Expression, Characterization, and Detection of Human Uridine Phosphorylase and Identification of Variant Uridine Phosphorolytic Activity in Selected Human Tumors

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ABSTRACT

Uridine phosphorylase (UPase) catalyzes the reversible phosphorolysis of uridine to uracil. We purified the enzyme from the murine colon 26 tumor using a two-step procedure through 5-amino-benzylacyclouridine affinity chromatography. Antibodies raised in rabbits against the purified protein revealed single bands in Western blots of normal human tissue and tumor extracts. The polyclonal antibody used to screen a human liver expression library allowed the isolation of a 1.2-kb clone that contained the entire open reading frame of the human UPase. The UPase cDNA has been expressed as a fusion protein in Escherichia coli using the pMal-C2 vector. The kinetic analysis demonstrated that the recombinant UPase preferentially uses uridine, 5-fluorouracil, and uracil as substrates, although lower levels of activity were observed with 2-deoxyuridine and thymidine.

Clinical samples of human tumors and adjacent normal tissues were assayed for phosphorolytic activity and sensitivity to 5-benzylacyclouridine (BAU), a potent inhibitor of the enzyme presently in Phase I-II clinical trial. Activity in normal tissues appeared to be low but very sensitive to BAU (∼90% inhibition at 10 μM). Tumors had generally 2-3-fold greater activity compared with adjacent normal tissues. In breast cancer specimens and head-neck squamous carcinomas, however, uridine cleavage was only partially inhibited (40-60%) by 10 or 100 μM BAU. The BAU-insensitive activity requires phosphate and pH conditions similar to the normal enzyme, and the new phosphorolytic activity was independent from thymidine phosphorylase. The BAU-insensitive phosphorolytic activity in selected tumors, coupled with the potent inhibitory activity of BAU against the "classical" uridine phosphorylase in normal human tissues, provides the rationale for combining BAU with 5-fluorouracil in the treatment of breast and head-neck tumors.

INTRODUCTION

The intracellular concentration of uridine is regulated by two major mechanisms: (a) transport of the pyrimidine nucleoside through the cell membranes; and (b) catabolism by nucleoside phosphorylases. The first mechanism is a non-energy-dependent, facilitated diffusion mechanism with broad substrate specificity toward synthetic and naturally occurring pyrimidine nucleosides (1-5). In addition to the nonconcentrative, facilitated diffusion mechanism, hepatic, renal, and gut epithelial cells were shown to possess a Na⁺-dependent transporter for nucleosides (6-11). We have demonstrated the generality of the expression of a concentrative, active transport mechanism for uridine in a variety of normal murine tissues (12). These observations changed the perception of the Na⁺-dependent uridine transport process from a rate phenomenon observed in isolated cells to a major physiological effect that afforded therapeutic opportunities. Perhaps the most significant finding in these studies was that intracellular concentrations of uridine in a wide variety of neoplastic cell lines did not exceed those in the media. It has been shown that some cell lines express the concentrative mechanism to a very limited degree (13) but that it is overwhelmed by the equilibration of intra- and extracellular nucleoside achieved by the very active, facilitated diffusion mechanism.

Intimately related to transport of nucleosides into cells are the processes responsible for their anabolism and catabolism. UPase (EC 2.4.2.3) catalyzes the reversible phosphorolysis of pyrimidine ribosides (except for cytidine) but also cleaves pyrimidine 2'- and 5'-deoxyribosides (14-18). Uridine phosphorylase is present in most tissues and in tumors (14, 16). The importance of pyrimidine nucleoside phosphorylases in the activation and catabolism of fluoropyrimidines is apparent (19, 20). Furthermore, uridine phosphorylase plays an important role in the homeostatic regulation of uridine concentrations in plasma (21-24). Several studies in animal models have demonstrated the ability of uridine to reduce the toxicity of 5-FU, allowing the administration of a higher dose of the fluoropyrimidine and resulting in an improved therapeutic index (21, 24). Because of the very short plasma half-life of uridine, large doses are necessary to achieve a significant therapeutic effect. Particularly in humans, high dose uridine is not well tolerated (25). We have completed a clinical trial that has demonstrated that a relatively small dose (800 mg/m²) of BAU, a potent inhibitor of UPase, can safely elevate plasma uridine concentration (26). We have recently started a Phase I clinical trial to seek modulation of 5-FU therapy by conservation of endogenous uridine via inhibition of UPase with BAU (16, 17). This approach, as suggested by our studies reported here, is potentially more selective, particularly in breast and oropharyngeal tumors, and should be better tolerated by the patients by reducing the toxic effects of chemotherapy to the normal tissues.

