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ORIGINAL ARTICLE

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5-(Phenylthio)acyclouridine: a powerful enhancer of oral uridine bioavailability: relevance to chemotherapy with 5-fluorouracil and other uridine rescue regimens

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Abstract *Purpose*: The purpose of this investigation was to evaluate the effectiveness of oral 5-(phenylthio)acyclouridine (PTAU) in improving the pharmacokinetics and bioavailability of oral uridine. PTAU is a potent and specific inhibitor of uridine phosphorylase (UrdPase, EC 2.4.2.3), the enzyme responsible for uridine catabolism. This compound was designed as a lipophilic inhibitor in order to facilitate its access to the liver and intestine, the main organs involved in uridine catabolism. PTAU is fully absorbed after oral administration with 100% oral bioavailability. *Methods*: Uridine (330, 660 or 1320 mg/kg) and/or PTAU (30, 45, 60, 120, 240 or 480 mg/kg) were orally administered to mice. The plasma levels of uridine, its catabolite uracil, and PTAU were measured using HPLC, and pharmacokinetic analysis was performed. *Results*: Oral PTAU up to 480 mg/kg per day is not toxic to mice. Oral PTAU at 30, 45, 60, 120 and 240 mg/kg has a prolonged plasma half-life of 2–3 h, and peak plasma PTAU concentrations (C_{max}) of 41, 51, 74, 126 and 161 μ*M* with AUCs of 70, 99, 122, 173 and 225 μmol h/l, respectively. Coadministration of uridine with PTAU did not have a significant effect on the pharmacokinetic parameters of plasma PTAU at any of the doses tested. Coadministration of PTAU (30, 45, 60 and 120 or 240 mg/kg) with uridine (330, 660 or 1320 mg/kg) elevated the concentration of plasma uridine over that following the same dose of uridine alone, a result of reduced metabolic clearance of uridine as evidenced by decreased plasma exposure (C_{max} and AUC) to uracil. Plasma uridine was elevated with the increase of uridine dose at each PTAU dose tested and no plateau was reached. Coadministration of PTAU at 30, 45, 60, 120 and 240 mg/kg improved the low oral bioavailability (7.7%) of uridine administered at 1320 mg/kg by 4.3-, 5.9-, 9.9-, 11.7- and 12.5-fold, respectively, and reduced the AUC of plasma uracil (1227.8 μmol h/l) by 5.7-, 6.8-, 8.2-, 6.3-, and 6.9-fold, respectively. Similar results were observed when PTAU was coadministered with lower doses of uridine. Oral PTAU at 30, 45, 60, 120 and 240 mg/kg improved the oral bioavailability of 330 mg/kg uridine by 1.7-, 2.4-, 2.6-, 5.2- and 4.3- fold, and that of 660 mg/kg uridine by 2.3-, 2.7-, 3.3-, 4.6- and 6.7-fold, respectively. *Conclusion*: The excellent pharmacokinetic properties of PTAU, and its extraordinary effectiveness in improving the oral bioavailability of uridine, could be useful to rescue or protect from host toxicities of 5-fluorouracil and various chemotherapeutic pyrimidine analogues used in the treatment of cancer and AIDS, as well as in the management of medical disorders that are remedied by the administration of uridine including CNS disorders (e.g. Huntington's disease, bipolar disorder), liver diseases, diabetic neuropathy, cardiac damage, various autoimmune diseases, and transplant rejection.

Keywords 5-(Phenylthio)acyclouridine · Uridine · Phosphorylase · Inhibitor · Chemotherapy

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Introduction

For over four decades, despite its clinical host toxicity and the extensive research undertaken to develop alternative drugs, 5-fluorouracil (FUra) remains among the

few "standard" anticancer drugs effective against solid tumors in humans such as colorectal, breast, and head and neck cancers. However, one of the major limitations of the anticancer activity of FUra in the clinic is the inability to increase its dose for better efficacy against the tumors. Increasing the administered dose has been hampered by host toxicity, mainly myelosuppression [33, 67, 86]. The chemotherapeutic merits of FUra have led several groups to attempt to develop biochemical modulators of FUra metabolism and/or toxicity to enhance its therapeutic index rather than searching for alternatives to FUra. Biochemical modulation involves different formulations of FUra or combinations of FUra with another drug and/or a natural compound that may modify FUra metabolism and/or toxicity.

The natural pyrimidine nucleoside uridine is one of the most promising biochemical modulators of FUra efficacy against solid tumors. It has been demonstrated that the combination of uridine with FUra exhibits definitive activity in preclinical models [45, 46, 55, 70]. Uridine has been shown to counteract FUra host toxicity without impairing FUra antitumor activity [45, 46, 55, 69, 70, 76]. In these studies, it was demonstrated that by using delayed uridine treatment, a single dose of FUra that leads to 50% mortality in mice, can be made completely tolerable, and that the maximum tolerated dose of FUra can be doubled [55]. Furthermore, animals treated with FUra plus uridine showed less depression of WBC and hematologic toxicities and faster recovery than animals receiving FUra alone. Concurrently, the combination resulted in a superior antitumor effect and allowed an increase of the FUra dose from 100 to 250–300 mg/kg [70]. Uridine has been also shown in clinical trials to alleviate bone marrow toxicity associated with FUra [54, 67, 83, 86], which would permit increasing the chemotherapeutic doses of FUra. Nevertheless, the use of oral uridine in patients is limited by its poor oral bioavailability (7–8%) and short plasma elimination half-life [2–4, 14, 47, 54, 58, 79, 83–86]. Therefore, substantial doses of uridine (10–12 g/m^2) [83] are required to elevate and maintain the uridine concentration at the necessary levels (about 75 μM) to safely increase the chemotherapeutic doses of FUra [56]. Unfortunately, such large doses of uridine have been found to only increase the plasma concentration to 50 μM, which is inadequate to increase the chemotherapeutic doses of FUra. This approach is also limited by toxic side effects including phlebitis, pyrogenic reactions, and diarrhea [16, 28, 67, 70, 83–86]. Prolonged intravenous infusions of uridine as an alternative to oral administration are also limited by high fever, cellulitis, thrombocytopenia, and superior vena cava syndrome [83, 84]. Most of these side effects are not induced by uridine per se, but rather by the accumulation of uridine catabolites [66, 67]. Therefore, it seems unlikely that combining FUra with uridine alone would improve the therapeutic index of FUra to a significant extent. Prodrugs of uridine (e.g. 2′,3′,4′-triacetyluridine or PN401) were synthesized to overcome the short half-life of uridine [3, 6]. However, such prodrugs also suffer from rapid degradation [3, 6, 44]. Hence, high doses must still be administered [3, 5, 6, 36, 44, 75] making the use of uridine prodrugs as single agents impractical in the clinic.

