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5-(*m*-Benzyloxybenzyl)barbituric Acid Acyclonucleoside, a Uridine Phosphorylase Inhibitor, and 2′,3′,5′-Tri-O-Acetyluridine, a Prodrug of Uridine, as Modulators of Plasma Uridine Concentration

IMPLICATIONS FOR CHEMOTHERAPY

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ABSTRACT. 5-(*m*-Benzyloxybenzyl)barbituric acid acyclonucleoside (BBBA), the most potent inhibitor known of uridine phosphorylase (UrdPase, EC 2.4.2.3), the enzyme responsible for uridine catabolism, and 2′,3′,5′-tri-O-acetyluridine (TAU), a prodrug of uridine, were used to investigate the possibility of improving the bioavailability of oral uridine in mice. Oral BBBA administered at 30, 60, 120, and 240 mg/kg increased the concentration of plasma uridine (2.6 ± 0.7 μM) by 3.2-, 4.6-, 5.4-, and 7.2-fold, respectively. After administration of 120 and 240 mg/kg BBBA, plasma uridine concentration remained 3- and 6-fold, respectively, higher than the plasma concentration at zero time (C_0) for over 8 hr. On the other hand, BBBA did not change the concentration of plasma uracil. TAU was far more superior than uridine in improving the bioavailability of plasma uridine. The relative bioavailability of plasma uridine released from oral TAU (53%) was 7-fold higher than that (7.7%) obtained by oral uridine. Oral TAU at 460, 1000, and 2000 mg/kg achieved area under the curve (*AUC*) values of plasma uridine of 82, 288, and 754 μmol · hr/L, respectively. Coadministration of BBBA with uridine or TAU further improved the bioavailability of plasma uridine resulting from the administration of either alone and reduced the C_{max} and *AUC* of plasma uracil. Coadministration of BBBA at 30, 60, and 120 mg/kg improved the relative bioavailability of uridine released from 2000 mg/kg TAU (53%) by 1.7-, 2.7-, and 3.9-fold, respectively, while coadministration of the same doses of BBBA with an equimolar dose of uridine (1320 mg/kg) increased the relative bioavailability of oral uridine (7.7%) by 4.1-, 5.3-, and 7.8-fold, respectively. Moreover, the *AUC* and C_{max} of plasma uridine after BBBA (120 mg/kg) coadministration with TAU were 3.5- and 11.5-fold, respectively, higher than those obtained from coadministration of BBBA with an equimolar dose of uridine. The exceptional effectiveness of the BBBA plus TAU combination in elevating and sustaining high plasma uridine concentration can be useful in the management of medical disorders that are remedied by administration of uridine as well as to rescue or protect from host-toxicities of various chemotherapeutic pyrimidine analogues. BIOCHEM PHARMACOL 51;12:1601–1611, 1996.

KEY WORDS. uridine; phosphorylase; inhibitor; prodrug; chemotherapy

The pyrimidine nucleoside, uridine, has been used successfully as a "protective" and/or "rescuing" agent against host-toxicity of various anti-cancer (e.g. 5-fluorouracil) [1–4] and anti-AIDS (e.g. 3′-azido-3′-deoxythymidine and 2′,3′-dideoxycytidine) [5–7] drugs without interfering with their chemotherapeutic efficacy. The use of uridine as an adjunct in therapy is not limited to the treatment of cancer and AIDS. Uridine was shown to protect from the toxicity of different anti-inflammatory and immunosuppressive agents used in the treatment of various auto-immune diseases and transplant rejection [8–11], and potentiate the anti-psychotic action of traditional neuroleptics [12, 13]. Uridine has also been used as a therapeutic agent in the treatment of several other medical disorders including: CNS disorders (e.g. cerebrovascular disorders and convulsions) [14–25], sleep promotion [26], muscle performance [27, 28], liver diseases [29–31], diabetic neuropathy [32], cardiac damage [33–38], and hereditary orotic aciduria [39]. However, because of its rapid clearance [40–48], it is necessary to administer substantial doses of uridine (10–12 g/m^2) [41] to attain and sustain the high plasma uridine concentrations (70 μM) [49] required to achieve the protective or rescuing effects. Unfortunately, such large doses of uridine also produce dose-limiting side-effects (e.g. phlebitis, pyrogenic reactions, and diarrhea) [42, 45, 50–52]. Therefore,

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alternative approaches to increase uridine bioavailability to the required concentrations must be sought.

Uridine is present in constant concentrations (1–5 μM) in the plasma of various species [53–57]. However, plasma uridine half-life is approximately 2 min [58]. Hence, the turnover of the plasma uridine must be rapid and efficient. Indeed, more than 90% of the circulating uridine is catabolized in a single pass through the liver by the activity of hepatic UrdPase† (EC 2.4.2.3), while constant amounts of uridine are synthesized *de novo* and released into the hepatic vein blood [40, 58]. Less than 2% of the uridine metabolized by the liver is salvaged and recovered in the uracil nucleotide pool in tissues of whole animals [55, 57, 59, 60], perfused rat liver [40, 58], and isolated liver cells [61]. The remainder is catabolized rapidly to products beyond uracil in the pyrimidine catabolic pathway [48, 62, 63].

One approach to maintain a high uridine concentration over a prolonged period is the use of UrdPase inhibitors to block the rapid catabolism of uridine to uracil. Inhibition of uridine catabolism by UrdPase inhibitors would lead to increased plasma uridine concentration as a result of the continuous *de novo* biosynthesis of uridine in the liver. Indeed, UrdPase inhibitors have been used to increase the concentration and half-life of plasma uridine [48, 49, 51, 62, 64–67] and the salvage or uridine by various tissues [49, 51, 68, 69].

