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TISSUE-SPECIFIC EXPANSION OF URIDINE POOLS IN MICE

EFFECTS OF BENZYLACYCLOURIDINE, DIPYRIDAMOLE AND EXOGENOUS URIDINE

JAMES W. DARNOWSKI,* ROBERT E. HANDSCHUMACHER,† ROSEMARY A. WIEGAND,† FREDERICK A. GOULETTE and PAUL CALABRESI

Department of Medicine, Brown University and Roger Williams General Hospital, Providence, RI; and †Department of Pharmacology, Yale University, School of Medicine, New Haven, CT, U.S.A.

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Abstract—The concentration of uridine (Urd) in murine tissues appears to be controlled by Urd catabolism, concentrative Urd transport, and the non-concentrative, facilitated diffusion of Urd. Previous reports document the tissue-specific disruption of these processes, and subsequently intracellular pools of free Urd in mice, by the administration of exogenous Urd (250 mg/kg) or the Urd phosphorylase (EC 2.4.2.3; uracil:ribose-1-phosphate phosphotransferase) inhibitor 5-benzylacyclouridine (BAU) (240 mg/kg). We now report the effect of combinations of BAU (120 mg/kg, p.o.), the nucleoside transport inhibitor dipyridamole (DP) (25 mg/kg, i.p.), and exogenous Urd (250 mg/kg, i.v.) on Urd pools in mice. This dose of BAU increased Urd pools 2- to 6-fold, in a tissue-specific manner, for up to 5 hr. DP increased Urd pools 3-fold in spleen, over a 4-hr period, but did not affect other tissues. Administration of BAU 1 hr prior to exogenous Urd resulted in a 50- to 100-fold expansion of tissue Urd pools which returned to normal within 4 hr, except in spleen, where the Urd concentration was normal after 6 hr. Administration of DP 1 hr prior to exogenous Urd caused a tissue-specific 40- to 100-fold increase in Urd pools which, except in spleen, returned to normal within 2 hr. The marked additive effects of these combinations were in contrast to those obtained following the administration of BAU 1 hr prior to DP. This regimen increased Urd pools from 4- to 9-fold, in a tissue-specific manner. In addition, Urd pools remained elevated for up to 9 hr, except in spleen where the Urd concentration was elevated for up to 15 hr. Analysis of enzyme activities indicated that DP does not enhance the inhibitory effect of BAU against murine liver Urd phosphorylase. However, DP did inhibit the plasma clearance of BAU, and this effect may partially explain the apparent synergistic effect of this combination. In spite of the prolonged and dramatic expansion of tissue Urd pools produced by BAU + DP, the total Ura nucleotide content in spleen, gut and colon tumor 38 (CT38) increased by less than 70% over a 12-hr period following administration of this combination. These findings are discussed in light of their biochemical and therapeutic implications.

Earlier reports from this laboratory demonstrated that murine tissues contain relatively high concentrations of free uridine (Urd‡) when compared to plasma [1]. Two biochemical processes appear to have an effect on the generation and maintenance of elevated intracellular Urd pools. First, the capacity of tissues to catabolize Urd via Urd phosphorylase (EC 2.4.2.3; uracil:ribose-1-phosphate phosphotransferase) is partially related to the size of tissue Urd pools. Tissues with relatively high Urd phosphorylase activity generally have low intracellular Urd pools. Second, the relative activities of various nucleoside transport systems appear to affect intratissue Urd pools. Findings from several laboratories suggest that, in addition to the well-defined and extensively studied facilitated transport systems [2–7], there exists at least two Na^+-dependent nucleoside transport systems with different substrate specificities [8–14]. These active transport systems are responsible for the unidirectional, concentrative transport of nucleosides. Further evidence suggests that these concentrative processes are distributed in a tissue-specific manner, with significant differences between epithelial and non-epithelial cell types [13, 14]. All nucleoside transport systems identified to date recognize Urd as a substrate. The relative activities of the concentrative and non-concentrative systems in tissues presumably correlate with their ability to maintain elevated intracellular Urd pools [1].

We have demonstrated previously that, in mice, tissue pools of Urd can be selectively expanded by increasing the plasma concentration of Urd or by inhibition of Urd catabolism [1]. Specifically, a single i.v. injection of Urd (250 mg/kg) generates a 5- to 70-fold tissue-specific expansion of Urd pools. This effect is of short duration; within 90 min Urd pools in plasma and all tissues are normal. Similarly, an i.v. injection of 5-benzylcyclouridine (BAU) (240 mg/kg), a potent and specific inhibitor of Urd phosphorylase [15, 16], results in a 5- to 15-fold

* To whom all correspondence should be addressed at: Department of Medicine, Roger Williams General Hospital, 825 Chalkstone Ave., Providence, RI 02908.