The limited clinical utility of regimens incorporating acute high doses of uridine (or uridine prodrugs), combined with the inadequacy of this approach for clinical use as an adjunct to long-term chemotherapy, has led to the proposition that administration of specific inhibitors of uridine catabolism could improve the oral bioavailability and plasma concentration of the administered uridine, as well as overcoming the inherent toxic side effects of uridine catabolites. Uridine is present at constant concentrations (1–5 μM) in the plasma of various species providing a source for nucleotide synthesis by the salvage pathways. However, the half-life of uridine in plasma is only 2 min. More than 90% of plasma uridine entering the liver by the portal vein is degraded in a single pass while constant amounts of uridine from de novo biosynthesis are released into the hepatic vein [30, 58, 61]. Less than 2% of the uridine metabolized by the liver is salvaged and recovered in the uracil nucleotide pool in tissues of whole animals [17, 37, 38, 61], perfused rat liver [30, 58], and isolated liver cells [37]. The remainder is rapidly catabolized by enzymes of the pyrimidine catabolic pathway [39, 59, 80].

Activity of hepatic uridine phosphorylase (UrdPase, EC 2.4.2.3) is the first step in the catabolism of plasma uridine delivered to the liver [21, 59]. Indeed, hepatic UrdPase activity exhibits a circadian rhythm which is the inverse of that of plasma uridine concentration [25, 64]. Furthermore, several studies by our group as well as others [1–4, 6, 18–23, 56, 59, 68, 71, 80] have shown that inhibition of UrdPase also inhibits the catabolism of uridine and subsequently causes a profound increase in the uridine concentration in plasma and different tissues. Inhibition of uridine catabolism also prevents the toxic side effects associated with high doses of uridine that result from the accumulation of uridine catabolites [68]. This modulation of uridine metabolism by UrdPase inhibitors has been used successfully to attain the effect of high doses of exogenous uridine without the clinical complications associated with the administration of high doses of uridine. However, the effectiveness and bioavailability of the currently available UrdPase inhibitors are limited by metabolism and inadequate pharmacokinetic properties [4, 6, 20, 23, 71, 80]. For example, the efficacy of 5-(benzyl)acyclouridine (BAU) and 5-(phenylselenenyl)acyclouridine (PSAU) in increasing plasma uridine is restricted only to lower doses of the inhibitor and does not reach the levels (about 75 μM) required to potentiate FUra activity [4, 23, 71, 80]. Furthermore, the bioavailability of BAU was severely limited by metabolism [20, 23, 71]. Additionally, the pharmacokinetics of PSAU are altered by coadministration of uridine, indicating that uridine may affect the elimination of PSAU [4, 6].

In order to overcome the limitations of these UrdPase inhibitors (e.g. BAU, PSAU, etc.), 5-(phenylthio)acy-

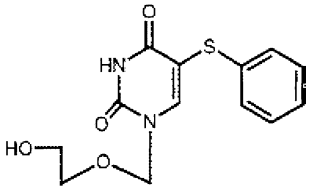

Fig. 1 Chemical structure of 5-(phenylthio)acyclouridine (PTAU)

clouridine (PTAU, Fig. 1) was designed. PTAU is a lipophilic inhibitor of UrdPase [26] and as such has better access to the liver and intestine, the main organs involved in uridine catabolism [30, 37, 39, 58, 59, 61]. We have previously demonstrated that PTAU is neither toxic nor metabolized in mice, has 100% oral bioavailability, and is an enhancer of plasma uridine concentration [1, 2]. In the present study we extended our previous investigations [1, 2] to explore the effects of a wider range of oral PTAU doses on the bioavailability and plasma pharmacokinetics of different doses of oral uridine in mice. Mice have been used successfully to investigate the in vivo effect of uridine and UrdPase inhibitors on the chemotherapy of various tumors including human tumors [1–6, 18, 23, 28, 45–47, 55, 56, 71]. Hence, the mouse model was used in our investigations.

Materials and methods

Chemicals

Heparinized Natelson pipettes, ammonium acetate, acetonitrile (HPLC grade), trichloroacetic acid (TCA), Gelman Acrodisc LC 13 PVDF 0.2 μm filters and ethyl ether (anesthetic grade) were obtained from Fisher Scientific (Pittsburgh, Pa.). Uridine, uracil, tri-*O*-n-octylamine, Freon (1,1,2-trichloro-trifluoroethane), hydroxypropylmethylcellulose (HPMC) and other chemicals were purchased from Sigma Chemical Company (St. Louis, Mo.). PTAU was synthesized as previously described [26].