Another approach to increase uridine bioavailability is to modify the structure of uridine to prevent its rapid catabolism by UrdPase and enhance its uptake into tissues where the modified uridine can be utilized. For this purpose, TAU (Fig. 1) has been designed and synthesized as a prodrug of uridine [70]. The acetyl groups of TAU increase the lipophilicity of uridine, thus enhancing its transport from the gastrointestinal tract to the blood stream and its reabsorption from the renal tubules, while rendering TAU resistant to catabolism by UrdPase [70]. Uridine is released from TAU by the action of plasma esterases. Furthermore, plasma has very little, if any, UrdPase activity; thus, the catabolism of uridine in plasma is minimal (unpublished data). This could eventually lead to a stable source for sustained delivery of high concentrations of uridine in plasma [70].

In the present study, we investigated the two approaches using BBBA (Fig. 1), the best known inhibitor of UrdPase [71–74], and/or TAU, as a prodrug of uridine, to improve the bioavailability and pharmacokinetics of plasma uridine in mice.

† *Abbreviations:* AUC, area under the curve; BBBA, 5-(*m*-benzyloxybenzyl)barbituric acid acyclonucleoside; C_0, plasma concentration at zero time; C_{max}, peak plasma concentration; Cl_T, total plasma clearance; DHUDase, dihydrouracil dehydrogenase, EC 1.3.1.2; HPMC, hydroxypropylmethylcellulose; *MRT*, mean residence time; $T_{1/2}$, elimination half-life; TAU, 2′,3′,5′-tri-O-acetyluridine; TCA, trichloroacetic acid; T_{max}, time to peak plasma concentration; UrdPase, uridine phosphorylase, EC 2.4.2.3; and V_{dss}, volume of distribution at steady state.

MATERIALS AND METHODS

Animals

Female CD-1 mice, 18–20 g, were purchased from Charles River Laboratories (Wilmington, MA) and housed 5/cage with food and water *ad lib.* under a normal light cycle (light, 6:00 a.m. to 6:00 p.m.; dark, 6:00 p.m. to 6:00 a.m.).

Chemicals

Uridine, tri-*n*-octylamine, freon (1,1,2-trichlorotrifluoroethane) and HPMC were purchased from the Sigma Chemical Co. (St. Louis, MO). Heparinized Natelson pipets, ammonium acetate, acetonitrile (HPLC grade), TCA, Gelman Acrodisc LC 13 PVDF 0.2 μm filters, and ethyl ether (anesthetic grade) were purchased from Fisher Scientific (Pittsburgh, PA). [6-^{14}C]Uracil (55 Ci/mol) and [2-^{14}C]uridine (56 Ci/mol) were purchased from Moravek Biochemicals, Inc. (Brea, CA). TAU was provided by Dr. Reid von Borstel, Pro-Neuron, Inc. (Rockfield, MD). BBBA was synthesized as described previously [71, 72].

Administration of Drugs

For oral administration, uridine (alone or with BBBA) was dissolved in double-distilled water. TAU (alone or with BBBA) was mixed well with HPMC in hot water (70°) and homogenized thoroughly using a polytron homogenizer (Brinkmann Instruments, Westbury, NY). The final concentration of HPMC was 0.75%. The drug suspension was mixed well before and periodically during dosing. HPMC was preferred over the commonly used methylcellulose because the latter must be cooled to 10° in order to hydrate completely. Drugs were administered orally (0.1 mL/10 g) using 18 gauge intubation needles (Popper & Sons, Inc., New Hyde Park, NY). For i.p. injection, uridine was dissolved in normal saline solution (0.9% NaCl) and injected at 0.1 mL/10 g. To avoid a possible circadian variation in UrdPase activity [75, 76], drugs were administered between 8:30 and 9:00 a.m. Control mice received the carrier solution without the drug(s).

Collection of Samples

At various time intervals (5, 10, 15, 30 min, 1, 2, 3, 4, 6, 8, 12, and 24 hr) after drug administration, 250 μL of whole blood was collected from the orbital sinuses of each of five mice (lightly anesthetized with ethyl ether) in heparinized Natelson pipets and placed on ice [75]. The whole blood from each mouse was then centrifuged (Fisher Microcentrifuge model 235 A) at 12,400 rpm for 5 min, and the plasma was recovered and immediately stored in a −20° freezer until analysis by HPLC.

Preparation of the Samples

Plasma was allowed to thaw on ice and then was deproteinized with 2 vol. of 15% TCA. After centrifugation

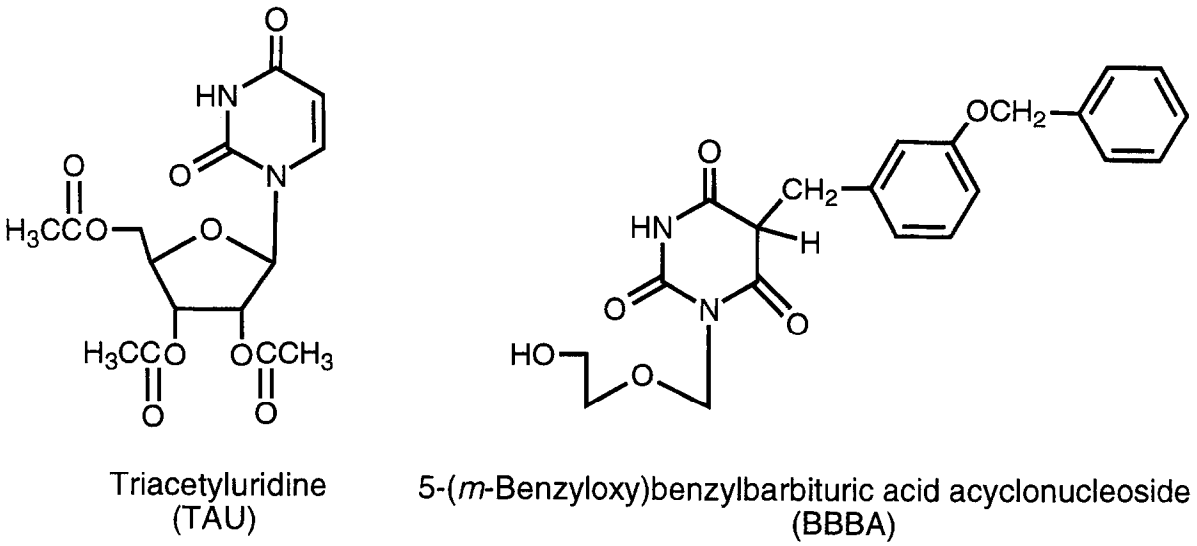

FIG. 1. Chemical structures of TAU and BBBA.

