

ORIGINAL ARTICLE

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Effects of 5-benzylacetyluridine, an inhibitor of uridine phosphorylase, on the pharmacokinetics of uridine in rhesus monkeys: implications for chemotherapy

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Abstract The effects of subcutaneous administration of 5-benzylacetyluridine (BAU), a uridine phosphorylase (UrdPase, EC 2.4.2.3) inhibitor, on uridine concentration in plasma and urine were evaluated in rhesus monkeys. Administration of BAU at 50, 100 and 250 mg/kg increased the plasma uridine baseline concentration 1.5-, 2.9-, and 3.2-fold, respectively. The basis for this moderate perturbation of plasma uridine by BAU was investigated using a tracer dose of 500 μ Ci 3 H-uridine. Administration of 3 H-uridine alone led to its rapid catabolism to uracil and dihydrouracil. Administration of 83.3 mg/kg BAU with 500 μ Ci 3 H-uridine resulted in a 2.5-fold enhancement of 3 H-uridine plasma levels and a substantial decrease in the plasma levels of uridine catabolites, suggesting inhibition of UrdPase activity by BAU in rhesus monkeys. Coadministration of 83.3 mg/kg BAU with 83.3 mg/kg uridine also reduced the plasma concentration of uracil and dihydrouracil, but it did not increase plasma uridine concentration above that of control animals receiving 83.3 mg/kg uridine alone. In animals receiving uridine alone at 83.3 or 25 mg/kg, approximately 10% of the administered dose was recovered in the urine within 6 h, with unchanged uridine being the major component. In contrast, administration of 83.3 mg/kg BAU increased the excretion of unchanged uridine to more than 32% of the total dose administered, even when the urinary excretion ratio of uracil to uridine was reduced ten-fold. Administration of

multiple doses (three times per day) of BAU alone (83.3 mg/kg) or in the presence of uridine (83.3 mg/kg) did not enhance plasma uridine concentration further. In addition, uridine pharmacokinetics were associated with a time-dependent relationship as evidenced by an increased total plasma clearance, renal clearance and volume of distribution, resulting in a substantial decrease in uridine peak concentration with time. These results indicate that administration of BAU inhibits UrdPase activity in rhesus monkeys as manifested by decreased uracil and dihydrouracil plasma levels, as well as a lower urinary excretion ratio of uracil to uridine, as compared to control animals. However, plasma levels of unchanged uridine were not substantially enhanced by BAU in spite of the large increase in urinary excretion of unchanged uridine. This phenomenon was also observed when uridine was coadministered with BAU, suggesting that plasma uridine concentration in monkeys may be strongly regulated by the renal system as evidenced by the “spillover” of excess plasma uridine into urine. In addition, the pharmacokinetics of uridine were dose-independent, but time-dependent. This investigation may provide insights into the clinical usefulness of BAU to protect against or rescue from host toxicity induced by FUra and other chemotherapeutic pyrimidine analogues whose toxicity can be alleviated by uridine.

Key words 5-Benzylacetyluridine · Uridine · Host protection

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Introduction

For over three decades, 5-fluorouracil (FUra) has remained one of the few “standard” drugs effective against solid tumors in humans. FUra is used mainly for the treatment of colorectal, ovarian, renal, breast, and head and neck cancers. However, the clinical utility of FUra is limited by its toxic effects, in particular,

myelosuppression [16, 20, 43]. Attempts have been made to modulate FUra-induced host toxicity with another drug or an "endogenous" compound that may alter FUra toxicity. Among these, uridine appears to be one of the most promising biochemical modulators of FUra toxicity.

In preclinical models uridine has been shown to protect against and/or rescue from FUra-induced host toxicity without impairment of its antitumor activity [23, 24, 33, 46]. However, attempts to implement such a regimen in the clinic have been limited by the rapid clearance of uridine [3, 17–20, 30, 43]. Therefore, high doses of uridine (gram doses) must be administered in order to elevate uridine concentration to levels adequate for protection. Unfortunately, the use of such high doses of uridine are hampered by toxic side effects, e.g. phlebitis, pyrogenic reactions and diarrhea [5, 12, 17, 19, 44, 45]. The limited clinical utility of a high-dose uridine regimen, combined with the inadequacy of this approach for clinical use as an adjunct to long-term chemotherapy, has led to the search for alternative approaches to increase plasma uridine levels. This goal could be accomplished by inhibiting the catabolism and/or clearance of uridine.