Animals

Female CD-1 mice (18–20 g) were obtained from Charles River Laboratories (Wilmington, Ma.) and housed five per cage with water and food ad libitum under a normal light cycle (light, 0600–1800 hours; dark, 1800–0600 hours) according to the guidelines established by the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Administration of drugs

Uridine and/or PTAU were mixed well with HPMC powder in hot water (80°C) and homogenized thoroughly using a Polytron homogenizer (Brinkmann Instruments, Westbury, N.Y.). The final concentration of HPMC was 0.75%. The drug solution was vortexed well before and periodically during dosing. HPMC was preferred over the commonly used methylcellulose because the latter must be cooled to (10°C) in order to hydrate it completely [1–6]. Drugs were administered (0.1 ml/10 g) using 18G intubation needles (Popper and Sons, New Hyde Park, N.Y.). Control mice received the carrier solution (0.75% HPMC). To avoid a possible circadian variation in UrdPase and dihydrouracil dehydrogenase (EC 1.3.1.2) activities [25, 64], all mice were injected at the same time (between 8:30 a.m. and 9:00 a.m.).

Determination of the toxicity of PTAU

Mice (five mice per group) were treated with 30, 60, 120, 240 and 480 mg/kg per day, administered orally for five consecutive days. Survival and body weight were monitored for 28 days to evaluate toxicity.

Effect of PTAU on the pharmacokinetics of oral uridine

Doses

Uridine was administered orally at 330, 660, or 1320 mg/kg alone or in combinations with PTAU at 30, 45, 60, 120 or 240 mg/kg.

Collection of samples

At various times (5, 10, 15 and 30 min, 1, 2, 3, 4, 6 and 8 h) after drug administration, 250 μl whole blood was collected from the orbital sinuses from each of five mice (lightly anesthetized with ethyl ether) into heparinized Natelson pipettes and placed on ice. The whole blood was then centrifuged (Fisher microcentrifuge model 235 A) at 12,400 rpm for 5 min, and the plasma recovered and immediately stored in a freezer at −20°C until analysis by high-performance liquid chromatography (HPLC).

Preparation of samples

Plasma was allowed to thaw on ice then was deproteinized with two volumes of 15% TCA. After centrifugation (16,000 *g*, 4°C) for 5 min, using a Fisher microcentrifuge, the supernatant acid-soluble material was neutralized by extraction with a 1:2 mixture of tri-*n*-octylamine in Freon. The neutralized supernatant was filtered through Gelman Acrodisc LC 13 PVDF 0.2 μm filter, prior to HPLC analysis [1–4, 6].

HPLC analysis

Samples were analyzed by HPLC using a computer-controlled Hewlett-Packard model 1050 liquid chroma-

tography 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, Colo.) connected in tandem. The mobile phase was composed of two buffers: buffer A was 50 m*M* ammonium acetate, 0.5% acetonitrile (pH 4.8), and buffer B was 50 m *M* ammonium acetate, 75% acetonitrile (pH 4.8) [1–4, 6]. Typically, 100 μl of treated plasma sample was analyzed with a multistep elution protocol. A 23-min isocratic elution in buffer A was followed by a 15-min linear gradient to 75% buffer B, then a 27-min isocratic elution in 75% buffer B was 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 38–55 min) [1]. The effluent was monitored by UV absorption at 243, 259.5, 262 and 254 nm. Under these conditions, uracil, uridine and PTAU eluted at 12, 26 and 48 min, respectively [1]. No metabolites of PTAU were detected in the plasma.

Uracil, uridine and PTAU were identified by UV absorption at their λ_{max} (259.5, 262 and 243 nm, respectively)/254 nm, and coelution with authentic standards. The recoveries of uracil and uridine were more than 98% using [6-^{14}C]uracil and [2-^{14}C]uridine. The areas under the curve for uracil, uridine and PTAU in the samples were calculated by the on-line computer. Standard curves for uracil, uridine or PTAU, prepared in double-distilled water, were obtained before and after each set of samples was analyzed, and were used to determine the concentrations of uracil, uridine or PTAU in the samples. Plots of area under the curve vs uracil, uridine or PTAU concentrations were linear between 1 and 3000 μ*M*.

Pharmacokinetic analysis of plasma uridine, uracil and PTAU

Plasma pharmacokinetic parameters of uridine, uracil and PTAU were estimated using a model-independent approach. The area under the plasma concentration-time curve (AUC) and the area under the first moment-time curve (AUMC) of up to 8 h were estimated according to the trapezoidal rule. Mean residence time (MRT) was calculated as AUMC/AUC. Plasma half-life ($t_{1/2}$) was defined as 0.693/k, where k, the slope of the terminal linear phase of the plasma concentration-time curve on a semilogarithmic scale, was generated by a linear regression analysis of the terminal phase data. The apparent total plasma clearance (CL) was estimated as dose/AUC. The apparent total volume of distribution (V_d) was calculated as CL/k. Both CL and V_d were weight-normalized. In addition, uridine and PTAU were only administered by the oral route and therefore the parameters termed "clearance" and "volume of distribution" represent the ratios of these parameters to its unknown absolute bioavailability. Other pharmacokinetic parameters included peak plasma concentration (C_{max}) and time to C_{max} (T_{max}), and were obtained directly from the plasma data. C_0 was the plasma baseline concentration of endogenous uridine and uracil observed at time 0 (8:30–9:00 a.m.).

Results

Toxicity of PTAU

Table 1 shows that oral administration of PTAU up to 480 mg/kg per day for five consecutive days did not cause any mortality, and did not significantly affect the mean body weight of mice at 4 weeks post-treatment. These results demonstrate the safety of PTAU administration at least at the doses tested.

Pharmacokinetics of PTAU

We have previously shown that PTAU is not metabolized and has 100% oral bioavailability in mice [1]. Table 2 and Fig. 2 show the pharmacokinetic parameters of various doses of PTAU and the effect of coadministration of various doses of uridine. Upon oral administration at 30, 45, 60, 120 and 240 mg/kg, PTAU exhibited a prolonged plasma half-life of 2–3 h resulting in peak plasma PTAU concentrations (C_{max}) of 41, 51, 74, 126 and 161 μ*M* with AUCs of 70, 99, 122, 173 and 225 μmol h/l, respectively. The coadministration of uridine with PTAU did not have a significant effect on the pharmacokinetic parameters of plasma PTAU at any of the concentrations tested.