‡ Abbreviations: Urd, uridine; Ura, uracil; FUrd, 5-fluorouridine; FUra, 5-fluorouracil; AZT, 3′-azidothymidine; BAU, 5-benzylacyclouridine (5-benzyl-1-[2′-hydroxyethoxymethyl]uracil); DP, dipyridamole; PCA, perchloric acid; and CT38, colon tumor 38.

increase in tissue Urd pools [1]. Although not of the same magnitude as that achieved after Urd administration, the duration of the effect with BAU is significantly longer and pools do not return to normal until 8–10 hr after dosing [1]. Since it has been demonstrated that elevated plasma and tissue pools of Urd can enhance the therapeutic effectiveness of agents, such as 5-fluorouracil (FUra) [17–19] and 3′-azidothymidine (AZT) [20], we have extended our previous studies and now report that tissue Urd pools and, to a lesser degree, tissue uracil (Ura) nucleotide pools can be selectively expanded for prolonged periods by inhibiting both Urd phosphorylase and the facilitated efflux of Urd. Preliminary aspects of these findings have been reported previously [21, 22].

MATERIALS AND METHODS

Mice/drug administration. All experiments utilized 6- to 12-week-old female C57BL/6 mice (hereafter called C57) obtained from Taconic Farms (NY). Colon tumor 38 (CT38) was transplanted, by subcutaneous injection, with 0.3 mL of a 10% tumor brei in the right axillary region [19, 23]. Unless otherwise noted, tumor-bearing mice (20–40 days post-transplant) were used in all experiments. For injection purposes, Urd was dissolved in normal saline and injected i.v. at a dose of 250 mg/kg. Because of its high bioavailability [24], and in anticipation of future clinical trials, BAU was dissolved in 20% ethanol (in saline) and administered at 120 mg/kg, orally. Injectable dipyridamole (DP) was diluted in saline and injected i.p. at 25 mg/kg. In all cases, 0.1 mL of drug solution was administered/10 g of body weight.

Chemicals. Urd was purchased from the Sigma Chemical Co. (St. Louis, MO). DP was the generous gift of Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, CT). [2-^{14}C]-5-Fluorouridine ([2-^{14}C]FUrd) (56 mCi/mmol) was purchased from Moravek Biochemicals (Brea, CA). BAU was supplied by Dr. S. Chu of Brown University. Whatman PE Sil G/UV flexible TLC plates, HPLC grade solvents and chemicals were purchased from Fisher Scientific (Medford, MA).

Methods. Tissue and plasma Urd pools were assayed after administration of either DP, BAU, or the combinations of DP $\xrightarrow{1hr}$ Urd, BAU $\xrightarrow{1hr}$ Urd or BAU $\xrightarrow{1hr}$ DP, with each agent administered at the dose and route of administration noted above. At various times after injection 250 μL of whole blood was collected from the orbital sinus in a heparinized Natelson pipette and placed on ice. The animals were then anesthetized with Metofane (Pitman-Moore, Inc., Washington Crossing, NJ) and a portion of the tumor, as well as intestine, liver and kidneys rapidly removed and frozen in liquid nitrogen. In addition to these tissues, and to circumvent mechanical problems involved in the isolation of murine bone marrow after liquid nitrogen processing, the spleen (as a representative hematopoietic organ) was also removed and processed as described above. The plasma and tissue samples were then processed further, and their Urd content was determined by HPLC methods exactly as previously described [1]. Alternatively, representative tissue samples were homogenized in a 1 vol. of 1.2 N perchloric acid (PCA) after liquid nitrogen treatment [25]. The homogenates were centrifuged, and the supernatant was removed and neutralized by the addition of 0.6 N KOH. After filtration, the samples were stored at −20° until HPLC analysis. In all instances, tissue Urd concentrations detected by both preparative methods were comparable.

To determine Ura nucleotide content, acid-soluble tissue extracts generated above were also assayed by a modification of previously reported HPLC methods [19, 26]. This analysis utilized a Rainin Modular HPLC system equipped with a Whatman Partisal SAX10 analytical (4.6 mm × 25.0 cm) column eluted at 1 mL/min with a linear 1 hr gradient of 0.02 M Na_2HPO_4 (pH 3.5) to 0.35 M NaH_2PO_4 + 0.7 M KCl (pH 3.5). The column effluent was monitored at 254 nm. Under these conditions, the retention times of UMP, UDP and UTP were 9.5, 22.5 and 43.0 min, respectively.