Uridine is present in the plasma of different species in relatively constant concentrations (1–5 μM) [9, 11] providing a source for nucleotide synthesis by the salvage pathways. However, the plasma half-life of uridine is only 2 min. More than 90% of plasma uridine entering the liver via the portal vein is degraded in a single pass, while constant amounts of uridine from de novo biosynthesis are released into the hepatic vein [13, 35, 37]. Hepatic uridine phosphorylase (UrdPase, EC 2.4.2.3) catalyses the first step in the degradative pathway of uridine [9, 21, 36] and its activity exhibits a circadian rhythm which is the inverse of the plasma uridine concentration [27, 40]. Therefore, inhibition of UrdPase might be expected to decrease the catabolism of uridine and increase plasma uridine levels as a result of the continuous de novo biosynthesis of uridine in the liver. Several acyclopyrimidines have been developed as specific inhibitors of UrdPase [4, 15, 26, 29, 31, 38, 39, 41, 42]. Among these inhibitors, 5-benzylacyclouridine (BAU) [10] has been shown to cause a marked increase in plasma uridine concentration and prolong the plasma half-life of uridine in mice [7, 8, 12, 34, 44]. This modulation in uridine metabolism by UrdPase inhibitors has been used successfully to raise plasma uridine levels in the same order of magnitude as high doses of exogenous uridine in experimental mouse models [7–9, 34], without the clinical toxicity observed with high doses of uridine [5, 12, 17, 19, 44, 45]. However, in contrast to mice [7, 8, 10, 12, 34, 44], low doses of BAU have been reported not to increase plasma uridine in humans [47] and cynomolgus monkeys [11]. This may result from variability in the substrate specificity of UrdPase [28] and its inhibition by BAU [1] in different species. Therefore, it was crucial to investigate the effect of different doses and regimens of BAU, alone

or in combination with uridine, on the disposition of uridine in a primate system. Such studies may elucidate the feasibility of the clinical use of BAU to protect against or rescue from host toxicity induced by FUra or other pyrimidine analogues (e.g. 3'-azido-3'-deoxythymidine [2, 49, 50] or 2',3'-dideoxycytidine [22]) which can be potentially alleviated by uridine. In the present study, we evaluated the effect of different concentrations of BAU alone or in combination with uridine, on levels of uridine and its catabolites in the plasma and urine of rhesus monkeys. A multiple dosing regimen of BAU was also assessed for its ability to attain higher plasma uridine levels. A preliminary report has been previously presented [25].

Materials and methods

Chemicals

BAU and its succinyl salt (succBAU) were synthesized by Dr. S. H. Chu of Brown University, Providence, RI. The chemical purity of the compounds was greater than 99% as confirmed by spectral and/or high-pressure liquid chromatography (HPLC) analysis. Unlabelled uridine was purchased from Sigma Chemical Co. (St. Louis, Mo.). [^3H]Uridine (28 Ci/mol) was purchased from Moravsek Biochemicals (Brea, Calif.) and was greater than 99% pure as ascertained by the HPLC technique described below. All other chemicals were of the highest quality commercially available.

Animals

Young adult rhesus monkeys (*Macaca mulatta*) weighing between 4.26 and 6.24 kg were used for the metabolic and pharmacokinetic studies. The animals were maintained at Yerkes Regional Primate Research Center of Emory University in accordance with the guidelines established by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Yerkes Center is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Doses and collection of samples

BAU, succBAU, and uridine were dissolved in sterile phosphate-buffered saline (pH 7.4) and administered subcutaneously. BAU was administered as succBAU unless otherwise specified. Blood samples (3 ml) were collected in heparinized tubes. When possible, urine was also collected by cystocentesis at similar time-points. The plasma and urine samples were frozen at -20°C until analysis by HPLC.

Single dose of BAU

Monkeys received one subcutaneous dose of 50, 100 or 250 mg/kg BAU. Samples of blood and urine were collected at 0, 0.25, 0.5, 1, 2, 4, 6 and 24 h after injection.

Single dose of BAU and uridine

Monkeys received one subcutaneous tracer dose of 500 μCi [^3H]—uridine with or without unlabelled uridine in the presence or

absence of 83.3 mg/kg BAU. Samples of blood and urine were collected at 0, 0.08, 0.17, 0.25 and 0.5 and 1, 2, 4 and 6 h after injection.

Multiple doses of BAU in the presence or absence of uridine

Monkeys received a subcutaneous loading dose of 250 mg/kg BAU in the presence or absence of 250 mg/kg uridine followed by seven doses each of 83.3 mg/kg BAU in the presence or absence of 83.3 mg/kg uridine at 24, 30, 36, 48, 54, 60 and 72 h. Samples of blood and urine were collected at 0, 2, 4, 6, 24, 26, 28, 30, 48, 50, 52, 54 and 72 h after the initial injection.