Effects of uridine alone and in combination with PTAU on plasma uridine concentration

The normal baseline concentrations (C_0) of plasma uridine and uracil at 8:30–9.00 a.m. in CD-1 mice were relatively constant, averaging 1.8 ± 0.2 and 6.9 ± 0.6 μ*M*, respectively. In previous studies, we investigated the bioavailability and pharmacokinetics of a wide range of oral doses of uridine (330–1320 mg/kg) [1–4, 6]. Therefore, when we studied the effects of PTAU as a modulator of plasma uridine concentration, we used the same

Table 1 Effect of oral administration of various doses of PTAU on body weight and survival of CD-1 mice. Values are means ± SD from at least five mice

Dose (mg/kg/day) ×5 days	Body weight (g)		Mortality
	Day 1	Day 28	
0	19.3 ± 0.5	23.6 ± 0.7	0
30	19.1 ± 0.5	22.5 ± 0.8	0
60	19.6 ± 0.5	22.6 ± 0.5	0
120	19.3 ± 0.7	22.8 ± 0.7	0
240	22.4 ± 1.2	24.3 ± 0.7	0
480	22.2 ± 1.1	20.6 ± 1.3	0

Table 2 Pharmacokinetic parameters of plasma PTAU in CD-1 mice after the administration of different doses of oral PTAU and the effect of coadministration of different doses of oral uridine. Values are means ± SD from at least five mice at each time point

PTAU (mg/kg)	Uridine (mg/kg)	C_{max} (μ*M*)	T_{max} (h)	AUC (μmol h/l)	V_d (l/kg)	MRT (h)	CL (l/h/kg)	$t_{1/2}$ (h)
30	0	40.1 ± 1.6	0.08 ± 0.00	87.4 ± 0.1	3.3 ± 4.1	2.3 ± 0.1	1.2 ± 0.1	2.1 ± 0.4
	330	34.7 ± 6.0	0.10 ± 0.04	55.2 ± 0.0	7.9 ± 1.5	2.3 ± 0.0	2.2 ± 0.1	2.4 ± 0.3
	660	45.3 ± 7.1	0.13 ± 0.05	48.4 ± 0.1	3.9 ± 1.4	1.7 ± 0.1	2.6 ± 0.4	1.0 ± 1.1
	1320	42.1 ± 5.5	0.12 ± 0.08	87.6 ± 0.1	5.6 ± 2.4	2.5 ± 0.1	1.4 ± 0.1	2.7 ± 1.0
Mean ± SD		40.5 ± 4.5	0.11 ± 0.02	69.7 ± 20.8	5.2 ± 2.0	2.2 ± 0.4	1.9 ± 0.7	2.0 ± 0.7
45	0	51.7 ± 2.1	0.08 ± 0.00	127.8 ± 0.1	2.5 ± 0.2	2.4 ± 0.1	1.2 ± 0.0	1.5 ± 0.0
	330	50.8 ± 2.0	0.08 ± 0.00	88.9 ± 0.2	7.1 ± 1.8	2.1 ± 0.2	2.1 ± 0.2	2.4 ± 0.6
	660	54.0 ± 2.3	0.08 ± 0.00	101.3 ± 0.3	3.8 ± 1.4	2.1 ± 0.3	1.8 ± 0.2	1.4 ± 0.4
	1320	48.2 ± 8.9	0.08 ± 0.00	77.1 ± 0.1	10.2 ± 2.4	2.4 ± 0.1	2.4 ± 0.1	3.0 ± 0.7
Mean ± SD		51.2 ± 2.4	0.08 ± 0.00	98.8 ± 21.7	5.9 ± 3.4	2.2 ± 0.2	1.9 ± 0.5	2.1 ± 0.8
60	0	68.1 ± 3.0	0.08 ± 0.00	170.9 ± 0.0	2.9 ± 0.3	2.4 ± 0.0	1.2 ± 0.0	1.7 ± 0.2
	330	71.7 ± 10.3	0.10 ± 0.04	100.2 ± 0.2	3.9 ± 1.0	2.0 ± 0.2	2.1 ± 0.2	1.4 ± 0.2
	660	85.3 ± 5.2	0.08 ± 0.00	105.6 ± 0.1	4.9 ± 0.5	1.8 ± 0.1	2.3 ± 0.2	1.4 ± 0.0
	1320	71.5 ± 7.2	0.08 ± 0.00	110.2 ± 0.2	5.8 ± 1.5	2.6 ± 0.2	2.2 ± 0.1	1.8 ± 0.4
Mean ± SD		74.1 ± 7.6	0.09 ± 0.01	121.7 ± 33.0	4.4 ± 1.3	2.2 ± 0.4	1.9 ± 0.5	1.6 ± 0.2
120	0	123.3 ± 22.3	0.10 ± 0.04	192.6 ± 13.2	5.7 ± 0.4	3.9 ± 0.3	1.5 ± 0.2	2.6 ± 0.3
	330	120.9 ± 5.4	0.08 ± 0.00	182.2 ± 7.3	5.3 ± 0.5	2.8 ± 0.4	1.9 ± 0.1	1.7 ± 0.4
	660	125.1 ± 11.8	0.08 ± 0.00	141.5 ± 7.6	5.7 ± 1.0	2.2 ± 0.4	2.6 ± 0.1	1.5 ± 0.5
	1320	133.8 ± 11.5	0.08 ± 0.00	175.5 ± 12.2	6.1 ± 1.8	3.7 ± 1.7	1.7 ± 0.2	2.6 ± 1.5
Mean ± SD		125.8 ± 12.7	0.09 ± 0.01	173.0 ± 10.1	5.7 ± 0.9	3.2 ± 0.7	1.9 ± 0.2	2.1 ± 0.7
240	0	145.2 ± 11.6	0.10 ± 0.04	234.4 ± 23.7	8.3 ± 1.4	3.2 ± 1.1	2.7 ± 0.5	2.1 ± 0.9
	330	171.6 ± 12.7	0.10 ± 0.04	210.8 ± 12.6	8.0 ± 0.9	2.5 ± 0.3	3.2 ± 0.2	1.6 ± 0.4
	660	171.9 ± 9.3	0.08 ± 0.00	228.7 ± 9.6	8.4 ± 1.1	3.2 ± 0.9	2.7 ± 0.4	2.5 ± 0.8
	1320	154.0 ± 9.4	0.08 ± 0.00	224.0 ± 6.8	9.7 ± 1.7	3.4 ± 0.8	2.9 ± 0.3	2.5 ± 0.8
Mean ± SD		160.7 ± 7.8	0.09 ± 0.01	224.5 ± 7.2	8.6 ± 0.9	2.9 ± 0.2	3.1 ± 0.5	2.2 ± 0.5