(16,000 g, 4°) for 5 min, using a Brinkmann Eppendorf Microcentrifuge, the supernatant acid-soluble material was neutralized by extraction with an equal volume of 1:2 mixture of tri-*n*-octylamine in freon. The neutralized supernatant was filtered through a Gelman Acrodisc LC 13 PVDF 0.2 μm filter, prior to HPLC analysis [75]. Under these conditions, the concentration of uridine released from TAU was not changed by the acid treatment or freezing during storage for up to 2 weeks (the longest duration of storage employed).

HPLC Analysis

Samples were analyzed by HPLC using a computer-controlled Hewlett-Packard model 1050 liquid chromatography apparatus equipped with an autosampler, a quaternary pump, and a multiple wavelength diode array base three channel UV detector. HPLC analysis was performed on two 5-μm Hypersil C_{18} reverse phase columns (250 × 5 mm) (Jones Chromatography, Littleton, CO) connected in tandem. Mobile phase was composed of two buffers, namely, Buffer A [50 mM ammonium acetate, 0.5% acetonitrile (pH 4.8)] and Buffer B [50 mM ammonium acetate, 60% acetonitrile (pH 4.8)]. Typically, 100 μL of treated plasma samples was analyzed with a multi-step elution protocol. A 23-min isocratic elution with Buffer A was followed by a 10-min linear gradient to 60% Buffer B, then a 22-min isocratic elution with 60% Buffer B, followed by a 20-min re-equilibration wash with 100% Buffer A. Flow rates were 1 mL/min, except for two 0.5 mL/min segments (8–23 min and 33–55 min). The effluent was monitored by UV absorption at 254 and 268 nm. Under these conditions, uracil, uridine, TAU and BBBA in the standards eluted at 13, 27, 47, and 48 min, respectively. Nevertheless, no TAU was recovered in plasma samples obtained as early as 5 min or at later time points. Instead, there were six metabolites, other than uridine and uracil, which we assumed to be the mono- and diacetyluridines. Incubation of TAU with plasma for various time periods supported this suggestion and showed the de-esterification of TAU to the six metabolites with measurable amounts of newly formed uridine but not uracil. BBBA, also, could not be recovered from plasma samples. A binding assay showed that BBBA has a high protein binding affinity. Only 0.5 and 6.5% of the drug were free after incubating 300 and 1000 μM concentrations, respectively, of BBBA with 25 mg/mL bovine serum albumin.

Uracil and uridine were identified by the ratio of their UV absorption at λ_{max} (259.5 and 262 nm, respectively)/254 nm, and co-elution with authentic samples. The recoveryof uracil and uridine was more than 98% using [6-^{14}C]uracil and [2-^{14}C]uridine. The *AUC* values for uracil and uridine in the sample were calculated by the on-line computer. The concentrations of uracil or uridine in the samples were determined using standard curves for uracil or uridine prepared in double-distilled water. Plots of area under the curve versus uracil or uridine concentrations were linear between 1 and 5000 μM.

Pharmacokinetic Analysis of Plasma Uridine and Uracil

The pharmacokinetic parameters of uridine and uracil were estimated as previously described [48] by compartmental model-independent methods using a SIPHAR/BASE program [77]. The *AUC* was estimated by the trapezoidal rule with extrapolation to time infinity using the terminal disposition slope (*K*) generated by a weighed non-linear least-squares regression of an exponential fit of the data [78], with the weighed square factor set as the reciprocal of the calculated concentration squared. $T_{1/2}$ values of uridine were calculated from 0.693/*K*. The Cl_T was calculated by dividing the administered dose by the *AUC* and normalized to the weight of the animals. The apparent V_{dss} was calcu-

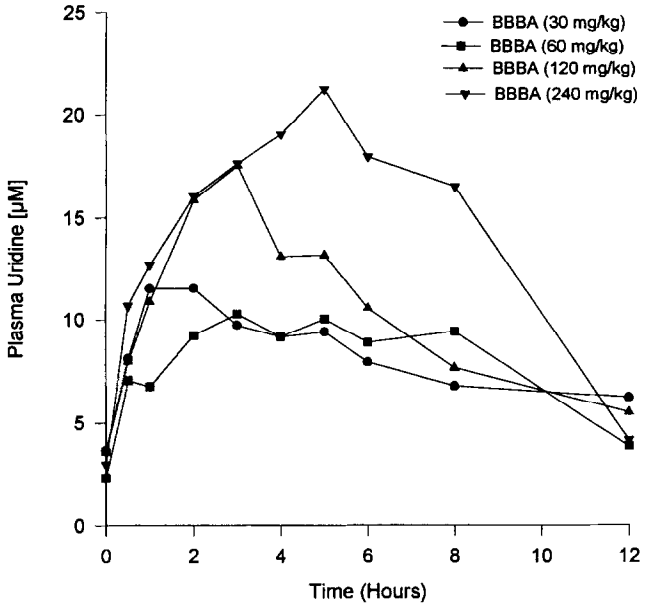

FIG. 2. Effect of different oral doses of BBBA on plasma uridine concentration in CD-1 mice. Each point represents the average from at least five mice.