MATERIALS AND METHODS

Experimental Tumors, Tissue Procurement, and UPase Assay. All normal human tissues and tumors were obtained from patients undergoing surgical removal of their malignancy through the Surgical Pathology Department of Yale New Haven Hospital (New Haven, CT), according to an appropriate clinical protocol approved by the local Institutional Review Board. Fresh tissues were obtained (within 5 min from the explant) and immediately frozen in liquid nitrogen after an on-call pathologist proceeded to localize the margin between the normal and the neoplastic portion of the specimen and separate the tissues. Characteristics and quality of the samples were confirmed by the same pathologist within 24 h after microscopic examination.

Colon tumor 26 was transplanted every 30-40 days as a s.c. brei in female BALB/c mice 6-8 weeks of age, excised from the animals once they reached a size of ~500 mg (usually 2 weeks after the transplant), and immediately placed in liquid nitrogen until homogenization.

Normal tissues and tumors were homogenized in 50 mm Tris-HCl (pH 7.5) containing 2 mm DTT, using a tissue mixer Ultra-Turrax (IKA, Staufen, Germany). The extract was then centrifuged at 100,000 × g for 1 h at 4°C. The supernatant was used to measure the UPase activity. The enzyme reaction was

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3 The abbreviations used are: UPase, uridine phosphorylase; 5-FU, 5-fluorouracil; BAU, 5-benzylacyclouridine; IPTG, isopropyl-1-thio-β-D-galactopyranoside; TPase, thymidine phosphorylase.
IDENTIFICATION OF BAU-INSENSITIVE URIDINE PHOSPHORYLASE

Identification of BAU-insensitive Uridine Phosphorylase

Carried out in a total volume of 100 µl of 50 mM Tris buffer (pH 7.5) containing 1 mM potassium phosphate, tissue extract, and 200 µM [5'-3H]uridine at 37°C for 30 min. Aliquots of 10 µl from each sample were applied to TLC silica plates. The TLC plates were developed with chloroform:methanol:acetic acid (85:15:5, v/v). The unlabelled markers were visualized under UV light, and the radioactivity in the appropriate areas was measured using a Beckman LS 7000 scintillation spectrometer. The protein concentration of the tissue extracts was determined by the method of Bradford (27).

Purification of Mouse UCPase by Using 5'-NH2-BAU Affinity Column.

Results

Antibody Production and Western Blot. Purified murine UPase was used to immunize three rabbits. For primary immunization, 100 µg of uridine phosphorylase were emulsified in complete Freund's adjuvant and inoculated s.c. at six sites in a shaved area of the back of the animal, using 100 µl of inoculum/site. Rabbits were then boosted s.c. 3–4 weeks later and every 3–6 weeks thereafter with antigen in incomplete Freund's adjuvant, using 100 µl of inoculum at one to three sites. After the first booster immunization, a small (5–10 ml) blood sample was drawn from the central ear artery using a 21-gauge butterfly cannula. Depending on antibody titer, large (30–50 ml) samples were drawn every 2–4 weeks thereafter.

Samples for SDS-PAGE were prepared by mixing three volumes of sample with 1 volume of buffer (0.25 M Tris-HCl (pH 6.8), 30% glycerol, 20% BME, and 0.277 M SDS) and heated at 100°C for 3 min. Protein from human normal tissue and tumors was separated by SDS-PAGE and transferred from the gel to nitrocellulose using a Bio-Rad trans-blot cell (Bio-Rad, Hercules, CA) with transfer buffer (20% methanol, 25 mM Tris-HCl, 192 mM glycine, and 0.1% SDS). Membranes were blocked, followed by incubation in a 1:1000 dilution of horseradish peroxidase-conjugated goat anti-rabbit antibody with appropriate washes between steps. Antigen detection was performed using the ECL method (Amerham, Arlington Heights, IL) as described by the manufacturer.