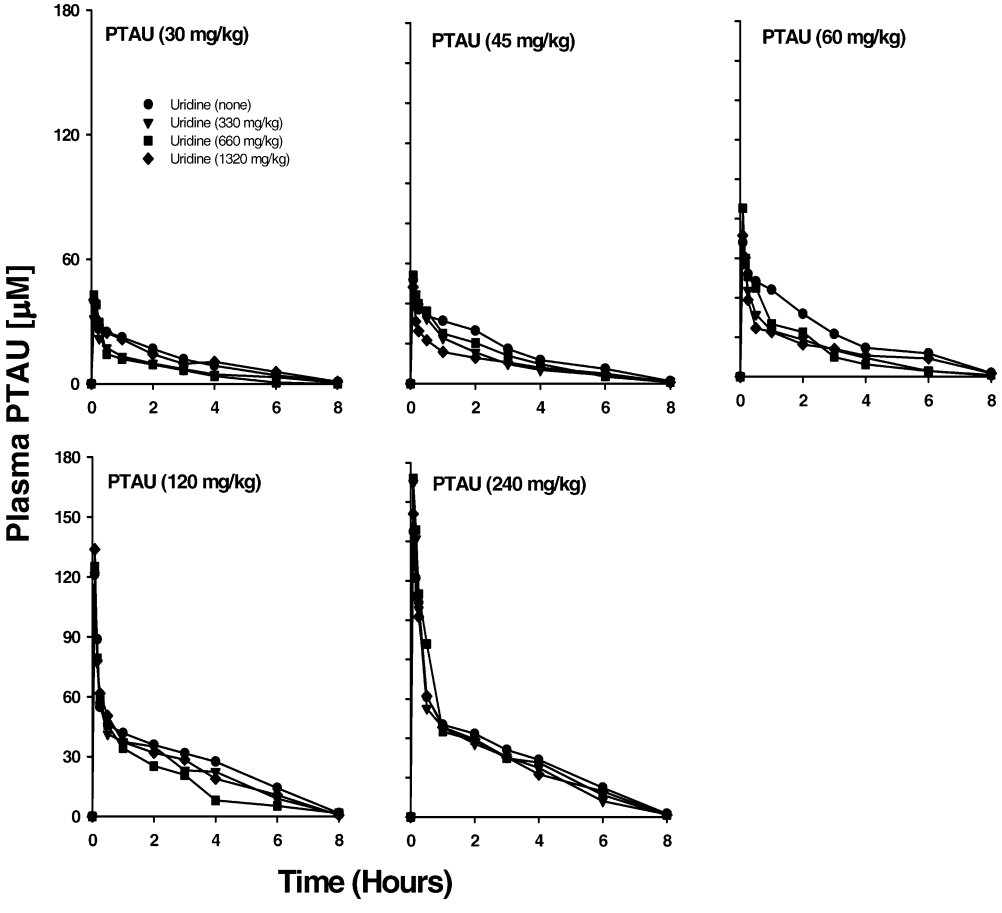

Fig. 2 Plasma concentration-time curves of PTAU in CD-1 mice after oral administration of PTAU and the effect of coadministration of different doses of oral uridine. Each point represents the mean concentration from five mice

Table 3 Effect of administration of different doses of oral PTAU alone or with uridine on the pharmacokinetics of plasma uridine and uracil in CD-1 mice. Values are means ± SD from at least five mice at each time point