lated as the product of the Cl_T and the *MRT* and normalized to the weight of the animals. The C_{max} and T_{max} values were estimated from the abscissa and the ordinate of the point with the highest ordinate on the computer-generated least squares curve depicting plasma concentration versus time. C_0 was the plasma concentration of endogenous uridine and uracil observed at zero-time (8:30 to 9:00 a.m.). Bioavailability of oral uridine was calculated as the percent of the *AUC* of plasma uridine resulting from oral administration of uridine/the *AUC* of plasma uridine resulting from i.p. administration of the same uridine dose. Relative bioavailability of uridine produced from the different oral regimens was expressed as the percent of the *AUC* of plasma uridine resulting from oral administration of uridine or TAU (alone or with BBBA)/the *AUC* of plasma uridine resulting from i.p. administration of the reference uridine concentration of 1320 mg/kg.

RESULTS

In the present study, the zero time (8:30 to 9:00 a.m.) concentrations of plasma uridine and uracil (C_0) in CD-1 mice were relatively constant, averaging 2.6 ± 0.7 and 7.4 ± 1.0 μM, respectively.

Administration of BBBA

Table 1 shows that oral administration of BBBA at 30, 60, 120, and 240 mg/kg increased the C_0 of plasma uridine by 3.2-, 4.6-, 5.4-, and 7.2-fold (C_{max}/C_0), respectively. Administration of the highest dose of BBBA (240 mg/kg) resulted in a plasma uridine C_{max} of 20.8 μM. Plasma uridine concentration remained 3- to 6-fold higher than control values for 8 hr after BBBA administration (Fig. 2). The *AUC* values were 104, 199, 227, and 280 μmol · hr/L, respectively (Table 1). There was no significant change in plasma uracil concentration following BBBA administration (data not shown).

Administration of Uridine

Previous studies have investigated the bioavailability and pharmacokinetics of wide range doses of oral uridine (350 to 5000 mg/kg) [48, 78]. Therefore, when we studied the effects of BBBA and TAU as modulators of plasma uridine concentration, we used only one dose of uridine as a reference dose for our investigations. We chose the uridine dose of 1320 mg/kg, which is approximately the median of previously studied doses. Administration of 1320 mg/kg of uridine by the i.p. route resulted in a uridine C_{max} of 2330 μM, a 1124-fold increase over zero time concentration (Table 2), at 5 min post-administration. However, this concentration dropped to 5 μM within 3 hr (data not shown). Plasma uracil concentration increased 61-fold, peaking to 536 μM at 0.6 hr (Table 2), and dropping to 19 μM within 3 hr, after which it was cleared from plasma (data not shown). The *AUC* values of plasma uridine and uracil were 1416 and 860 μmol · hr/L, respectively (Table 2).

Oral administration of the same dose of uridine (1320 mg/kg) resulted in a C_{max} of plasma uridine and uracil of 20 and 207 μM at 1.0 and 2.4 hr, respectively. Plasma uridine concentration remained at least 3-fold higher than basal concentration for up to 4 hr, while uracil was slowly cleared from plasma (Fig. 3A). The *AUC* values of plasma uridine and uracil were 109 and 1421 μmol · hr/L (Table 2). The V_{dss} and the Cl_T of plasma uridine were 210 L/kg and 52 L/hr/kg, respectively. These values were 50- and 14-fold

TABLE 1. Effect of oral administration of different concentrations of BBBA on the pharmacokinetics of plasma uridine in CD1 mice

BBBA (mg/kg)	C_{max} (μM)	Fold change (C_{max}/C_0)	T_{max} (hr)	*AUC* (μmol · hr/L)
30	11.6 ± 3.7	3.18 ± 0.34	1.9 ± 0.14	104 ± 28.3
60	10.4 ± 3.6	4.61 ± 0.65	3.9 ± 1.28	199 ± 68.1
120	15.5 ± 3.4	5.39 ± 2.02	2.9 ± 0.66	227 ± 44.4
240	20.8 ± 11.2	7.19 ± 1.43	3.2 ± 0.04	280 ± 15.7

C_{max}, peak plasma concentration; C_0, zero time plasma concentration; T_{max}, time to peak plasma concentration; and *AUC*, area under the curve. Values are means ± SD from at least 5 mice at each time point.

TABLE 2. Effect of administration of uridine alone and in combination with different concentrations of BBBA on the pharmacokinetics of plasma uridine and uracil in CD1 mice

Uridine (mg/kg)	BBBA	C_{max} (μM)	Fold change (C_{max}/C_0)	T_{max} (hr)	AUC (μmol· hr/L)	V_{dss} (L/kg)	MRT (hr)	Cl_T (L/hr/kg)	$T_{1/2}$ (hr)
				Uridine					
Intraperitoneal									
1320	0	2330 ± 1120	1124 ± 112	0.08 ± 0.00	1416 ± 830	4.2 ± 3.1	1.1 ± 0.6	3.6 ± 1.3	0.29 ± 0.03
Oral									
1320	0	20.1 ± 4.2	7.5 ± 0.6	1.00 ± 0.28	109 ± 54.3	210.0 ± 13.9	4.6 ± 1.5	52.0 ± 8.5	0.26 ± 0.11
1320	30	74.2 ± 10.7	23.3 ± 1.8	1.00 ± 0.03	446 ± 164	58.7 ± 4.6	5.2 ± 1.2	12.4 ± 3.8	0.27 ± 0.05
1320	60	86.0 ± 35.7	28.2 ± 8.1	0.70 ± 0.23	577 ± 119	56.3 ± 20.2	6.2 ± 1.2	9.2 ± 1.5	0.14 ± 0.09
1320	120	141 ± 0.8	36.1 ± 9.9	0.41 ± 0.31	848 ± 259	36.1 ± 1.6	5.8 ± 1.5	6.3 ± 1.5	0.06 ± 0.08
				Uracil					
Intraperitoneal									
1320	0	536 ± 197.0	61.0 ± 26.3	0.55 ± 0.00	860 ± 362				
Oral									
1320	0	207 ± 80.0	27.1 ± 7.1	2.40 ± 0.09	1421 ± 519				
1320	30	179 ± 21.2	27.5 ± 1.4	1.75 ± 1.07	872 ± 880				
1320	60	40.6 ± 35.5	4.9 ± 3.5	3.00 ± 0.86	467 ± 57.4				
1320	120	29.1 ± 6.7	3.5 ± 0.4	1.75 ± 118	307 ± 16.3				