Cloning of the Human UPase.

Specificity of Anti-UPase Polyclonal Antibody. The purified protein was used to immunize three rabbits using standard methodology. After the first boost, we obtained a highly specific polyclonal antibody for human UPase, which we used to screen a commercial human liver expression library constructed in the Lambda ZAP II vector and hosted in the XL-1-Blue MRF strain of E. coli (Stratagene, La Jolla, CA). The primary screening yielded 100 µg of murine UPase in total volume of 100 µl, which was dialyzed with 1:1000 dilution of a high-titer stock of clone 1/1 resulting in at least eight well-defined clones with negligible background. Using a commercially available methodology, the ExAssist/SOLR System (Stratagene), the α-bluetick sequence was amplified from the Lambda ZAP II vectors. After evaluating by Western blot, the expression of UPase in the control SOLR cell extracts and in the extract of SOLR cells containing the putative recombinant plasmids after IPTG induction, the double-strand DNA insert of the positive clone was sequenced by the Protein and Nucleic Acid Chemistry Facility of the Yale Cancer Center.

To confirm the obtained sequence, two degenerate primers were synthesized based on a published murine UPase cDNA sequence (28) to amplify the murine UPase by RT-PCR. Total RNA from mouse colon tumor was purified by TRIzol reagent (Stratagene) and used as template. A human liver cDNA library constructed in a Lambda ZAP II vector (Stratagene) was screened at an estimated sequence identity with our previously identified cDNA.

The clone was sequenced by the Protein and Nucleic Acid Chemistry Facility of the University of California, San Diego (UCSD). The cDNA was subsequently inserted into a Prokaryotic Expression Vector (pMal-C2 vector (New England Biolabs, Beverly, MA) at the EcoRI-HindlV cut sites, which encodes for a maltose binding fusion protein. A single DH5α colony transformed with the plasmid containing the UPase gene was grown overnight in rich medium (tryptone, yeast extract, NaCl, and glucose) containing 100 µg/ml ampicillin. The overnight culture was diluted 1:100 in rich medium containing ampicillin and grown until A600 reached 0.5. Growth was induced with 0.3 mM IPTG for 2 h at 37°C. Bacteria were pelleted by centrifugation for 15 min, 7000 x g at 4°C. Pellets were resuspended in column buffer (20 mM Tris-HCl (pH 7.5)), 200 mM NaCl, 1 mM EDTA, and 10 mM β-mercaptoethanol. Lysis was achieved by using a French Press, and the lysate was clarified by centrifuging at 10,000 x g (1 h at 4°C) in a JA-20 rotor (Sorvall, Newtown, CT). Supernatants were applied to an amyllose resin column, which binds the maltose binding protein component of the fusion protein. The fusion protein bound to the column eluted using 10 mM maltose in column buffer and then cleaved by 0.1% factor Xa at 4°C for 2 h. The fusion protein cleavage mixture was dialyzed with 20 mM Tris-HCl, 25 mM NaCl (pH 7.5; three changes of 100 volumes for 2 h) and then applied to a DEAE column. Maltose binding protein and trace contaminants were retained by the DEAE resin, and pure recombinant UPase was collected in the flow-through. The pure protein was then concentrated using Centriplus microconcentrators (Amicon, Beverly, MA).

RESULTS

UPase Purification. We have prepared an affinity matrix using an NH3 derivative of BAU (5'-amino-benzylacyclouridine) coupled through N-hydroxy-succinimide chemistry to an agarose matrix (Affigel-10). Particle-free extracts of murine colon tumor 26 have been used as our initial sources of uridine phosphorylase. Using a 0.2-mL column to which ~5 µmol of amino-BAU were coupled (the remainder of the sites were blocked with ethanolamine), up to 20 ml of the supernatant from a 5:1 homogenate could be stripped of phosphorylase activity. After thorough washing, the column was eluted with 20 mM uridine to yield a single major protein band at Mₗ ~32,000–35,000, which contained 20–40% of the original activity. When combined with a preliminary DEAE column purification (adsorption and elution with 0.2 M NaCl), a homogeneous protein was obtained as judged by SDS-PAGE (Fig. 1) and isoelectric focusing (data not shown). With only two steps, we were able to generate a 7000-fold purification with a >20% yield (Table 1).