The effects of BAU and DP on Urd phosphorylase activity were assayed in the cytosol fraction of murine liver. Homogenates were prepared and enzyme activity was assessed in the presence of various concentrations of BAU ± 100 μM DP by TLC methods previously described in detail [1].

The effect of DP on the plasma clearance of BAU was studied in groups of fourteen C57 mice. After BAU administration mice were divided into two groups, one of which received DP 1 hr after BAU. Whole blood and tissues were obtained and processed as described above. BAU was quantitated by reverse-phase HPLC methods previously described [24].

The effect of BAU on the plasma clearance of DP was monitored by a modification of previously reported methods [27]. Protein-free DP levels in plasma were determined in groups of fourteen C57 mice treated with BAU and DP by the regimen described above. At various times after DP administration 500 μL of whole blood was obtained and the plasma separated by centrifugation. Free DP in plasma was quantitated by adding 200 μL of plasma to a prewashed and dried Centrifree-YMT (Amicon) ultrafiltration unit. After centrifugation at 2000 *g* for 30 min, DP in the recovered sample was quantitated utilizing a Rainin Modular HPLC system equipped with a Dynamax C-18 analytical column (4.6 mm × 25.0 cm) eluted with a mixture (7:3, v/v) of MeOH in 5 mM heptane sulfonic acid–1.0% glacial acetic acid (pH 6.0) at 1.25 mL/min. The column effluent was monitored with a Gilson model 121 fluorometer (excitation wavelength = 290 nm, emission cutoff filter = 470 nm). Under these conditions the retention time for DP was 11 min.

RESULTS

We have reported that a single i.v. injection of BAU (240 mg/kg) in mice increases plasma and tissue Urd pools up to 15-fold over a 10-hr period [1]. In the present study we employed oral BAU, at a dose of 120 mg/kg, to increase the plasma concentration of Urd 8-fold, to approximately 24 μM, and tissue Urd pools 2- to 6-fold. Urd pools peaked 2–3 hr after injection and returned to normal within

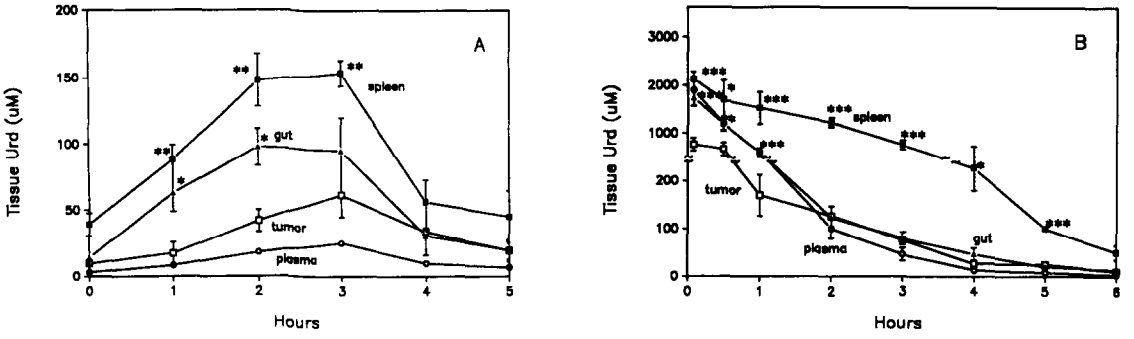

Fig. 1. (A) Effect of BAU (120 mg/kg, p.o.) on the Urd concentration in various tissues and plasma obtained from CT38-bearing C57 female mice. (B) Effect of BAU (120 mg/kg, p.o.) $\xrightarrow{1\,hr}$ Urd (250 mg/kg, i.v.) on the Urd concentration of various tissues and plasma obtained from CT38-bearing C57 female mice. In this figure, as well as in Figs. 2 and 3, immediately after whole blood was obtained, mice were anesthesized lightly and selected tissues were harvested into liquid nitrogen. The Urd content of the acid-soluble tissue extracts was determined by HPLC methods. For clarity, only spleen, gut, tumor and plasma are presented. Each point is the mean ± SEM of 3–5 determinations. Key: (*) $P \leq 0.05$, (**) $P \leq 0.02$ and (***) $P \leq 0.01$ (vs tumor) as determined by Student's *t*-test.