C_{max}, peak plasma concentration; C_0, zero time plasma concentration; T_{max}, time to peak plasma concentration; *AUC*, area under the curve; Cl_T, total plasma clearance; $T_{1/2}$ elimination half-life; V_{dss}, volume of distribution at steady state; and *MRT*, mean residence time. Values are means ± SD from at least 5 mice at each time point.

higher than those produced by the i.p. route (Table 2). The bioavailability of oral uridine was estimated to be 7.7%.

Administration of TAU

TAU was administered orally at 460, 1000, and 2000 mg/kg (molar equivalent to uridine doses of 300, 660, and 1320 mg/kg, respectively). The C_{max} of plasma uridine and uracil reached 78, 330, and 507 μM; and 265, 342, and 665 μM, respectively. These concentrations were 28-, 164-, and 252-fold, and 37-, 51-, and 72-fold higher than the C_0 of plasma uridine and uracil, respectively. The *AUC* values of plasma uridine were 82, 288, and 754 μmol · hr/L, while those of plasma uracil were 267, 610, and 2115 μmol · hr/L, respectively (Table 3 and Fig. 3B). The relative bioavailability of uridine released from oral TAU was 53%. Administration of TAU at the tested doses did not induce any noticeable toxicity (e.g. hypothermia, diarrhea, or weight loss) in the treated animals.

Coadministration of BBBA with Uridine

BBBA, at 30, 60, and 120 mg/kg increased the C_{max} of plasma uridine (20 μM), achieved by 1320 mg/kg of oral uridine alone, by 3.7-, 4.3-, and 7.0-fold, respectively (Table 2 and Fig. 3A). At the highest dose used (120 mg/kg), BBBA caused the plasma uridine C_{max} to reach 141 μM, 1 hr after coadministration, and to remain over 40 μM for 8 hr (Fig. 3A). Coadministration of 30, 60, and 120 mg/kg BBBA, increased also the *AUC* of plasma uridine (109 μmol · hr/L) by 4.1-, 5.3-, and 7.8-fold, respectively, and decreased the V_{dss} (210 L/kg) as well as Cl_T (52 L/hr/kg) by 3.6-, 3.7-, and 5.8-fold, and 4.2-, 5.6- and 8.3-fold, respectively (Table 2). Thus, as shown in Fig. 4, coadministration of 30, 60, and 120 mg/kg BBBA increased the relative bioavailability of oral uridine from 7.7 to 31, 41, and 60%, respectively.

Plasma uracil concentration was also affected by the coadministration of BBBA: the higher the dose of BBBA, the lower the uracil concentration. Coadministration of BBBA at 30, 60, and 120 mg/kg with uridine decreased plasma uracil C_{max} from 207 to 179, 41, and 29 μM, respectively, with a corresponding reduction in the *AUC* from 1421 to 872, 467, and 307 μmol · hr/L, respectively (Table 2 and Fig. 3A).

Coadministration of BBBA with TAU

Coadministration of BBBA at 30, 60, and 120 mg/kg with TAU (460 mg/kg) increased the C_{max} of plasma uridine (78 μM), achieved by TAU alone, by 1.3-, 2.9-, and 2.5-fold, and reduced the C_{max} of plasma uracil (265 μM) by 5.2-, 8.7-, and 12.6-fold, respectively. BBBA also expanded the *AUC* of plasma uridine released from TAU (82 μmol · hr/L) by 1.7-, 4.4-, and 4.3-fold, respectively. This increase in plasma uridine *AUC* was accompanied by a concomitant 2.2-, 3.2-, and 3.6-fold decrease in plasma uracil *AUC* values (Table 3). A similar trend was observed when these same concentrations of BBBA were coadministered with a higher dose of TAU (1000 mg/kg). The C_{max} of plasma uridine (330 μM) was increased by 1.8-, 2.0-, and 3.2-fold, while that of uracil (342 μM) was decreased by 1.2-, 2.4-, and 3.0-fold, respectively. Consequently, the *AUC* of plasma uridine (288 μmol · hr/L) was increased by 2.4-, 2.8-, and 6.3-fold, while that of plasma uracil (610 μmol · hr/L) was reduced by a 1.1-, 1.3-, and 1.8-fold, re-