Sample analysis

Non-radioactive specimens were processed to determine uridine levels as previously described [27]. Non-radioactive plasma (500 μ l) was extracted with 1 ml 15% ice-cold trichloroacetic acid at 5°C and proteins were removed by centrifugation. The supernatant was neutralized with an equal amount of a 1:1 mixture of ice-cold tri-*N*-octylamine and 1,1,2-trichlorotrifluoroethane (freon). The phases were allowed to separate and 50- μ l aliquots of the top phase were analyzed by HPLC using a Hewlett-Packard model 1050 liquid chromatograph equipped with a manual injector and a fixed wavelength spectrophotometer. Reversed-phased chromatography was performed using a Hypersil ODS 5- μ m column (Jones Chromatography, Littleton, Colo. Urine samples were filtered through a 0.45- μ m pore size Acro LC 13 filter (Gelman Sciences, Ann Arbor, Mich.) to remove proteins and 50- μ l aliquots were analyzed by HPLC. Isocratic elution was performed at 1 ml/min with 10 mM phosphoric acid containing 30 μ M heptane sulfonic acid, pH 3.1. The column temperature was maintained at 25°C, and the absorbance was measured at 254 nm. Under these conditions, uridine eluted at 13–14 min. Standard curves from 4–400 μ M were linear, with $r^2 > 0.98$, and were used to determine the concentrations of uridine in the samples by interpolation.

Radioactive plasma and urine samples were filtered through a 0.45- μ m pore size Acro LC 13 filter and aliquots were analyzed by HPLC. Isocratic elution was performed at 1 ml/min with PIC 8 (5 mM tetrabutylammonium hydrogen sulfate and 1.5 mM potassium phosphate, pH 8.0). The column temperature was maintained at 25°C, and the absorbance was measured at 254 nm. Under these conditions, uridine eluted at 14–15 min, uracil at 10–12 min, and dihydrouracil at 8–9 min. The radioactive eluent from the column was directed via a low dead-volume connection line into a model 2112 Redirac fraction collector (LKB Instruments, Rockville, Md), and timed fractions of 0.5 ml were collected into mini-scintillation vials. After adding 5 ml of Econo-Safe scintillation fluor (Research Products International Corp., Mount Prospect, Ill.), radioactivity was measured using a Beckman model LS 5000TA liquid scintillation counter (Beckman Instruments, Fullerton, Calif.) equipped with an automatic quenching correction program. Intra- and interday percentage coefficients of variation were less than 10%, at concentrations between 4 and 400 μ M. The quantitation detection limit was 1.0 μ M.

Pharmacokinetic analysis

The pharmacokinetic parameters of uridine, uracil and dihydrouracil were estimated by compartmental model-independent methods using a SIPHAR/Base program [14]. The area under the curve (AUC) was determined by the trapezoidal rule with extrapolation to time infinity using the terminal disposition slope (K) generated by a weighted nonlinear least-squares regression of an exponential fit of the data [32], with the weighted square factor set

Table 1 Effect of a single subcutaneous administration of different doses of BAU alone on plasma uridine levels in rhesus monkeys (C_{\max} peak plasma concentration, C_{\max}/C_0 peak plasma concentration over baseline plasma concentration at $t = 0$, T_{\max} , time to peak plasma concentration)

Monkey	C_{\max} (μ M)	C_{\max}/C_0	T_{\max} (hr)
50 mg/kg BAU			
AP-31	12.2	1.21	2.00
RCz-1	14.3	1.25	2.00
RDp-1	9.1	1.15	0.50
Mean \pm SD	11.9 ± 2.62	1.20 ± 0.05	1.50 ± 0.87
50 mg/kg BAU ^a			
Rzo-1	13.9	1.70	2.00
Rsp-1	9.66	1.27	4.00
Mean \pm SD	11.8 ± 3.00	1.49 ± 0.31	3.00 ± 1.41
100 mg/kg BAU ^a			
RCz-1	14.5	2.93	2.00
RDp-1	22.4	2.95	6.00
Mean \pm SD	18.4 ± 5.56	$2.94 \pm 0.01^*$	4.00 ± 2.83
250 mg/kg BAU ^a			
RHz-1	10.7	3.54	6.00
RTu-1	10.6	2.81	4.00
Mean \pm SD	10.7 ± 0.09	$3.18 \pm 0.52^*$	5.00 ± 1.41