5 hr (Fig. 1A). As previously reported [1], the Urd content of CT38 remained low among the tissues surveyed.

The administration of oral BAU 1 hr prior to Urd (250 mg/kg, i.v.) dramatically increased both the magnitude and the duration of the effect on tissue Urd pools when compared to Urd alone [1]. The Urd concentration in plasma increased 650-fold to approximately 1800 μM and tissue Urd pools increased 50- to 100-fold within 5 min after Urd injection (Fig. 1B). The Urd concentration in plasma and all tissues returned to normal within 4 hr except in spleen, where the Urd concentration was normal after 6 hr. Comparing these data with Fig. 1A suggests that the combination of BAU and exogenous Urd in this regimen does not increase the duration of the effect beyond that produced by BAU alone.

We next examined the effects of DP in combination with intravenous Urd or oral BAU. Injection of DP (25 mg/kg) alone resulted in a 3-fold increase in the Urd content in spleen (Fig. 2A), presumably by virtue of its ability to inhibit the efflux of intracellular Urd [13]. Urd pools in plasma and the other tissues surveyed, however, did not expand after administration of this agent. In spleen the effect was of similar duration to that of BAU alone; Urd pools did not return to normal until 4 hr after dosing.

When DP was administered 1 hr prior to Urd, the peak concentration of Urd detected in plasma was similar to that achieved after BAU + Urd, and in Urd concentration in tissues increased 40- to 100-fold (Fig. 2B). Again, however, tissue Urd pools quickly fell and, in all tissues except spleen, were normal within 2 hr. Urd pools in spleen returned to normal 5 hr after Urd administration. A comparison of the results in panels A and B of Fig. 2 suggests that the combination of DP and Urd does not extend the duration of effect beyond that of DP alone.

Given the effective inhibition of Urd catabolism by BAU and tissue Urd efflux with DP, it was reasonable to expect that the combination of these agents would have an even more profound effect. Indeed, the administration of BAU followed 1 hr later by DP resulted in a significant expansion of tissue Urd pools (Fig. 3) even though the Urd concentration in plasma only increased 8-fold to approximately 23 μM. Furthermore, Urd levels in most tissues surveyed did not return to normal until 9 hr after dosing. A comparison of the results in Fig. 3 with those in Figs. 1A and 2A indicates that the combination of BAU and DP considerably increased the area under the tissue [Urd] vs time curve beyond that produced by either agent. Also note that although the Urd concentration in CT38 did increase, it remained relatively low.

To determine, more specifically, the basis for the apparent synergistic action of BAU and DP, we examined the effect of DP on the pharmacokinetics of BAU. When DP was administered 1 hr after BAU, the plasma clearance of BAU was inhibited for approximately 30 min after DP injection, resulting in a relatively constant BAU plasma concentration during this period. Subsequently, BAU clearance paralleled that in control animals (Fig. 4). Consequently, the plasma concentration of BAU remained above 10 μM, a minimally effective plasma concentration [24], for approximately 300 min after BAU administration, representing a 39% increase in the duration of Urd phosphorylase inhibition. DP also elevated, by up to 20%, the tissue concentration of BAU, as compared to plasma (data not shown). Thus, the combination of BAU and DP extended the plasma half-life of BAU and increased retention at its intracellular site of action. Enzymatically, DP, even at 100 μM, neither inhibited murine Urd phosphorylase (data not shown) nor enhanced the inhibitory activity of BAU. Administration of BAU 1 hr prior to DP did not affect the plasma clearance of DP (data not shown).

Although major increases in tissue Urd were seen with the BAU and DP regimen, its effect on the total Ura nucleotide content (UXP) in tissues was significantly less pronounced. In all of the tissues