(mg/kg)	PTAU (mg/kg)	C_{max} (μ*M*)	Fold change (C_{max}/C_0)	T_{max} (h)	AUC (μmol h/l)	V_d (l/kg)	MRT (h)	CL (l/h/kg)	$t_{1/2}$ (h)
Uridine									
0	30[a]	8.7 ± 0.3	5.7 ± 0.8	2.00 ± 0.00	34.6 ± 0.8				
	45[a]	11.4 ± 0.7	6.5 ± 1.4	2.00 ± 0.00	41.5 ± 1.4				
	60[a]	14.8 ± 1.1	10.4 ± 2.5	2.00 ± 0.00	49.5 ± 2.5				
	120	17.0 ± 1.8	6.0 ± 1.7	1.80 ± 0.50	67.7 ± 4.1				
	240	18.8 ± 2.5	9.5 ± 4.2	2.30 ± 0.50	82.7 ± 4.4				
330	0	11.2 ± 1.5	6.1 ± 2.1	1.19 ± 0.63	50.3 ± 2.6	159 ± 103	2.8 ± 0.2	22.0 ± 2.5	4.3 ± 2.1
	30	29.1 ± 3.9	18.0 ± 7.0	1.50 ± 0.58	87.1 ± 1.8	64.4 ± 21.9	2.5 ± 0.2	18.7 ± 1.5	2.4 ± 0.6
	45	40.6 ± 3.7	22.0 ± 3.9	1.00 ± 0.00	122 ± 11.5	30.8 ± 2.3	2.4 ± 0.1	12.7 ± 1.0	1.7 ± 0.3
	60	46.4 ± 4.6	29.5 ± 9.8	1.25 ± 0.50	132 ± 7.6	27.6 ± 5.1	2.2 ± 0.0	11.4 ± 0.7	1.7 ± 0.2
	120	69.9 ± 5.9	31.5 ± 8.3	1.30 ± 0.50	261 ± 26.8	13.8 ± 0.8	2.7 ± 0.2	5.1 ± 0.5	3.2 ± 0.9
	240	75.7 ± 15.2	33.8 ± 10.7	1.50 ± 0.60	217 ± 33.6	16.8 ± 3.2	2.9 ± 0.4	5.8 ± 0.7	1.5 ± 0.3
660	0	13.5 ± 1.6	6.3 ± 3.6	1.14 ± 0.71	52.9 ± 5.5	163 ± 87.5	2.9 ± 0.2	39.7 ± 11.7	2.5 ± 0.9
	30	42.6 ± 4.8	22.9 ± 5.5	1.00 ± 0.00	121 ± 8.9	64.8 ± 14.1	2.4 ± 0.1	25.8 ± 2.1	1.7 ± 0.4
	45	54.2 ± 4.8	32.6 ± 8.9	1.00 ± 0.00	143 ± 13.7	42.1 ± 5.6	2.3 ± 0.1	21.2 ± 2.1	1.4 ± 0.1
	60	55.2 ± 5.4	34.6 ± 16.1	1.00 ± 0.00	173 ± 14.9	38.0 ± 11.3	2.4 ± 0.1	17.2 ± 1.9	1.5 ± 0.4
	120	88.0 ± 7.8	47.6 ± 2.3	1.00 ± 0.00	241 ± 10.7	30.8 ± 0.8	2.7 ± 0.1	11.0 ± 0.4	1.5 ± 1.1
	240	97.5 ± 6.7	41.4 ± 6.6	1.00 ± 0.00	355 ± 4.6	20.2 ± 1.4	2.7 ± 0.2	7.5 ± 0.1	1.3 ± 0.1
1320	0	15.6 ± 1.0	7.8 ± 2.8	0.74 ± 0.28	69.7 ± 3.3	177 ± 79.0	2.9 ± 0.2	52.0 ± 6.0	2.6 ± 0.9
	30	105 ± 6.2	54.6 ± 15.6	1.13 ± 0.63	302 ± 19.8	40.3 ± 12.8	2.2 ± 0.1	19.0 ± 1.5	1.5 ± 0.4
	45	132 ± 8.2	70.6 ± 17.7	2.00 ± 0.00	415 ± 5.7	22.0 ± 1.7	2.4 ± 0.1	13.6 ± 1.0	1.1 ± 0.1
	60	169 ± 20.9	82.6 ± 27.5	3.00 ± 0.00	691 ± 61.7	23.1 ± 3.2	3.3 ± 0.1	8.1 ± 0.7	2.1 ± 0.2
	120	199 ± 5.2	147 ± 84.5	3.00 ± 0.00	817 ± 81.9	24.7 ± 2.8	4.2 ± 0.1	5.9 ± 0.6	2.2 ± 0.0
	240	209 ± 12.6	81.7 ± 15.2	3.00 ± 0.00	869 ± 75.3	22.8 ± 1.2	4.0 ± 0.3	5.7 ± 0.6	1.8 ± 0.3
Uracil									
0	30	11.1 ± 0.3	1.5 ± 0.3	2.00 ± 0.00	71.1 ± 0.9				
	45	10.6 ± 0.2	1.5 ± 0.4	1.75 ± 0.50	68.7 ± 1.9				
	60	10.9 ± 0.2	1.5 ± 0.3	1.75 ± 0.50	71.7 ± 1.3				
	120	14.2 ± 0.8	2.0 ± 0.7	1.60 ± 0.80	74.0 ± 5.2				
	240	15.3 ± 1.6	2.2 ± 0.4	0.90 ± 0.30	81.0 ± 6.8				
330	0	59.2 ± 3.8	9.0 ± 3.1	1.25 ± 0.29	287 ± 6.4				
	30	35.1 ± 4.4	5.9 ± 1.3	1.50 ± 0.58	145 ± 4.2				
	45	31.1 ± 2.1	4.6 ± 0.5	1.25 ± 0.50	124 ± 5.9				
	60	30.4 ± 2.0	5.2 ± 0.7	1.25 ± 0.50	112 ± 4.6				
	120	52.6 ± 4.3	8.2 ± 0.3	1.80 ± 0.50	201 ± 2.2				
	240	69.7 ± 4.4	7.6 ± 1.5	2.80 ± 0.50	294 ± 23.0				
660	0	133 ± 9.9	19.3 ± 5.0	1.78 ± 0.50	635 ± 49.5				
	30	46.6 ± 6.9	7.7 ± 1.1	2.25 ± 0.50	218 ± 9.1				
	45	59.4 ± 5.4	12.6 ± 2.7	2.75 ± 0.50	190 ± 13.9				
	60	44.6 ± 9.2	7.4 ± 1.8	3.00 ± 0.00	158 ± 10.3				
	120	60.1 ± 3.7	7.6 ± 1.8	2.80 ± 0.50	271 ± 9.1				
	240	71.1 ± 9.2	10.2 ± 2.3	2.00 ± 0.00	272 ± 21.6				
1320	0	275 ± 27.2	41.4 ± 8.4	3.00 ± 0.01	1228 ± 102				
	30	74.1 ± 9.8	12.1 ± 2.3	1.00 ± 0.00	215 ± 8.3				
	45	73.4 ± 2.51	7.3 ± 4.3	1.00 ± 0.00	180 ± 11.3				
	60	60.8 ± 3.3	9.3 ± 1.4	0.88 ± 0.25	149 ± 3.6				
	120	62.3 ± 2.7	8.7 ± 0.1	1.00 ± 0.00	195 ± 11.7				
	240	59.8 ± 17.9	8.6 ± 1.8	0.90 ± 0.30	178 ± 23.10				