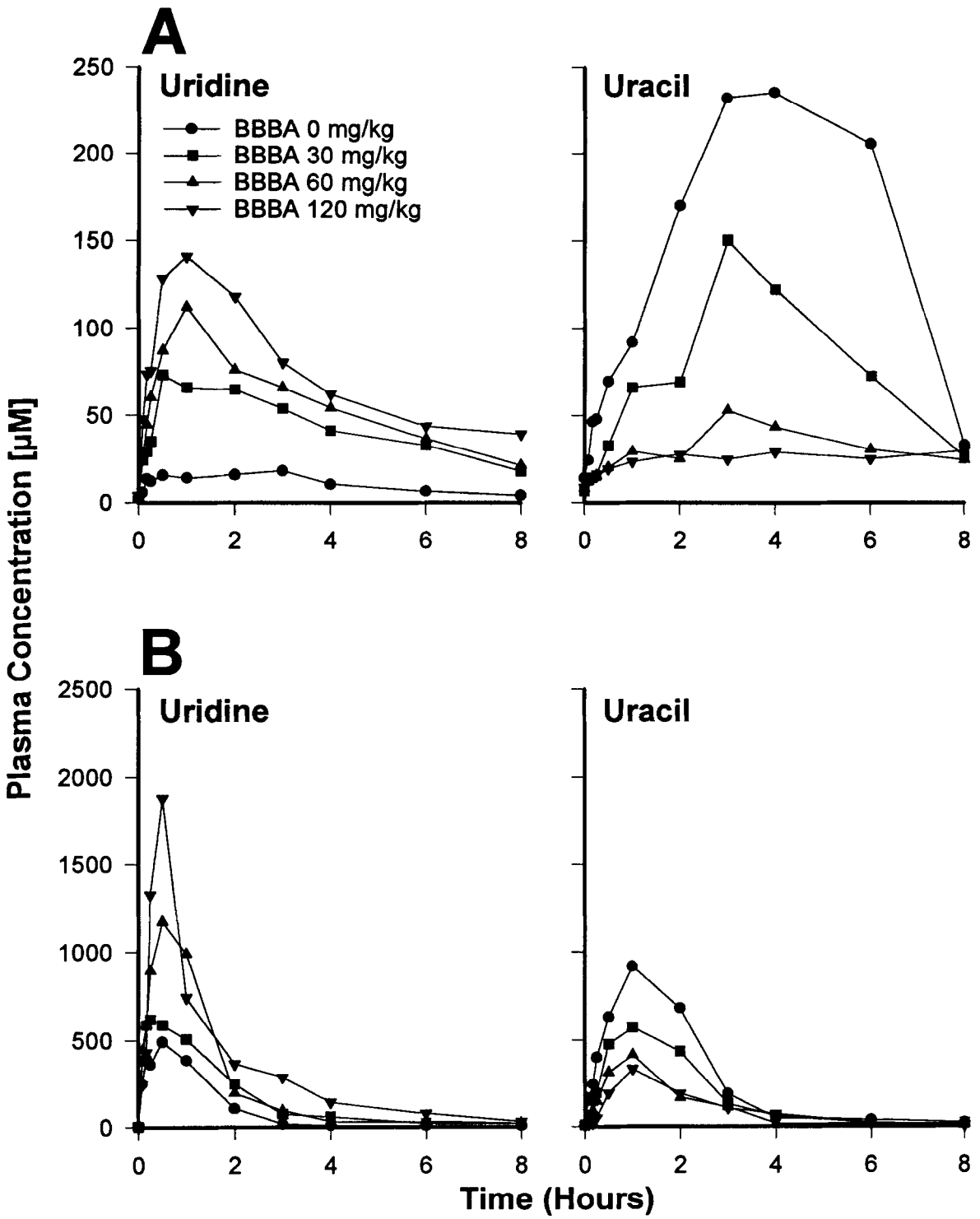

FIG. 3. Effect of oral coadministration of different doses of BBBA with (A) uridine (1320 mg/kg) or (B) a molar equivalent dose of TAU (2000 mg/kg) on plasma concentration of uridine and uracil in CD-1 mice. Each point represents the average from at least five mice.

spectively. At the highest dose of TAU (2000 mg/kg) tested, coadministration of BBBA further increased he C_{max} of plasma uridine (507 μM) by 1.2-, 2.3-, and 3.2-fold and decreased that of uracil (665 μM) by 1.2-, 2.1-, and 2.5-fold, respectively. Coadministration of BBBA also increased the AUC of plasma uridine (754 μmol · hr/L) by 1.6-, 2.7-, and 3.9-fold and decreased that of uracil (2115 μmol · hr/L) by 1.4-, 2.4-, and 2.7-fold, respectively. Figure 4 shows the effects of different doses of BBBA on the relative bioavailability of uridine released from oral TAU.

DISCUSSION

The present results indicated that the bioavailability of oral uridine is only 7.7%. This is in agreement with other results

TABLE 3. Effect of oral administration of TAU alone and in combination with BBBA on the pharmacokinetics of plasma uridine and uracil in CD1 mice