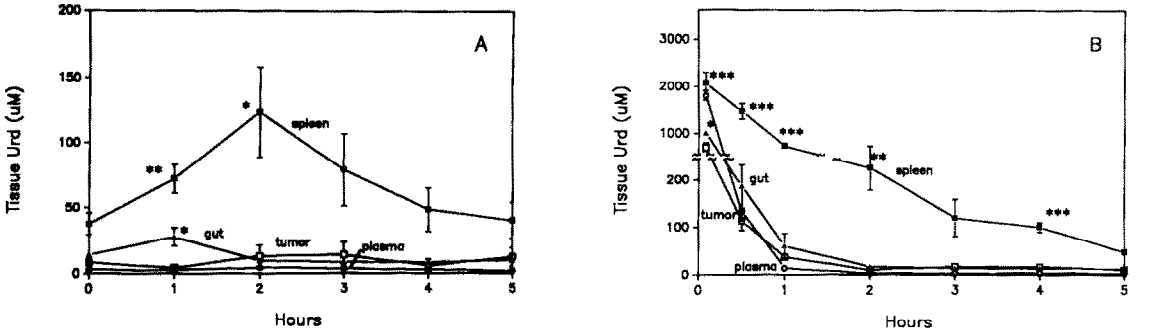

Fig. 2. (A) Effect of a single i.p. injection of DP, at a dose of 25 mg/kg, on the Urd concentration in various tissues and plasma obtained from CT38-bearing C57 female mice. (B) Effect of DP (25 mg/kg, i.p.) —1 hr→ Urd (250 mg/kg, i.v.) on the Urd concentration of various tissues and plasma obtained from CT38-bearing C57 female mice. In the studies represented in this figure, whole blood and tissues were harvested, prepared and assayed as described in the text. Each point is the mean ± SEM of 3–5 determinations. Key: (*) $P \leq 0.05$, (**) $P \leq 0.02$ and (***) $P \leq 0.01$ (vs tumor) as determined by Student's *t*-test.

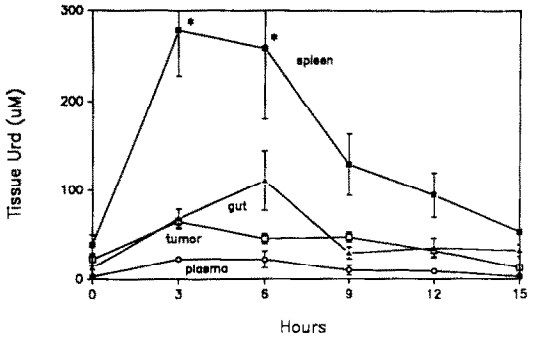

Fig. 3. Effect of BAU (120 mg/kg, p.o.) —1 hr→ DP (25 mg/kg, i.p.) on the Urd concentration in selected tissues and plasma obtained from CT38-bearing C57 female mice. Tissues and plasma were harvested, prepared and assayed by HPLC methods as described in the text. Each point is the mean ± SEM of 3–5 determinations. Key: (*) $P \leq 0.05$ (vs tumor) as determined by Student's *t*-test.

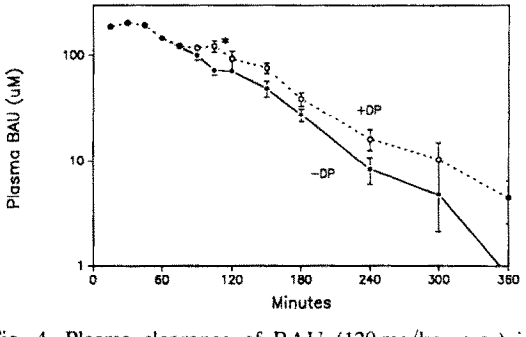

Fig. 4. Plasma clearance of BAU (120 mg/kg, p.o.) in CT38-bearing C57 female mice when administered alone (●) or according to the following regimen: BAU —1 hr→ DP (25 mg/kg, i.p.) (○). At various times after BAU administration 250 μL of whole blood was obtained, processed and analyzed to determine its BAU concentration as described in the text. Each point is the mean ± SEM of 5–6 determinations. Key: (*) $P \leq 0.05$ as determined by Student's *t*-test.

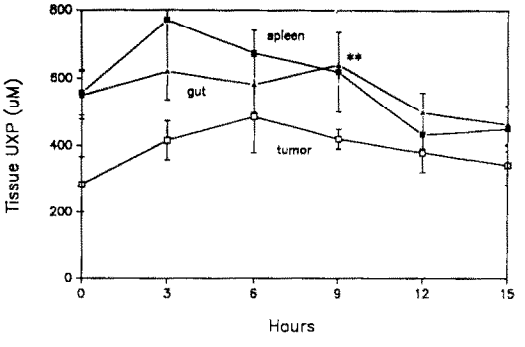

Fig. 5. Effect of BAU (120 mg/kg, p.o.) —1 hr→ DP (25 mg/kg, i.p.) on the total Ura nucleotide concentration in gut, spleen, and CT38 tissue obtained from C57 female mice. Under anesthesia, tissues were removed and frozen in liquid nitrogen. Acid-soluble tissue extracts were prepared and analyzed as described in the text. In these experiments, the UMP concentration in tissues ranged from 50 to 100 μM, UDP from 125 to 175 μM and UTP averaged approximately 300 μM, except in tumor, where the UTP content was approximately 50 μM. Each point is the mean ± SEM of 3 determinations. Key: (**) $P \leq 0.02$ (vs tumor) as determined by Student's *t*-test.

surveyed, UXP pools increased by less than 70% following drug administration (Fig. 5). Although changes in the total tissue pool of Ura nucleotide pools were minor, increases were detected for up to 12 hr after dosing. Analysis of the individual Ura nucleotide pools revealed increases that ranged from 35% to 2-fold for up to 12 hr after dosing (data not shown).