[a]Data from reference [2]

doses of uridine. The bioavailability of these oral doses of uridine was 7.7% [2–4]. The data in Table 3 demonstrate that oral administration of uridine at 330, 660 and 1320 mg/kg resulted in concentrations of plasma uridine that were approximately 6.1-, 6.3- and 7.8-fold higher, respectively, over the endogenous level (1.8 ± 0.2 μ*M*). Coadministration of PTAU with uridine further increased the C_{max} and AUC of plasma uridine. This increase was dose-dependent (Figs. 3 and 4). Coadministration of 30, 45, 60, 120 and 240 mg/kg oral PTAU with 330 mg/kg uridine increased the AUCs of plasma uridine (50.3 μmol h/l) resulting from the administration of 330 mg/kg uridine alone by 1.7-, 2.4-, 2.6-, 5.2- and 4.3-fold, respectively, while decreasing the CL (22.0 l/h/kg) by 1.2-, 1.7-, 1.9-, 4.3- and 3.8-fold, respectively (Table 3, Fig. 3). Similar results were obtained by increasing the PTAU dose coadministered with 660 mg/kg oral uridine. Coadministration of PTAU at 30, 45, 60, 120 and 240 mg/kg with 660 mg/kg uridine increased the C_{max} (13.5 μ*M*) by 3.2-, 4.0-, 4.1-, 6.5- and 7.2-fold and the AUC (52.9 μmol h/l) by 2.3-, 2.7-, 3.3-, 4.6- and 6.7-fold, respectively (Table 3, Fig. 3) over those achieved by 660 mg/kg uridine alone. Coadministration of 30, 45, 60, 120 and 240 mg/kg PTAU

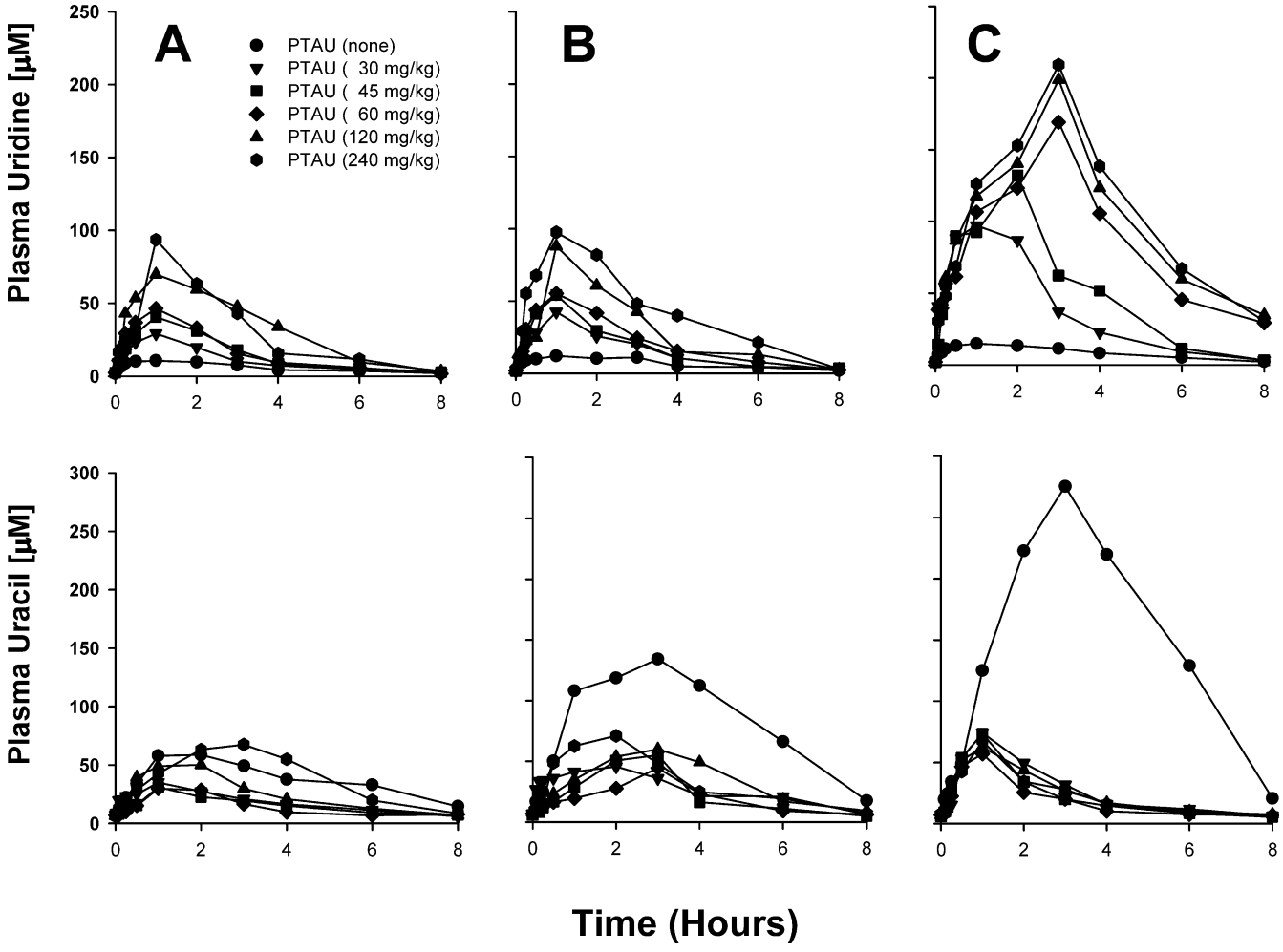

Fig. 3 Plasma concentration versus time curves of uridine and uracil in CD-1 mice after oral administration of different doses of uridine (**a** 330 mg/kg, **b** 660 mg/kg, **c** 1320 mg/kg) and the effect of coadministration of different doses of PTAU. Each point represents the mean concentration from five mice

