc. Am. Assoc. Cancer Res., *26:* 14, 1985.
19. Meynial, D., Malet-Martino, M. C., Lopez, A., and Martino, R. Phosphorolysis of 5′-deoxy-5-fluorouridine in human plasma, serum and blood platelets. J. Pharm. Pharmacol., *38:* 426–431, 1985.
20. Marsh, J. C., and Perry, S. Thymidine catabolism by normal and leukemic human leukocytes. J. Clin. Invest., *43:* 267–278, 1964.
21. Zimmerman, M. Selective inhibition by deoxyglucosyl thymine of thymidine phosphorylase not catalyzing deoxyribosyl transfer. Biochem. Biophys. Res. Commun., *16:* 600–603, 1964.
22. Lazarus, H., Barell, E. F., Oppenheim, S., and Krishan, A. Divergent properties of two human lymphocytic cell lines isolated from a single specimen of peripheral blood. *In Vitro*, *5:* 303–310, 1974.
23. Fox, R. M., Piddington, S. K., Tripp, E. H., Dudman, N. P., and Tattersall, M. H. N. Thymidine sensitivity of cultured leukemic lymphocytes. Lancet, *2:* 391–393, 1979.
24. Piper, A. A., and R. M. Fox. Biochemical basis for the differential sensitivity of human T- and B-lymphocyte lines to 5-fluorouracil. Cancer Res., *42:* 3753–3760, 1982.
25. Chu, M. Y. W., Naguib, F. N. M., Iltzsch, M. H., el Kouni, M. H., Chu, S. H., Cha, S., and Calabresi, P. Potentiation of 5-fluoro-2′-deoxyuridine antineoplastic activity by the uridine phosphorylase inhibitors benzylacyclouridine and benzyloxybenzylacyclouridine. Cancer Res., *44:* 1852–1856, 1984.
26. Vertongen, F., Fondu, P., Van den Heule, B., Cauchie, C., and Mandelbaum, I. M. Thymidine kinase and thymidine phosphorylase activities in various types of leukaemia and lymphoma. Tumour Biol., *5:* 303–311, 1984.
27. Vezzoni, P., Giardini, R., Lombardi, L., Rilke, F., Lucchini, R., Vezzoni, M., and Clerici, L. Multienzymatic analysis of human malignant lymphomas. Correlation of enzymatic data with pathologic and ultrastructural findings in Burkitt's and lymphoblastic lymphomas. Cancer (Phila.), *54:* 489–499, 1984.
28. Naguib, F. N. M., Niedzwicki, J. G., Iltzsch, M. H., Wiemann, M. C., el Kouni, M. H., and Cha, S. Effects of *N,N*-dimethylformamide and sodium butyrate on enzymes of pyrimidine metabolism in cultured human tumor cells. Leukemia Res., *11:* 855–861, 1987.
29. Heidelberger, C., Greisbach, L., Cruz, O., Schnitzer, R. J., and Grunberg, E. Fluorinated pyrimidines VI. Effects of 5-fluorouridine and 5-fluoro-2′-deoxyuridine on transplanted tumors. Proc. Soc. Exp. Biol. Med., *97:* 470–475, 1958.
30. Mukherjee, K. L., Boohar, J., Wentland, D., Ansfield, F. J., and Heidelberger, C. Studies on fluorinated pyrimidines. XVI. Metabolism of 5-fluorouracil-2-C^{14} and 5-fluoro-2′-deoxyuridine-2-C^{14} in cancer patients. Cancer Res., *23:* 49–66, 1963.
31. Birnie, G. D., Kroeger, H., and Heidelberger, C. Studies of fluorinated pyrimidines. XVIII. The degradation of 5-fluoro-2′-deoxyuridine and related compounds by nucleoside phosphorylase. Biochemistry, *2:* 566–572, 1963.
32. LaCreta, F. P., Warren, B. S., and Williams, W. M. Effects of pyrimidine nucleoside phosphorylase inhibitors on hepatic fluoropyrimidine elimination in the rat. Cancer Res., *49:* 2567–2573, 1989.
33. Chu, S. H., Chen, Z. H., Rowe, E. C., Naguib, F. N. M., el Kouni, M. H., and Chu, M. Y. Synthesis and biological activity of hydroxymethyl analogs of 5-benzylacyclouridine and 5-benzyloxybenzylacyclouridine. Nucleosides & Nucleotides, *3:* 303–311, 1984.
34. Chu, S. H., Weng, Z. Y., Chen, Z. H., Rowe, E. C., Chu, E., Naguib, F. N. M., el Kouni, M. H., Cha, S., and Chu, M. Y. Synthesis of 5-benzyl and 5-benzyloxybenzyl 2,2′-anhydrouridines and related nucleoside analogs as inhibitors of uridine phosphorylase. Nucleosides & Nucleotides, *7:* 91–102, 1988.
35. Monks, A., Ayers, O., and Cysyk, R. L. Effect of 5-benzylacyclouridine, a potent inhibitor of uridine phosphorylase, on the metabolism of circulating uridine by the isolated rat liver. Biochem. Pharmacol., *32:* 2003–2009, 1983.
36. Darnowski, J. W., and Handschumacher, R. E. Tissue-specific enhancement of uridine utilization and 5-fluorouracil therapy in mice by benzylacyclouridine. Cancer Res., *45:* 5364–5368, 1985.
37. Peters, G. J., van Groeningen, C. J., Laurensse, E. J., Lankelma, J., Leyva, A., and Pinedo, H. M. Uridine-induced hypothermia in mice and rats in relation to plasma and tissue levels of uridine and its metabolites. Cancer Chemother. Pharmacol., *20:* 101–108, 1987.