Specificity of Anti-UPase Polyclonal Antibody. The purified protein was used to immunize three rabbits using standard methodology. After the first boost, we obtained a highly specific polyclonal antibody...
antibody. Serum dilutions of 1:500 and 1:1,000 were able to recognize <5 ng of the purified murine protein. The same polyclonal antibody was able to detect UPase present in crude extracts of normal human liver and paired liver tumor (Fig. 2). The major single band present in a human liver tumor specimen had an intensity by densitometry that was 2-fold higher than the normal tissue. This ratio was consistent with the enzymatic activity that was 46.8 nmol/h/mg protein in tumor and 20.2 nmol/h/mg protein in normal tissue. Similar results were obtained with other tumors and paired normal tissues (Fig. 3). Again, the antibody recognized a single protein of ~32,000–35,000, and the densitometry data correlated with the difference in enzymatic activity between tumor and paired normal tissue.

Cloning of Human UPase. Human UPase CDNA was cloned from a human liver expression library using the specific polyclonal antibody obtained against the murine colon 26 protein as described in “Materials and Methods.” A ~1.2-kb CDNA fragment was isolated and tested positive, by Western blot, for expression of UPase in the extract of SOLR cells containing the putative recombinant plasmid after IPTG induction (29). Based on a published sequence for the murine protein (28), the same human liver expression library was screened with a probe obtained by using two degenerate primers for the murine enzyme. A positive clone was isolated having an insert size of ~36.8 kb. The cDNA sequence that we obtained was identical in the longer 5' untranslated region.

The human UPase appears to have little in common with other sequenced proteins. It contains the sequence SHGMGIPSISIMLHELS (amino acids 107–122) that represents the signature motif for the purine and other phosphorylase family 1. The same consensus pattern [GST]-x-G-[LIVM]-G-x-[PA]-S-x-[GSTA]-I-x (3)-E-L has been found present in purine nucleoside phosphorylase (EC. 2.4.2.1) from most bacteria (31). This enzyme catalyzes the cleavage of inosine and guanosine to the respective bases, and in 5'-methylthioadenosine phosphorylase (EC. 2.4.2.28) from Sulfolobus solfataricus (32), this protein regulates polyamine metabolism and the salvage of adenine and methionine. The phosphorylase family 1 signature pattern present in the human UPase appears highly conserved throughout different species as shown in Table 2.

Expression and Kinetic Characterization of Human UPase. The recombinant human UPase obtained after cleavage of the maltose binding portion of the fusion protein by factor Xa and purification on a DEAE column showed high substrate specificity for uridine, 5-fluorouracil, and uracil (Table 3). Lower levels of activity were obtained with 2-deoxyuridine and thymidine when used as substrates. No enzymatic activity was detected in the presence of cytidine. Analysis of the kinetic parameters of the recombinant human UPase determined by steady-state kinetic methods indicated similar $K_m$ and $V_{max}$ values for uridine and uracil, with higher affinity but lower $V_{max}$ for ribose-1-phosphate (Table 4).

The enzymatic activity was pH dependent with an optimal range between 7 and 7.75 and maximum activity at pH 7.5 (Fig. 4). Both enzymatic reactions, catabolic (uracil formation) and anabolic (uridine formation), were competitively inhibited by benzylcyclouridine with a $K_i$ of 7.3 μM (Fig. 5).