^aAdministered as the succinyl salt of BAU

* $p < 0.05$ versus 50 mg/kg of succinyl salt of BAU, two-tailed paired *t*-test

as the reciprocal of the calculated concentration squared. Elimination half-life ($t_{1/2}$) values of uridine, uracil and dihydrouracil were calculated from 0.693/ K . The total plasma clearance (Cl_T) was calculated by dividing the administered dose by the AUC and the weight of the monkey. The apparent volume of distribution (V_d) was calculated as the product of the Cl_T and mean residence time (MRT) divided by the weight of the monkey. The renal clearance (Cl_R) was determined by dividing the amount of selected entity excreted in urine during a specified time period by the AUC value in plasma corresponding to the same time period. The baseline plasma concentration (C_0), peak plasma concentration (C_{\max}) values and time to peak plasma concentration (T_{\max}) values were observed experimental values for uridine, uracil, and dihydrouracil. The catabolite/uridine urinary excretion ratio was calculated by dividing the renal clearance (Cl_R) of uracil or dihydrouracil by the renal clearance of uridine. For the multiple dose studies, the initial parameters were obtained from an analysis of the first four time-points and were normalized over the 72 h time course, taking into account the dosing levels at each administration. The AUC was determined by the trapezoidal rule as above; however, the regression of the exponential curve was non-weighted.

Results

Effect of increasing doses of BAU on plasma uridine levels.

The effects of a single subcutaneous administration of different doses of BAU on plasma uridine concentration are shown in Table 1. Plasma uridine level (C_{\max}/C_0) was enhanced 1.5- and 2.9-fold following the administration of 50 and 100 mg/kg of BAU,

respectively. However, 250 mg/kg BAU did not increase plasma uridine concentration further as compared to that achieved with 100 mg/kg BAU. The increase in plasma uridine concentration induced by BAU was detected within 30 min and uridine levels remained elevated for 4–6 h (data not shown). No significant difference was observed between plasma uridine concentrations resulting from the administration of BAU or succBAU. Therefore, the more water-soluble succBAU was used throughout the remainder of the experiments.

Effect of BAU on plasma kinetics of ^3H -uridine, ^3H -uracil and ^3H -dihydrouracil

In an attempt to understand the basis for the lack of uridine perturbation in plasma by BAU, a tracer dose (500 μCi) of [^3H]-uridine was administered in the presence or absence of 83.3 mg/kg BAU. In particular, the use of radioactive uridine permitted the quantitation of uridine's catabolic products, uracil and dihydrouracil, and therefore the determination of whether or not BAU effectively inhibits UrdPase activity in monkeys. After administration of ^3H -uridine alone, unchanged uridine was detected only at the 5- and 10-min bleeding time-points with a C_{max} of 3.8 nCi/ml, demonstrating a rapid catabolism to uracil and dihydrouracil. Hence, the AUC and plasma elimination $t_{1/2}$ of ^3H -uridine were not evaluable. ^3H -Uracil and ^3H -dihydrouracil were rapidly detected and achieved maximum plasma levels of 29 and 32.6 nCi/ml within 0.5 and 4 h, respectively (Fig. 1A). As illustrated in Fig. 1B, concomitant administration of 83.3 mg/kg BAU with a tracer amount of 500 μCi of ^3H -uridine led to a 2.5-fold increase in the plasma concentration of radiolabelled uridine, whereas the C_{max} of ^3H -uracil and ^3H -dihydrouracil decreased more than 9- and 5-fold, respectively, suggesting that BAU inhibits UrdPase activity in vivo. Further evidence for enzyme inhibition by BAU was demonstrated by a measurable AUC for ^3H -uridine (14.3 nCi h/ml) and a 9-fold decrease in AUC of ^3H -uracil (from 53.5 to 6.0 nCi h/ml) as well as a 6-fold decrease in AUC of ^3H -dihydrouracil (from 150.8 to 24.4 nCi h/ml).

Effect of coadministration of BAU with uridine

No information is currently available on the role of exogenous uridine on the concentration and metabolism of uridine in monkeys, or whether or not BAU effects can be enhanced by exogenous uridine. Table 2 shows that administration of 25 mg/kg uridine led to a 14-fold increase in uridine plasma levels (C_{max}/C_0). Increasing the uridine dose to 83.3 mg/kg resulted in a 74-fold higher level of plasma uridine and apparent elimination $t_{1/2}$, Cl_T and V_d values of the parent