DISCUSSION

A tissue-specific expansion of Urd pools can be achieved by the administration of exogenous Urd, by the disruption of the facilitated diffusion transport mechanism for Urd with DP, or by inhibition of Urd catabolism with BAU. These findings support the

hypothesis that Urd pools in tissues appear to be governed by opposing biochemical or transport processes [1, 28]. Combinations of BAU + Urd or DP + Urd produced generally additive effects on tissue Urd pools. The combination of BAU and DP, however, exerted a synergistic effect, presumably a consequence, in part, of the direct inhibition of BAU transport across cell membranes by DP. This effect disrupted both the plasma clearance and tissue distribution of BAU. DP also limited the facilitated diffusion of physiological nucleosides across the cell membrane. This latter function served to "trap" Urd in normal cells after it was transported into the cell by the Na^+-dependent active transport system in a manner analogous to its effect on other therapeutically relevant nucleoside analogues [29]. Thus, DP can increase Urd pools in tissues whose capacity to transport nucleosides by Na^+-dependent systems is relatively great compared to their facilitated diffusion capacity (i.e. gut and spleen). When combined with BAU, this effect of DP was accentuated in tissues which possess high Urd phosphorylase activity (i.e. gut). Thus, the apparent tissue specificity of this combination may result from biochemical effects at two different target sites, namely nucleoside efflux and intracellular Urd catabolism. In themselves, however, these effects only partially explain the apparent synergistic activity of BAU + DP.

Perhaps relevant is the observation that the significant expansions in tissue Urd pools resultant from BAU and DP failed to proportionately increase tissue pools of Ura nucleotides. These findings are consistent with our previous studies with BAU alone [19]. However, it is of interest to note that the duration of the effect of BAU + DP on tissue Urd and UXP pools was similar. These observations may support the hypothesis of Reichard and coworkers [30, 31] who present data which indicate that "substrate cycles," mediated by deoxyribonucleoside kinase and nucleotidase activities, link pyrimidine deoxyribonucleoside and nucleotide pools. In our study, the prolonged effect of BAU + DP on tissue Urd pools may reflect enhanced catabolism of elevated Ura nucleotides to Urd. Further biochemical evidence that Urd and UXP pools may be linked has been presented by Moyer *et al.* [32], who demonstrated that inhibition of Urd salvage, *in vivo*, with 3-deazauridine, significantly, albeit transiently, inhibits the incorporation of [^{3}H]Urd into Ura nucleotides. In addition, Karle *et al.* [33] have reported that incubation of L1210 cells in media containing physiological concentrations of Urd (1–20 μM) significantly inhibits *de novo* synthesis but increases Ura nucleotide pool size by up to 50%. The findings in the present study also suggest that the degree to which pyrimidine salvage and Ura nucleotide pools are linked may be tissue specific.

Therapeutically, the expansion of Urd pools in normal tissues with BAU + DP may represent a previously unappreciated therapeutic opportunity which exploits biochemical differences in Urd transport and metabolism between normal and neoplastic cells and tissues. The apparent absence, or nonfunctional nature, of the concentrative Urd transport system(s) in neoplastic cell lines [1, 4, 5, 7, 34] can explain our finding that Urd pools in CT38, though increased after BAU + DP treatment, remained low relative to normal tissues. This observation may therefore explain the findings in various murine models suggesting that FUra-induced host toxicity can be reduced by the administration of BAU or exogenous Urd [17, 18, 21, 22]. We have also reported that BAU can reduce AZT-associated myelosuppression, without inhibition of its antiviral effectiveness [35, 36]. Therefore, clinical evaluation of this approach to enhance the therapeutic index of agents not only like FUra and AZT, but also of the other fluoropyrimidines and agents which disrupt Urd metabolism, such as *N*-(phosphonacetyl)-L-aspartate (PALA) [37, 38] and pyrazofurin [39, 40] warrants further study.