Activity and BAU Sensitivity of UPase Present in Clinical Specimens. UPase activity was evaluated in fresh tumor specimens and adjacent normal tissues of patients after surgical resection of their malignancies. The enzymatic activity was variable among the different tissue specimens, but overall it was 2–3-fold higher in tumors compared with the paired normal tissue (Fig. 6). For the tissues that we were able to collect, the most clinical specimens, breast and colon, the difference in activity between tumor and normal tissues was statistically significant with Ps of 0.012 and 0.021, respectively. In all normal tissues and most tumor specimens evaluated, the UPase activity was very sensitive to the effect of BAU, with 90–95% inhibition in the presence of 10 μM BAU. Breast, head-neck, and ovarian tumors showed partial sensitivity to the inhibitor with ~40% of residual phosphorylolytic activity still present after the addition of 100 μM BAU (Fig. 7).

The contribution of TPase activity to the BAU-insensitive phosphorylolytic activity was evaluated using inhibitors of TPase. Two

![Figure 2](cancerres.aacrjournals.org) Western blot analysis of UPase in liver tumor and paired normal liver tissue extracts. Tissue extract proteins were separated on a 7.5% SDS-PAGE gel and analyzed for enzymatic activity as described in "Materials and Methods." M W, molecular weight in thousands.

![Figure 3](cancerres.aacrjournals.org) Western blot analysis of UPase present in tumor and adjacent normal tissue extracts. Tumor tissue and paired normal tissue extracts were analyzed by Western blot, after SDS-PAGE separation, and for enzymatic activity as described in "Materials and Methods." M W, molecular weight in thousands.

### Table 1 Purification of UPase from colon 26 murine tumor

<table>
<thead>
<tr>
<th>Specific activity (nmol/mg/hr)</th>
<th>Purification (X)</th>
<th>Yield (%)</th>
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<tr>
<td>Tissue extract</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>DEAE column (NaCl eluate)</td>
<td>77.5</td>
<td>6</td>
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<tr>
<td>BAU column (uridine eluate)</td>
<td>89.600</td>
<td>6,945</td>
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### Table 2 Sequence identities and homologies for the phosphorylase family 1 signature pattern in UPases from different species

<table>
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<tr>
<th>Species</th>
<th>Signature Pattern</th>
<th>107</th>
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<th>107</th>
<th>66</th>
<th>107</th>
<th>66</th>
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<tr>
<td>Human</td>
<td>SHGMGIPSISIMLHELS</td>
<td>122</td>
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<td>122</td>
<td>122</td>
<td>122</td>
<td>122</td>
<td>81</td>
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<tr>
<td>Mouse</td>
<td>SHGMGIPSISIMLHELS</td>
<td>123</td>
<td>123</td>
<td>123</td>
<td>123</td>
<td>81</td>
<td>122</td>
<td>81</td>
</tr>
<tr>
<td>E. coli</td>
<td>S G+G PS SI + EL</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella aerogenes</td>
<td>SHGMGIPSISIMLHELS</td>
<td>122</td>
<td>122</td>
<td>86</td>
<td>122</td>
<td>81</td>
<td>122</td>
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<tr>
<td>Salmonella typhimurium</td>
<td>SHGMGIPSISIMLHELS</td>
<td>122</td>
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<td>86</td>
<td>122</td>
<td>81</td>
<td>122</td>
<td>81</td>
</tr>
</tbody>
</table>
breast tumor extracts were incubated in the presence of 100 μM BAU and 100 μM 5-bromo-6-aminouracil, an inhibitor of TPase (33). The addition of the TPase inhibitor did not potentiate the inhibitory effect of BAU in one tumor specimen and caused only a 15% reduction in the other (Table 5). Approximately 30–35% of the initial phosphorolytic activity remained insensitive to classical phosphorylase inhibitors.

To confirm this finding, the tissue extract of a breast tumor was evaluated for both uridine and TPase activities before being adsorbed on a BAU affinity matrix. The activity of both enzymes was then determined in the flow-through in the presence of 100 μM of the respective inhibitor. The affinity column extracted ~80% of the UPase activity, and the addition of BAU to the eluate marginally reduced the residual activity. None of the initial TPase activity remained on the column, and 100 μM 5-bromo-6-aminouracil caused a 75% reduction of the enzymatic capacity (Fig. 8). These experiments confirm that TPase does not significantly contribute to the BAU-insensitive phosphorolytic activity present in breast tumor tissues.