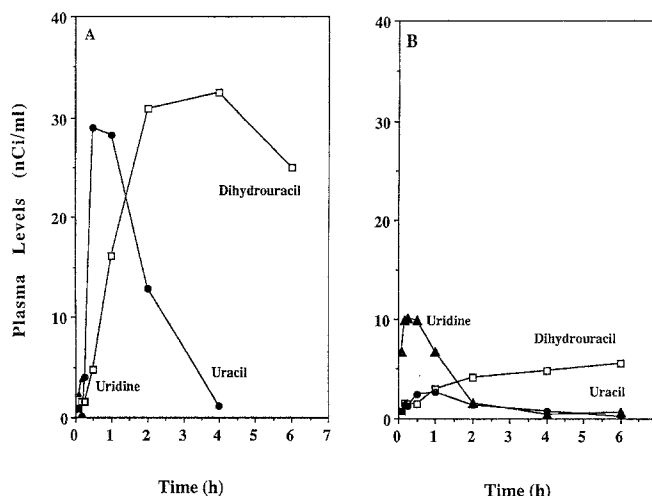


Fig. 1A, B Plasma concentration-time curves of ^3H -uridine (▲), ^3H -uracil (●) and ^3H -dihydrouracil (□) after subcutaneous administration of 500 μCi ^3H -uridine in the absence (A) and in the presence of 83.3 mg/kg BAU (B)

compound were dose-independent. It should be noted that despite a 4-fold reduction in plasma concentration of uracil and dihydrouracil which is consistent with inhibition of UrdPase activity, coadministration of BAU (83.3 mg/kg) with 83.3 mg/kg uridine did not increase the C_{max}/C_0 of plasma uridine beyond that obtained by administration of uridine alone. Furthermore, BAU did not alter the pharmacokinetic parameters of uridine.

Effect of BAU on urinary excretion of uridine and its catabolites, uracil and dihydrouracil

To determine why the plasma uridine concentration was not increased by a potent inhibitor of UrdPase such as BAU, and to investigate the fate of administered uridine in monkeys, the urinary excretion of uridine and its catabolites, uracil and dihydrouracil, was investigated in the monkeys which received 25 and 83.3 mg/kg uridine in the absence or presence of 83.3 mg/kg BAU. Within 6 h after administration of 25 and 83.3 mg/kg uridine, total urinary recovery (total radioactivity) averaged 11 and 13% with unchanged ^3H -uridine accounting for 6.0 ± 1.8 and $10.3 \pm 4.2\%$, respectively (Table 3). The Cl_R of uridine and dihydrouracil did not change following administration of 25 and 83.3 mg/kg uridine. In contrast, the Cl_R of uracil decreased 4.5-fold from 230 to 51 ml/h per kg resulting in a lower uracil/uridine urinary excretion ratio. The mean relative Cl_R/Cl_T ratio indicated that more than 90% of the administered dose of uridine underwent non-renal elimination in the rhesus monkeys.

In contrast, administration of 83.3 mg/kg of BAU with 83.3 mg/kg uridine, increased total urinary recovery of radioactivity to 33.4%, with more than 97% being accounted for by unchanged uridine.

Table 2 Pharmacokinetic parameters of plasma uridine and its catabolites uracil and dihydrouracil after subcutaneous administration of 25 mg/kg and 83.3 mg/kg uridine with a tracer amount of 500 μ Ci of 3 H-uridine in the absence and presence of a dose of 83.3 mg/kg of BAU in rhesus monkeys. Blood samples were collected at specified times as detailed in Materials and methods. C_{\max} , peak plasma concentration, C_{\max}/C_0 peak plasma concentration over plasma baseline concentration at $t = 0$, T_{\max} , time to peak plasma concentration, $t_{1/2}$ plasma elimination half-life, Cl_T plasma total clearance, V_d volume of distribution, NE not evaluable

Monkey	Compound	C_{\max} (μ M)	C_{\max}/C_0	T_{\max} (h)	Apparent $t_{1/2}$ (h)	Cl_T (l/h/kg)	V_d (l/kg)
3 H-uridine (25 mg/kg)							
RSe-2	Uridine	69.0	12.3	0.17	1.04	1.13	1.58
	Uracil	54.3	NE	0.25	1.34		
	Dihydrouracil	35.1	NE	6.00	NE		
RSg-2	Uridine	61.9	16.3	0.17	1.18	1.25	2.14
	Uracil	15.0	NE	0.56	0.59		
	Dihydrouracil	28.0	NE	4.00	NE		
Mean \pm (SD)	Uridine	65.5 (5.0)	14.3 (2.8)	0.17 (0.00)	1.11 (0.10)	1.19 (0.08)	1.86 (0.40)
	Uracil	34.7 (27.8)	NE	0.41 (0.22)	0.97 (0.53)		
	Dihydrouracil	31.6 (5.0)	NE	5.00 (1.41)	NE		
3 H-uridine (83.3 mg/kg)							
RRt-1	Uridine	175.0	106.7	0.50	0.27	0.88	1.60
	Uracil	36.9	NE	1.00	0.28		
	Dihydrouracil	122.8	NE	6.00	NE		
RZq-2	Uridine	140.6	40.2	1.00	1.60	0.63	1.45
	Uracil	62.6	NE	1.00	3.73		
	Dihydrouracil	220.2	NE	6.00	NE		
Mean \pm (SD)	Uridine	157.8 (24.3)	73.5 (47.0)	0.75 (3.5)	0.94 (0.94)	0.76 (0.18)	1.55 (0.07)
	Uracil	49.7 (18.2)	NE	1.00 (0.0)	2.01 (2.44)		
	Dihydrouracil	171.5 (68.9)	NE	6.00 (0.0)	NE		
3 H-uridine (83.3 mg/kg) plus BAU ^a (83.3 mg/kg)							
CF-77	Uridine	142.7	49.7	0.50	0.26	1.00	1.23
	Uracil	12.2	NE	1.00	0.34		
	Dihydrouracil	96.3	NE	6.00	NE		
RSr-1	Uridine	190.4	66.3	0.50	0.35	0.37	0.95
	Uracil	12.2	NE	2.00	0.56		
	Dihydrouracil	56.1	NE	6.00	NE		
Mean \pm (SD)	Uridine	166.5 (33.8)	58.0 (11.7)	0.50 (0.00)	0.31 (0.06)	0.87 (0.19)	1.09 (0.20)
	Uracil	12.2 (0.01)	NE	1.50 (0.71)	0.45 (0.16)		
	Dihydrouracil	76.2 (28.4)	NE	6.00 (0.00)	NE		