38. Martin, D. S., Stolfi, R. L., and Sawyer, C. Use of oral uridine as a substitute for parenteral uridine rescue of 5-fluorouracil therapy, with and without the uridine phosphorylase inhibitor 5-benzylacyclouridine. Cancer Chemother. Pharmacol., *24:* 9–14, 1989.
39. Darnowski, J. W., and Handschumacher, R. E. Enhancement of fluorouracil therapy by the manipulation of tissue uridine pools. Pharmacol. Ther., *41:* 381–392, 1989.
40. Chu, S-H., Wiemann, M. C., Chu, M. Y., and Calabresi, P. Modulation of FUdR toxicity by two benzylacyclouridine analogs (Abstract). Proc. Am. Assoc. Cancer Res., *28:* 410, 1987.
41. Langen, P., Etzold, G., Barwolff, D., and Preussel, B. Inhibition of thymidine phosphorylase by 6-aminothymine and derivatives of 6-aminouracil. Biochem. Pharmacol., *16:* 1833–1837, 1976.
42. Woodman, P. W., Sarrif, A. M., and Heidelberger, C. Inhibition of nucleoside phosphorylase cleavage of 5-fluoro-2′-deoxyuridine by 2,4-pyridinediol derivatives. Biochem. Pharmacol., *29:* 1059–1063, 1980.
43. Desgranges, C., Razaka, G., Rabaud, M., Bricaud, H., Balzarini, J., and De Clercq, E. Phosphorolysis of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) and other 5-substituted-2′-deoxyuridines by purified human thymidine phosphorylase and intact blood platelet. Biochem. Pharmacol., *32:* 3583–3590, 1983.
44. Liermann, B., Matthes, E., and Langen, P. Human tissues degrade uridine much less

Downloaded from http://aacrjournals.org/cancerres/article-pdf/53/16/3687/2451328/cr0530163687.pdf by guest on 24 August 2022

than thymidine. Possible consequence for 5-fluorouracil therapy. Biochem. Pharmacol., *33:* 721–724, 1984.
45. el Kouni, M. H., Naguib, F. N. M., Niedzwicki, J. G., Iltzsch, M. H., and Cha, S. Uridine phosphorylase from *Schistosome mansoni.* J. Biol. Chem., *263:* 6081–6086, 1988.
46. Naguib, F. N. M., el Kouni, M. M., and el Kouni, M. H. Differences in characteristics and substrate specificity of human and mouse uridine (UrdPase) and thymidine (dThdPase) phosphorylases: relevance to chemotherapy (Abstract). FASEB J., *1:* A198, 1992.
47. el Kouni, M. H., Naguib, F. N. M., Park, K. S., Cha, S., Darnowski, J. W., and Soong, S-J. Circadian rhythm of hepatic uridine phosphorylase activity and plasma concentration of uridine in mice. Biochem. Pharmacol., *40:* 2479–2485, 1990.
48. Naguib, F. N. M., Soong, S-J., and el Kouni, M. H. Circadian rhythm of orotate phosphoribosyltransferase, pyrimidine nucleoside phosphorylases and dihydrouracil dehydrogenase in mouse liver: possible relevance to chemotherapy with 5-fluoropyrimidines. Biochem. Pharmacol., *45:* 667–673, 1993.
49. Naguib, F. N. M., el Kouni, M. H., Chu, S. H., and Cha, S. New analogues of benzylacyclo-uridines, specific and potent inhibitors of uridine phosphorylase from human and mouse livers. Biochem. Pharmacol., *36:* 2195–2201, 1987.
50. Wilkinson, G. N. Statistical estimations in enzyme kinetics. Biochem. J., *80:* 324–332, 1961.
51. Cleland, W. W. The statistical analysis of enzyme kinetic data. Adv. Enzymol., *29:* 1–32, 1967.
52. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., *72:* 248–254, 1976.
53. Monks, A., and Cysyk, R. L. Uridine regulation by the isolated rat liver: Perfusion with an artificial oxygen carrier. Am. J. Physiol., *242:* R465-R470, 1982.
54. Moyer, J. D., Oliver, J. T., and Handschumacher, R. E. Salvage of circulating pyrimidine nucleosides in the rat. Cancer Res., *41:* 3010–3017, 1981.
55. Gasser, T., Moyer, J. D., and Handschumacher, R. E. Novel single-pass exchange of circulating uridine in rat liver. Science (Washington DC), *213:* 777–778, 1981.
56. Holstege, A., Leser, H-G., Pausch, J., and Gerok, W. Uridine catabolism in Kupffer cells, endothelial cells, and hepatocytes. Eur. J. Biochem., *149:* 169–173, 1985.
57. Holstege, A., Pausch, J., and Gerok, W. Effect of 5-diazouracil on the catabolism of circulating pyrimidines in rat liver and kidneys. Cancer Res., *149:* 169–173, 1985.
58. Berlinger, W. G., Stene, R. A., Spector, R., and Al-Jurf, A. S. Plasma and cerebrospinal fluid nucleosides and oxypurines in acute liver failure. J. Lab. Clin. Med., *110:* 137–144, 1987.
59. Heidelberger, C. *In:* A. C. Sartorelli and D. G. Johns (eds.), Antineoplastic and Immunosuppressive Agents, Part II, pp. 193–231. Berlin: Springer-Verlag, 1975.
60. Kit, S. Thymidine kinase, DNA synthesis and cancer. Mol. Cell Biochem., *11:* 161, 1976.
61. Naguib, F. N. M., Levesque, D. L., Wang, E-C., Panzica, R. P., and el Kouni, M. H. 5-Benzylbarbituric acid derivatives, potent and specific inhibitors of uridine phosphorylase. Biochem. Pharmacol., in press, 1993.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/53/16/3687/2451328/cr0530163687.pdf by guest on 24 August 2022