**DISCUSSION**

Using a specific polyclonal antibody for UPase, we have isolated from a human liver expression library a cDNA clone containing a 0.4-kb 5' untranslated region, the UPase coding region, and a 3' untranslated sequence with a poly(A) tail. The coding region of the isolated cDNA is identical to the one obtained from a HTC-116 human colorectal tumor library with a mouse UPase cDNA probe (30). Except for a 15-amino acid sequence characteristic for the phosphorylase family 1, human UPase does not appear to share any common sequence or homology with other known proteins. The purified recombinant human enzyme has kinetic characteristics similar to other previously purified mammalian UPases (14, 34, 35).

Our studies have also indicated a higher enzymatic activity in tumor tissues as compared with paired normal tissue. These data are in contrast with a report from Maehara et al. (36), indicating no difference in UPase activity between tumor and normal tissues. We also dispute the finding from the same group that the main pyrimidine nucleoside phosphorylase in human is TPase with activity 20-fold higher than UPase (36). In our experience, we have observed a high degree of variation in the ratio of TPase to UPase activity with an overall ratio of 2 (data not shown). In many tissues, particularly breast, the ratio was actually slightly in favor of UPase. Our study suggests that TPase does not significantly contribute to the elevated UPase activity in tumor. Except for breast and head-neck tumors, a relatively low concentration of BAU (10 μM) was able to reduce the enzymatic activity equally in normal and tumor samples by 90–95%. Because BAU does not inhibit TPase at such a low concentration, we have to conclude that the contribution of this enzyme to the higher UPase activity in tumors is not significant.

The increased uridine catabolism in tumor and the lack of an active transport of the nucleoside in neoplastic cells are crucial observations that have led us to the careful examination of the use of "uridine rescue" after 5-FU-based chemotherapy.

The drug regimen of 5-FU followed by uridine has been initially implemented to test the hypothesis that the antitumor effect of 5-FU is primarily due to its inhibition of thymidylate synthase and the host toxicity primarily related to 5-FU incorporation into RNA (37). Using several tumor models in vivo, uridine has been shown to protect against the toxicity of 5-FU, thereby permitting the use of higher doses of 5-FU without compromising its antitumor effect (38, 39).

A number of clinical trials have been conducted to determine the clinical pharmacology of uridine in patients (25, 40) and also to examine the ability of uridine rescue to actually prevent toxic effects of 5-FU alone or in combination with methotrexate and N-(phosphonoacetyl)-L-aspartic acid, thereby allowing dose escalation of 5-FU (41). These studies have indicated that patients tolerated combination...
therapy with uridine up to a dose of 750 mg/m² of 5-FU, with 25% experiencing moderate mucositis (grade II). In a previous clinical trial without uridine, four of six patients could not tolerate a 600 mg/m² dose of 5-FU because of mucositis, diarrhea, and a decrease in performance status. In another study, 5-FU treatment was continued in the presence of uridine at a weekly dose of 5-FU that caused dose-limiting myelosuppression. In most of the patients who had previously developed leukopenia, the WBC count increased markedly despite continued 5-FU administration. In patients developing thrombocytopenia, uridine failed to rescue the 5-FU toxicity. Uridine, however, is difficult to administer p.o. because of a very low bioavailability (7–12%). The extremely large doses of nucleoside that must be given p.o. cause diarrhea because of the dose-limiting toxicity in both single-dose and multiple-dose regimens. On the other hand, prolonged i.v. infusions of uridine caused high fever. With intermittent infusions, fever was no longer dose limiting. Problems still remained that pre-

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**Fig. 6.** UPase activity present in tumor and paired normal tissue extracts. Normal human tissues and tumors obtained from patients undergoing surgical procedures were homogenized in 50 mM Tris-HCl (pH 7.5), and the extract was assayed for enzymatic activity in the presence of 1 mM potassium phosphate and 200 μM [5-³H]uridine at 37°C as described in "Materials and Methods." Bars, SD.