^aAdministered as the succinyl salt of BAU

Furthermore, while the dihydrouracil/uridine excretion ratio remained unchanged, the uracil/uridine urinary excretion ratio was decreased 10-fold, and the mean relative CL_R/CL_T ratio indicated that the metabolic elimination decreased from 90% to less than 70%. These observations are consistent with inhibition of UrdPase by BAU.

Effect of multiple doses of BAU and uridine on plasma levels of uridine

The data shown in Tables 1–3 indicate that a single dose of BAU reduced in vivo uridine catabolism in monkeys; however, plasma uridine concentrations were not increased. This lack of increase in plasma uridine concentration appears to result from enhanced renal excretion, as evidenced by increased amounts of uridine in urine. Therefore, we investigated whether or not the urinary excretion of uridine could be saturated by prolonged coadministration of high doses of uridine with

BAU. The effect of a high dose of BAU (250 mg/kg) in the absence or presence of a loading dose of uridine (250 mg/kg), followed by subsequent multiple administration of BAU (83.3 mg/kg) with or without uridine (83.3 mg/kg), three times per day for 4 days was studied. Figure 2 illustrates the plasma uridine concentrations with these regimens. A baseline plasma concentration of $2.5 \pm 0.7 \mu$ M uridine ($n = 5$) was observed. Following the administration of 250 mg/kg BAU, plasma uridine levels rose 3.4-fold (Fig. 1A) which is consistent with the data reported in Table 1 using different animals. Further administration of BAU did not substantially increase plasma uridine concentrations beyond 10μ M (Fig. 1A), and the characteristics of uridine pharmacokinetics did not change over the 4-day administration schedule (data not shown).

Coadministration of a single loading dose of 250 mg/kg BAU with 250 mg/kg uridine raised plasma uridine levels by over 100-fold, but plasma uridine concentrations declined to normal levels within 12 h (Fig. 1B). Continuous coadministration of 83.3 mg/kg

Table 3 Urinary excretion of uridine and its catabolites uracil and dihydrouracil after subcutaneous administration of 25 mg/kg and 83.3 mg/kg uridine with a tracer amount of 500 μ Ci 3 H-uridine in the absence and presence of BAU in rhesus monkeys (CL_R renal clearance)

Monkey	Compound excreted	Percentage of total dose administered	CL_R (ml/h/kg)	Urinary excretion ratio (% catabolite/-uridine)
3 H-uridine (25 mg/kg)				
RSe-2	Uridine	4.7	49.4	
	Uracil	5.5	166.9	338
	Dihydrouracil	1.5	2.19	4
RSg-2	Uridine	7.3	95.6	
	Uracil	2.2	293.4	307
	Dihydrouracil	0.6	3.5	4
Mean \pm SD	Uridine	6.0 ± 1.8	72.5 ± 32.7	
	Uracil	3.8 ± 2.3	230.2 ± 89.5	322.5 ± 21.9
	Dihydrouracil	1.0 ± 0.6	2.9 ± 0.9	4.0 ± 0.0
3 H-uridine (83.3 mg/kg)				
RRt-1	Uridine	7.3	67.2	
	Uracil	0.2	36.3	54
	Dihydrouracil	0.3	3.9	6
RZq-2	Uridine	13.3	81.3	
	Uracil	3.1	64.7	80
	Dihydrouracil	1.1	1.3	2
Mean \pm SD	Uridine	10.3 ± 4.2	74.3 ± 4.3	
	Uracil	1.7 ± 2.0	50.5 ± 20.1	67.0 ± 18.4
	Dihydrouracil	0.7 ± 0.6	2.6 ± 1.8	4.0 ± 2.8
3 H-uridine (83.3 mg/kg) plus BAU ^a (83.3 mg/kg)				
CF-77	Uridine	32.5	330.0	
	Uracil	0.3	21.6	7
	Dihydrouracil	0.2	16.8	5
RSr-1	Uridine	32.3	238.0	
	Uracil	1.1	15.8	7
	Dihydrouracil	0.4	12.4	5
Mean \pm SD	Uridine	32.4 ± 0.1	284.0 ± 65.0	
	Uracil	0.7 ± 0.5	18.7 ± 4.1	7.0 ± 0.0
	Dihydrouracil	0.3 ± 0.1	14.6 ± 3.1	5.0 ± 0.0