TAU (mg/kg)	BBBA (mg/kg)	C_{max} (μM)	Fold change (C_{max}/C_0)	T_{max} (hr)	AUC (μmol· hr/L)
		Uridine			
460	0	78.2 ± 21.8	28.2 ± 1.8	0.20 ± 0.04	82.4 ± 41.0
460	30	104 ± 30.9	36.6 ± 9.9	0.13 ± 0.02	144 ± 72.9
460	60	225 ± 68.3	105 ± 14.3	0.25 ± 0.03	366 ± 32.6
460	120	192 ± 13.4	95.6 ± 29.9	0.45 ± 0.14	358 ± 10.4
1000	0	330 ± 102	164 ± 10.5	0.25 ± 0.04	288 ± 85.4
1000	30	582 ± 256	290 ± 55.1	0.25 ± 0.11	693 ± 118
1000	60	670 ± 95	333 ± 104	0.25 ± 0.03	796 ± 73.2
1000	120	1045 ± 329	520 ± 325	0.50 ± 0.03	1814 ± 994
2000	0	507 ± 298	252 ± 22.3	0.41 ± 0.22	754 ± 355
2000	30	621 ± 223	309 ± 128	0.31 ± 0.04	1239 ± 468
2000	60	1173 ± 503	584 ± 19.5	0.50 ± 0.02	2016 ± 493
2000	120	1618 ± 706	805 ± 17.8	0.50 ± 0.09	2931 ± 824
		Uracil			
460	0	265 ± 70.4	37.1 ± 21.5	0.29 ± 0.02	267 ± 50.8
460	30	50.8 ± 42.2	8.3 ± 5.0	0.46 ± 0.09	123 ± 77.0
460	60	30.4 ± 6.1	6.1 ± 0.3	0.45 ± 0.02	84.3 ± 20.8
460	120	21.0 ± 3.7	3.5 ± 0.1	0.25 ± 0.10	74.4 ± 18.5
1000	0	342 ± 79.9	50.7 ± 2.0	0.62 ± 0.00	610 ± 158
1000	30	275 ± 51.4	40.7 ± 0.3	0.52 ± 0.03	536 ± 98.1
1000	60	141 ± 24.6	28.2 ± 9.9	0.70 ± 0.01	466 ± 91.0
1000	120	112 ± 94.4	24.2 ± 14.8	0.69 ± 0.10	338 ± 125
2000	0	665 ± 287	71.6 ± 13.9	1.00 ± 0.10	2,115 ± 839
2000	30	536 ± 190	57.7 ± 8.6	0.76 ± 0.17	1,466 ± 375
2000	60	313 ± 130	44.2 ± 5.7	0.76 ± 0.03	892 ± 405
2000	120	266 ± 56.8	36.3 ± 5.3	1.00 ± 0.03	775 ± 208

C_{max}, peak plasma concentration; C_0, zero time plasma concentration; T_{max}, time to peak plasma concentration; and AUC, area under the curve. Values are means ± SD from at least 5 mice at each time point.

from mice [79] and humans [45]. The present data also demonstrated that TAU is a superior substitute for uridine. The relative bioavailability of uridine following oral TAU administration (53%) was 7-fold higher than that achieved by oral uridine (7.7%). Not only did TAU increase the concentration of plasma uridine, but it also reduced the time required to attain the maximum concentration. Oral TAU produced a plasma uridine C_{max} of 507 μM at 0.4 hr, while an equimolar dose of oral uridine resulted in a C_{max} of only 20 μM at 1 hr (Tables 2 and 3).

The low bioavailability of oral uridine can be attributed mainly to the first pass effect and reflects the contribution of the intestine and liver to uridine catabolism. It was reported previously that there is an inverse relationship between plasma uridine concentration and its hepatic clearance, i.e. increasing the uridine concentration entering the liver is accompanied by a decrease in hepatic clearance [63], until uridine reaches a concentration of approximately 50 μM (the threshold or hepatic maximum for uridine clearance), after which a constant amount of uracil is discharged into the circulation [63]. This threshold or hepatic maximum for uridine clearance results from saturation of the transport system in the liver and/or catabolism of uridine by hepatic UrdPase activity [63]. These factors could also apply to the intestine which is a major organ responsible for the low bioavailability of oral uridine [45, 79, and the present results]. In this regard, it should be noted that UrdPase activity in the intestine is the highest in all studied organs of the body. In mice, intestinal UrdPase activity (47,308 ± 1,498 pmol/min/mg protein) was 146-fold higher than that of the liver (unpublished results). Such high activity of uridine catabolism in the intestine and liver is considered among the principal components of the rapid disappearance of uridine from plasma following its oral administration [48, 55, 58, 62]. This view is supported by the respective 50- and 15-fold increase in the V_{dss} and Cl_T of oral uridine when compared to i.p. uridine (Table 2).

The better efficiency of oral TAU over uridine in delivering uridine to the plasma can also be ascribed to the extent of the extravascular catabolism of uridine by UrdPase. TAU, unlike uridine, is resistant to catabolism by UrdPase. It is also more lipophilic which enhances its absorption from the gastrointestinal tract and reabsorption from the renal tubules [70]. Therefore, a large portion of administered TAU is transported or diffused into the plasma unchanged and/or as mono- or diester derivatives. In plasma, these uridine esters act as depots releasing uridine by plasma esterases over a longer period of time than when oral uridine is used. Furthermore, our unpublished results indicate that, in contrast to other sites in the body, plasma has a negligible UrdPase activity (6 pmol/min/mg protein). Indeed, incubation of TAU with plasma resulted in the lib-

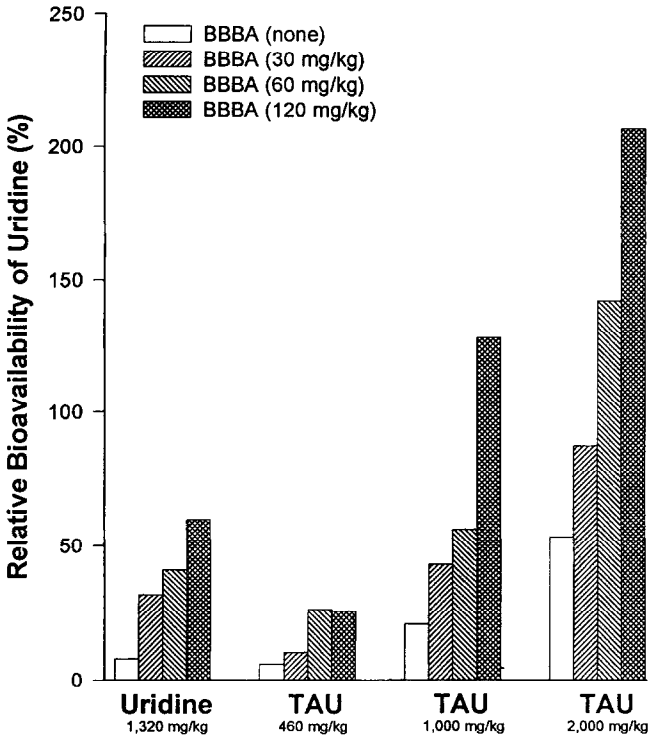

FIG. 4. Relative bioavailability of uridine from the oral administration of uridine or TAU, alone or with different doses of BBBA in CD-1 mice. Relative bioavailability is calculated as the percent of *AUC* of plasma uridine resulting from the oral administration of the compound(s) under study/*AUC* of plasma uridine resulting from the i.p. administration of 1320 mg/kg uridine. Each value represents the average from at least five mice.