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**Fig. 7.** Inhibitory effect of BAU on UPase activity present in tumor and paired normal tissue extracts. Tumor and adjacent normal tissues extracts were assayed for UPase activity in the absence and in the presence of 10 and 100 μM BAU as described in "Materials and Methods." Bars, SD.
include extensive clinical studies: phlebitis, cellulitis, and in some patients, superior vena cava syndrome.

BAU has been shown by our group to cause a 5–50-fold expansion of plasma and tissue uridine (42, 43). These observations have suggested the use of BAU as a modulator of 5-FU therapy in place of or as an augmentor of uridine modulation. In a colon tumor model, BAU has been shown to decrease 5-FU toxicity with an actual improvement in antitumor effect (21). Other studies by Martin et al. (24) have indicated that an improved therapeutic index of 5-FU is achieved in some model breast tumors with a combination of BAU and uridine administered after 5-FU. A recent Phase I clinical study that we have conducted has shown that BAU elevates plasma uridine pools without significant toxic effects to the patients (26).

Our present studies have indicated that both bone marrow and gut mucosal specimens possess low phosphorolytic activity compared with tumor tissues, and this activity is completely inhibited by a low concentration of BAU. These data suggest a specific benefit of the uridine rescue approach for these tissues (gut and bone marrow) that are the primary targets of drug toxicities.

Our experience with murine tumor models possessing only the BAU-sensitive phosphorolytic activity indicates that neoplastic tissues contain uridine concentrations below other normal tissues. Tumor concentrations are usually in equilibrium with the plasma level because of the inefficient transport of the nucleoside and increased catabolism related to high UPase activity. In view of our discovery of a BAU-insensitive phosphorolytic activity, we can predict that, with those tumors containing the dual enzymatic activity (breast and squamous cell carcinomas), the differential with the other normal tissues could actually be enhanced, leading to increased specificity and selectivity of the uridine rescue.

The generally higher level of uridine degradation activity and expression of considerable BAU-insensitive activity in selected human tumors provides further encouragement for the use of BAU and uridine as modifiers for 5-FU chemotherapy in the clinic. Given the similar distribution of BAU in all tissues (26), higher residual uridine cleavage would be expected to remain in those tissues with higher phosphorylase activity and/or BAU-insensitive activity. Consequently, these tissues will have lower concentrations of uridine to compete with 5-FU cytotoxicity. In regimens using uridine and BAU as comodulators, this may be even more important because elevated concentrations of uridine will be accessible to all cells. Selective and favorable enhancement of 5-FU cytotoxicity toward tumors could thereby be achieved, because they would have lower uridine concentrations consequent to the very active BAU-insensitive cleavage activity as well as a limited potential to generate or sustain uridine concentration gradients.

Our experiments partially confirm a report indicating a contribution of TPase to the degradation of uridine in some tumor specimens (35). Data from el-Kouni et al. (35) have indicated that 15–17% of the phosphorolytic degradation of uridine in liver was due to TPase activity, and our study in some breast cancer specimens is in agreement with those values. Our observation, however, is that the BAU-insensitive phosphorylase activity is independent from the classical uridine and TPase activities. The BAU-insensitive enzyme shows cross-reactivity with the UPase polyclonal antibody, and Northern blots of various breast tumors probing with normal UPase cDNA under low stringency conditions revealed an additional band present in breast tumor tissues, suggesting an alternative form of UPase, which shares significant homology to “classical” UPase. Studies are in progress to isolate and characterize this new protein.

ACKNOWLEDGMENTS

We thank Dr. S-H. Chu from Brown University for kindly providing several inhibitors of UPase and TPase.

REFERENCES


Table 5 Effect of “classical” phosphorylase inhibitors on the phosphorolytic activity present in breast tumor extracts

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Breast tumor no. 1</th>
<th>Breast tumor no. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μM BAU</td>
<td>35.5*</td>
<td>49.0</td>
</tr>
<tr>
<td>100 μM BAU + 100 μM 5-Br-6-NH₂-uracil</td>
<td>32.5</td>
<td>34.5</td>
</tr>
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</table>

* Values are expressed as a percentage of residual phosphorolytic activity using [³H]uridine (200 μM) as substrate.


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