^aAdministered as the succinyl salt of BAU

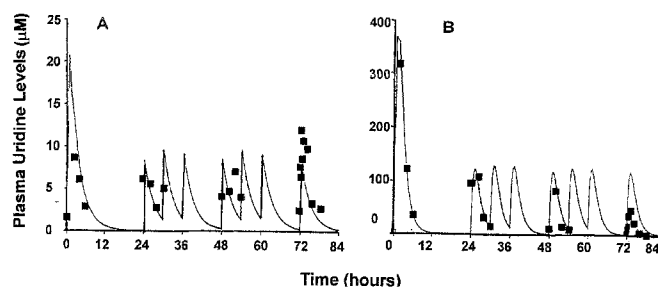


Fig. 2 A, B Effect of multiple administrations of BAU in the absence or presence of uridine on plasma concentration of uridine in representative rhesus monkeys. Two monkeys received a subcutaneous administration of 250 mg/kg BAU in the absence (A) and presence of a loading dose of 250 mg/kg uridine (B) followed by seven injections of 83.3 mg/kg BAU in the absence (A) or presence of 83.3 mg/kg uridine (B) at 24, 30, 36, 48, 54, 60 and 72 h. Each point represents the experimental concentration of uridine in plasma and the solid line represents the computer-fitted lines generated by using a SI-PHAR/Base program [14]

uridine and BAU, on the other hand, elevated plasma uridine levels to approximately 100 μ M at 24 h, but the peak concentration of plasma uridine decreased with time, with C_{max} values of 81 and 43 μ M at 48 and 72 h, respectively. Analysis of uridine pharmacokinetic

parameters after the 72-h dose revealed that the plasma elimination $t_{1/2}$ of 0.89 h remained within the same range when compared to animals receiving a single dose of that regimen (Table 2), but the CL_T , CL_R , and V_d were increased approximately 3-fold with values of 3.4 l/h per kg, 650 ml/h per kg and 4.5 l/kg, respectively. Therefore, this trend does not appear to be associated with an increase in urinary elimination of uridine as evidenced by an unchanged CL_T/CL_R ratio but rather reflects a time-dependent phenomenon for uridine pharmacokinetics. This disposition was confirmed in another animal which received a similar regimen of uridine and BAU (data not shown).

Discussion

The aim of the present study was to investigate the effects of BAU on uridine concentration in biological fluids of rhesus monkeys. High doses of uridine have been used to protect against or rescue from host toxicity induced by anticancer agents such as FUra [23, 24, 33] and anti-HIV drugs such as 3'-azido-3'-deoxythymidine [2, 49, 50] and 2',3'-dideoxycytidine

[22]. However, administration of high doses of uridine can result in toxic side effects including phlebitis and pyrogenic reactions [5, 12, 17, 19, 44, 45]. Therefore, it has been postulated that the UrdPase inhibitor, BAU, may increase endogenous uridine plasma concentrations to levels required to improve the selectivity of these therapeutic agents without the limiting toxicities of high doses of exogenous uridine [7, 8, 10, 12, 34, 44]. A non-human primate model was selected in our investigations since it represents a step closer to humans as compared to previously investigated animals. Furthermore, the disposition and metabolism of some pyrimidine analogues has been demonstrated to be similar in rhesus monkeys and humans [6]. In this context, plasma uridine levels (1.6–10 μM) in rhesus monkeys are within the same range as those reported in cancer patients [7].