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REFERENCES

1. Darnowski JW and Handschumacher RE, Tissue uridine pools: Evidence *in vivo* of a concentrative mechanism for uridine uptake. *Cancer Res* **46**: 3490–3494, 1986.
2. Oliver JM and Paterson ARP, Nucleoside transport. A mediated process in human erythrocytes. *Can J Biochem* **49**: 262–270, 1971.
3. Cass CE and Paterson ARP, Mediated transport of nucleosides in human erythrocytes and specificity toward pyrimidine nucleosides as permeants. *J Biol Chem* **247**: 3314–3320, 1972.
4. Bowen D, Diasio RB and Goldman ID, Distinguishing between membrane transport and intracellular metabolism of fluorodeoxyuridine in Erhlich ascites tumor cells by application of kinetic and high performance liquid chromatographic techniques. *J Biol Chem* **254**: 5333–5339, 1979.
5. Wohlhueter RM, Marz R and Plagemann PGW, Thymidine transport in cultured mammalian cells. Kinetic analysis, temperature dependence and specificity of the transport system. *Biochim Biophys Acta* **553**: 262–270, 1979.
6. Jarvis SM and Young JD, Nucleoside translocation in sheep reticulocytes and fetal erythrocytes: A proposed model for the nucleoside transporter. *J Physiol (Lond)* **324**: 47–66, 1982.
7. Belt JA, Heterogeneity of nucleoside transport in mammalian cells. Two types of transport activity in L1210 and other cultured neoplastic cells. *Mol Pharmacol* **24**: 479–484, 1983.
8. Kuttesch JF Jr, Robins MJ and Nelson JA, Renal transport of 2′-deoxytubercidin in mice. *Biochem Pharmacol* **31**: 3387–3394, 1982.
9. Trimble ME and Coulson R, Adenosine transport in perfused rat kidney and renal cortical membrane vesicles. *Am J Physiol* **246**: F794–F803, 1984.
10. LeHir M and Dubach UC, Uphill transport of pyrimidine nucleosides in renal brush border vesicles. *Pflugers Arch* **404**: 238–243, 1985.
11. Schwenk M, Hegazy E and Lopez del Pino V, Uridine uptake by isolated intestinal epithelial cells of guinea pig. *Biochim Biophys Acta* **805**: 370–374, 1984.
12. Spector R and Huntoon S, Specificity and sodium dependence of the active nucleoside transport system in choroid plexus. *J Neurochem* **42**: 1048–1052, 1984.
13. Darnowski JW, Holdridge C and Handschumacher RE, Concentrative uridine transport by murine splenocytes:

Kinetics, substrate specificity, and sodium dependency. *Cancer Res* **47**: 2614–2619, 1987.
14. Crawford CR, Ng CY, Ullman B and Belt JA, Nucleoside transport in L1210 murine leukemia cells. Evidence for three transporters. *J Biol Chem* **265**: 9732–9736, 1990.
15. Niedzwicki JG, Chu SH, el Kouni MH, Rowe EC and Cha S, 5-Benzylacyclouridine and 5-benzyloxybenzylacyclouridine, potent inhibitors of uridine phosphorylase. *Biochem Pharmacol* **31**: 1857–1861, 1982.
16. Naguib FN, el Kouni MH, Chu SH and Cha S, New analogues of benzylacyclouridines, specific and potent inhibitors of uridine phosphorylase from human and mouse livers. *Biochem Pharmacol* **36**: 2195–2201, 1987.
17. Martin DS, Stolfi RL, Sawyer RC, Spiegelman S and Young CW, High-dose 5-fluorouracil with delayed uridine "rescue" in mice. *Cancer Res* **42**: 3964–3970, 1982.
18. Klubes P and Cerna I, Use of uridine rescue to enhance the antitumor selectivity of 5-fluorouracil. *Cancer Res* **43**: 5364–5368, 1983.
19. Darnowski JW and Handschumacher RE, Tissue-specific enhancement of uridine utilization and 5-fluorouracil therapy in mice by benzylacyclouridine. *Cancer Res* **45**: 5364–5368, 1985.
20. Sommadossi J-P, Carlisle R, Schinazi RF and Zhou Z, Uridine reverses the toxicity of 3'-azido-3'-deoxythymidine in normal human granulocyte-macrophage progenitor cells *in vitro* without impairment of antiretroviral activity. *Antimicrob Agents Chemother* **32**: 997–1001, 1988.
21. Darnowski JW and Handschumacher RE, Manipulation of tissue uridine pools with benzylacyclouridine and dipyridamole modifies fluorouracil therapy. *Proc Am Assoc Cancer Res* **29**: 474, 1988.
22. Darnowski JW, Falcone A, Brunnetti I, Chu M, Chu S and Calabresi P, Tissue uracil nucleotide pools: Potential role in fluorouracil chemotherapy. *Proc Am Assoc Cancer Res* **30**: 564, 1989.
23. Corbett TH, Griswold DP, Roberts BJ, Peckham J and Schabel FM Jr, A mouse colon-model for experimental therapy. *Cancer Chemother Rep* **5**: 169–186, 1975.
24. Darnowski JW and Handschumacher RE, Benzylacyclouridine. Pharmacokinetics, metabolism and biochemical effects in mice. *Biochem Pharmacol* **37**: 2613–2618, 1988.
25. Plagemann PGW and Woffenden C, Na^+-dependent and -independent transport of uridine and its phosphorylation in mouse spleen cells. *Biochim Biophys Acta* **981**: 315–325, 1989.
26. Pogolotti AL Jr, Nolan PA and Santi DV, Methods for the complete analysis of 5-fluorouracil metabolites in cell extracts. *Anal Biochem* **117**: 178–186, 1981.
27. Chan TCK, Markman M, Cleary S and Howell SB, Plasma uridine changes in cancer patients treated with the combination of dipyridamole and *N*-phosphonacetyl-L-aspartate. *Cancer Res* **46**: 3168–3172, 1986.
28. Darnowski JW and Handschumacher RE, Enhancement of fluorouracil therapy by the manipulation of tissue uridine pools. *Pharmacol Ther* **41**: 381–392, 1989.
29. Grem JL and Fischer PH, Alteration of fluorouracil metabolism in human colon cancer cells by dipyridamole with a selective increase in fluorodeoxyuridine monophosphate levels. *Cancer Res* **46**: 6191–6199, 1986.
30. Nicander B and Reichard P, Evidence for the involvement of substrate cycles in the regulation of deoxyribonucleoside triphosphate pools in 3T6 cells. *J Biol Chem* **260**: 9216–9222, 1985.
31. Bianchi V, Pontis E and Reichard P, Interrelations between substrate cycles and *de novo* syntheses of pyrimidine deoxyribonucleoside triphosphates in 3T6 cells. *Proc Natl Acad Sci USA* **83**: 986–996, 1986.
32. Moyer JD, Malinowski N and Cysyk RL, The effect of 3-deazauridine and dipyridamole on uridine utilization by mice. *Eur J Cancer Clin Oncol* **22**: 323–327, 1986.
33. Karle JM, Anderson LW and Cysyk RL, Effect of plasma concentrations of uridine on pyrimidine biosynthesis in cultured L1210 cells. *J Biol Chem* **259**: 67–72, 1984.
34. Dagnino L, Bennett LL Jr and Paterson ARP, Concentrative transport of nucleosides in L1210 mouse leukemia cells. *Proc Am Assoc Cancer Res* **28**: 15, 1987.
35. Falcone A, Darnowski JW, Ruprecht RM, Chu SH, Brunetti I and Calabresi P, Differential effect of benzylacyclouridine on the toxic and therapeutic effects of azidothymidine in mice. *Blood* **76**: 2216–2221, 1990.
36. Calabresi P, Falcone A, St. Clair MH, Wiemann MC, Chu SH and Darnowski JW, Benzylacyclouridine reverses azidothymidine-induced marrow-suppression without impairment of anti-HIV activity. *Blood* **76**: 2210–2215, 1990.
37. Collins KD and Stark GR, Aspartate transcarbamylase. Interactions with the transition state analogue *N*-(phosphonacetyl)-L-aspartate. *J Biol Chem* **246**: 6599–6609, 1971.
38. Mann GB, Hortobagyi GN, Buzdar AU, Yap H-Y and Valdivieso M, A comparative study of PALA, PALA plus 5-FU, and 5-FU in advanced breast cancer. *Cancer* **56**: 1320–1324, 1985.
39. Gutowski GE, Sweeney MJ, DeLong DC, Hamill RL, Gerzon K and Dyka RW, Biochemical and biological effects of the pyrazofurins (pyrazomycins): Initial clinical trials. *Ann NY Acad Sci* **255**: 544–551, 1975.
40. Cadman E and Benz C, Uridine and cytidine metabolism following inhibition of *de novo* pyrimidine synthesis by pyrazofurin. *Biochim Biophys Acta* **609**: 372–383, 1980.