eration of the mono- and diacetyl derivatives of TAU as well as uridine, but not uracil. Hence, higher and sustained levels of plasma uridine were observed after administration of TAU than when uridine was used. These results suggest that the difference between oral uridine and TAU in delivering uridine to the plasma is due primarily to extravascular uridine catabolism by UrdPase. This postulation is further supported by the finding that coadministration of the UrdPase inhibitor BBBA (120 mg/kg) with uridine enhanced the relative bioavailability of oral uridine by 7.8-fold, while its coadministration with an equimolar concentration of TAU increased the relative bioavailability of plasma uridine by only 3.9-fold (Fig. 4).

BBBA, the most potent inhibitor of UrdPase [71–73], is a powerful enhancer of plasma uridine concentration. Oral administration of BBBA produced a dose-dependent increase in the *AUC* and C_{max} of plasma uridine (Table 1). Although uridine C_{max} achieved by BBBA was similar to that attained by oral administration of another UrdPase inhibitor, 5-benzylacyclouridine (BAU) [49, 80], the effect of BBBA on plasma uridine concentration was more prolonged. BBBA at 120 and 240 mg/kg maintained plasma uridine concentration 3- and 6-fold higher than the zero time concentration for 8 hr after administration (Fig. 2). On the other hand, uridine concentration dropped to near zero time concentration 6 hr post-administration of the same doses of BAU [49, 80].

Coadministration of BBBA with uridine or TAU increased the relative bioavailability of uridine in a dose-dependent fashion (Fig. 4), presumably due to inhibition of UrdPase as indicated by the increase in the *AUC*, C_{max} and C_{max}/C_0 and decrease in V_{dss} and Cl_T of plasma uridine, as well as decrease in the *AUC* and C_{max} of plasma uracil (Tables 2 and 3 and Fig. 3). However, the combination of BBBA with TAU was superior to that with uridine in increasing plasma uridine concentration and bioavailability. Figure 4 demonstrates that coadministration of BBBA (30, 60, and 120 mg/kg) with uridine (1320 mg/kg) increased the relative bioavailability of oral uridine (7.7%) by 4.1-, 5.3-, and 7.8-fold, while coadministration of the same doses of BBBA with a molar equivalent dose of oral TAU (2000 mg/kg) improved the relative bioavailability of uridine released from TAU (53%) by 1.7-, 2.7-, and 3.9-fold, respectively. The superiority of the BBBA and TAU combination is also evident from the fact that the *AUC* of plasma uridine observed after administration of 1320 mg/kg uridine with 120 mg/kg BBBA could be achieved by the administration of TAU (1000 mg/kg) with BBBA (60 mg/kg), i.e. the molar equivalent of half the doses of uridine and BBBA, respectively.

The marked increase of plasma uracil concentration following the administration of uridine or TAU (Tables 2 and 3) could be attributed to the saturation of uracil catabolism. DHUDase (EC 1.3.1.2), the rate-limiting enzyme of uracil catabolism in the liver [81], is a saturable enzyme and inhibited by increasing concentration of its substrate, uracil [81]. Degradation by UrdPase of a large amount of the administered uridine or the uridine released from TAU would increase uracil formation. When uracil concentration reaches the critical saturating limit (*ca.* 75 μM), it inhibits DHUDase [81]. This would lead to the delivery of increasing amounts of uracil to the plasma, hence, the observed rise in plasma uracil concentration and *AUC* after administration of uridine or TAU. It should be noted, however, that oral TAU increased the C_{max} and *AUC* of plasma uracil above that achieved by administration of an equimolar dose of oral uridine. This observation is not unexpected since administered uridine is subject to the sequential activities of intestinal and hepatic UrdPase and DHUDase. These activities would lead not only to reduction of uridine bioavailability but also to a decreased uracil pool. On the other hand, TAU and its mono- and diacetyl derivatives are not subject to UrdPase activity, hence the expansion of available uridine which will be reabsorbed and eventually metabolized to increase plasma uracil concentration.

The lack of significant alterations in plasma uracil concentration following the administration of BBBA alone indicates that the doses of BBBA used were not sufficient to inhibit totally UrdPase and uridine catabolism. Consequently, the remaining UrdPase activity converts uridine to

uracil, which in turn is subject to DHUDase activity. However, the concentration of this newly formed uracil appears not to be high enough to disturb the homeostatic mechanisms maintaining the constancy of plasma uracil concentration, including the saturation and inhibition of DHUDase. As a result, plasma uracil concentration remained unchanged.

In conclusion, the specific UrdPase inhibitor BBBA alone increased plasma uridine concentration and bioavailability in a dose-dependent manner. TAU, a prodrug of uridine, proved to be an excellent substitute for uridine in achieving a greater bioavailability of plasma uridine. Combining BBBA with uridine or TAU, for oral administration, secured and maintained higher levels of plasma uridine than either alone. However, the combination of BBBA plus TAU was more effective in accomplishing this goal. Therefore, the combination of BBBA plus TAU can provide a better substitute for the massive doses of uridine required to achieve the high levels of uridine necessary to rescue or protect from host-toxicities of certain anti-cancer and antiviral pyrimidine analogues, without the toxic side-effects associated with such doses of uridine. The combination of BBBA plus TAU can also replace uridine in the treatment of other pathological disorders that can be remedied by administration of uridine.

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