In contrast to previous studies [7–9, 12, 34, 36, 44], the more water-soluble succBAU was used in the present study. Table 1 demonstrates that this formula did not change BAU efficacy as an inhibitor of UrdPase activity. Subcutaneous administration of BAU at doses between 50 and 250 mg/kg had only a moderate effect (a maximum threefold increase) on uridine plasma levels in rhesus monkeys. Of note, this effect was in the same range after administration of either 100 or 250 mg/kg BAU. Therefore, it is unlikely that administration of BAU alone could generate uridine levels approximating 100 μM which may be required for protection against FUra host toxicity [34].

These results contrast with previous studies in mice where no plateau effect was observed, and a linear relationship was demonstrated between peak plasma uridine concentrations and BAU doses up to 240 to 300 mg [7, 8]. These data have been recently confirmed by Davis et al. [11]. However, that study also indicated that BAU effects on plasma uridine are species dependent [11]. A modest increase (twofold) in plasma uridine has been observed in rats but none has been detected in cynomolgus monkeys after intravenous administration of 30 mg/kg of BAU [11]. Administration of higher doses of BAU in monkeys were not studied and the reasons for the lack of uridine perturbation were unclear. The differences in the ability of BAU to increase uridine peak plasma levels between rodents and monkeys have been suggested to relate to a variation in UrdPase specific activities between the various species [11]. In our study, however, administration of a tracer dose of 500 μCi of ^3H -uridine in combination with 83.3 mg/kg BAU resulted in a substantial decrease in ^3H -uracil (ninefold) and ^3H -dihydrouracil (fivefold) plasma levels as compared to control animals (Fig. 1), consistent with inhibition of UrdPase activity in rhesus monkeys using this BAU dosing regimen.

Differences in the efficacy of BAU in raising plasma uridine may also reflect a variability between species in regulating uridine concentration. Our data show that administration of exogenous uridine is an effective

modulator of plasma uridine levels in rhesus monkeys, as previously demonstrated in humans [3, 17–20, 30, 43]. Indeed, administration of 83.3 mg/kg uridine, which is equivalent to 960 mg/m² resulted in uridine plasma levels approximating 160 μM (Table 2), in accordance with the levels achieved in humans with similar doses of uridine [19, 20, 30, 43]. Pharmacokinetic parameters of uridine, including plasma elimination $t_{1/2}$, V_d and CL_T were independent of the dose (Table 2), as previously demonstrated in humans [19, 20, 30, 43].

Despite a fourfold reduction in the plasma concentration of uridine catabolites, demonstrating inhibition of UrdPase activity, coadministration of 83.3 mg/kg BAU with 83.3 mg/kg uridine did not further increase uridine plasma levels beyond 160 μM (Table 2). Evaluation of urinary excretion of uridine and its catabolites confirmed the inhibition of UrdPase activity, as assessed by a reduction in the uracil/uridine urinary excretion ratio, and also revealed a major enhancement of uridine Cl_R after BAU administration (Table 3). These results suggest that, although exogenous uridine can elevate plasma uridine concentrations and BAU is effective in inhibiting uridine catabolism, their effectiveness is limited by the enhanced excretion of unchanged uridine in the urine, probably reflecting a saturation of its renal reabsorption process.

Multiple administration of BAU did not show evidence of a cumulative effect on plasma uridine levels (Fig. 2). In fact, multiple doses of BAU and uridine (83.3 mg/kg three times per day) resulted in decreased uridine C_{max} values. This is in contrast to previous studies in mice in which no differences were observed after administration of such a combination with a similar 8-h interval [34]. It is important to note that this decrease in uridine AUC over time was not related to enhanced “spillover” of excess uridine, but reflected a time-dependent relationship of uridine pharmacokinetics in monkeys. The reason for this phenomenon is unclear. However, such mechanisms as increased incorporation of uridine nucleotides in nucleic acids and/or induction of catabolic enzymes may be involved and warrant further investigation.

In conclusion, the present study indicates that the doses of BAU used in monkeys were sufficient to inhibit UrdPase, as manifested by increased uridine levels in plasma and urine and decreased concentrations of its catabolites, uracil and dihydrouracil. The plasma concentration of uridine in monkeys appears to be regulated mainly by the renal system, as evidenced by the “spillover” of excess plasma uridine into the urine. Thus, as observed with glucose, uridine may have a tubular maximum which limits reabsorption when its concentration is elevated. Therefore, an inhibitor of renal excretion could be used in combination with BAU and uridine (or a prodrug of uridine) to maintain a prolonged increase in plasma uridine level. Preliminary data demonstrate that dilazep, a nucleoside

transport inhibitor, enhances plasma uridine concentration by inhibiting uridine renal excretion [48]. Such manipulation of plasma and tissue uridine pools may improve the therapeutic efficacy of pyrimidine analogues in the treatment of cancer and other diseases by lowering the incidence of host toxicity.

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