decreased CL (39.7 l/h/kg) of 660 mg/kg uridine alone by 1.5-, 1.9-, 2.3-, 3.6- and 5.3-fold, respectively (Table 3). When the low dose of PTAU (30 mg/kg) was coadministered with 1320 mg/kg uridine, the C_{max} of plasma uridine reached 105 μ*M*, 1.0 h after coadministration and remained higher than control until 8 h. Coadministration of 30 mg/kg PTAU also increased the AUC of plasma uridine (69.7 μmol h/l) resulting from the administration of 1320 mg/kg uridine alone by 4.3-fold, while decreasing the CL (52 l/h/kg) by 2.7-fold (Fig. 3, Table 3). Increasing the coadministered dose of PTAU to 45, 60, 120 and 240 mg/kg further improved the pharmacokinetic parameters of plasma uridine. The plasma uridine C_{max} reached 132, 169, 199 and 209 μ*M* while the AUC increased by 6.0-, 9.9-, 11.7- and 12.5-fold, respectively. The CL further decreased by 3.8-, 6.4-, 8.8-, and 9.1-fold, respectively (Table 3).

Plasma uracil pharmacokinetic parameters were accordingly affected by coadministration of PTAU. Coadministration of PTAU with uridine decreased plasma uracil C_{max} and AUC (Fig. 3, Table 3). For example, coadministration of PTAU at 30, 45, 60, 120 and 240 mg/kg with uridine at 1320 mg/kg decreased plasma uracil C_{max} from 275 to 74, 73, 61, 62 and 60 μ*M*, respectively, with a reduction in the AUC from 1228 to 215, 180, 149, 195 and 178 μmol h/l, respectively (Table 3).

Figure 4 depicts the combined effects of coadministration of 30, 45, 60, 120 and 240 mg/kg oral PTAU with different doses of uridine on the C_{max} and AUC of plasma uridine and uracil. PTAU increased the C_{max} and AUC of uridine while decreasing those of uracil in an inverse manner. More importantly, it should be noted that at each dose of PTAU, there was an incremental elevation in plasma uridine concentration by increasing the dose of uridine, and no plateau was reached. However, the maximum effect appeared to be reached at the PTAU dose of 120 mg/kg.

Discussion

Our present (Tables 1–3, Figs. 2–4) and previous [1, 2, 26] studies demonstrate that oral PTAU is a potent

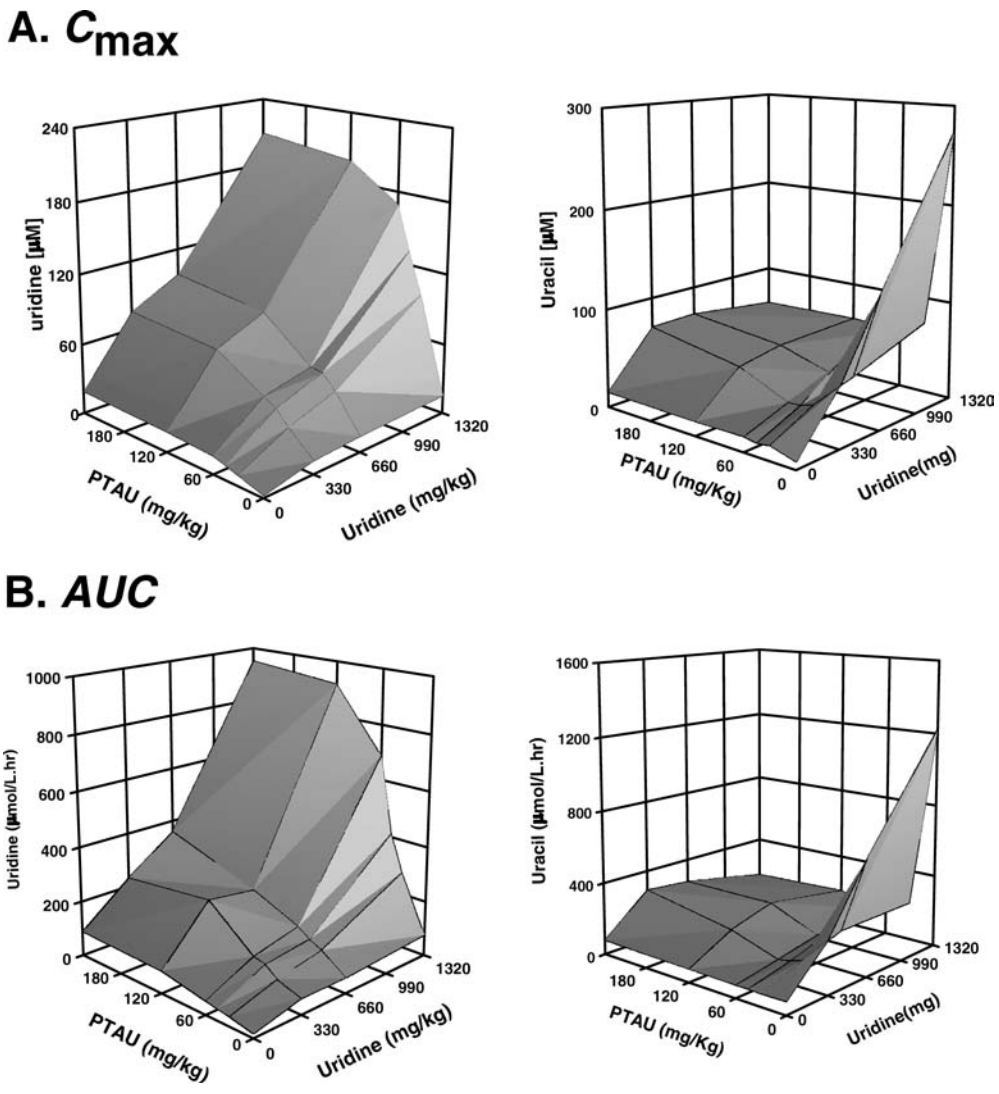

Fig. 4 The effect of coadministration of different doses of PTAU with different doses of uridine on the (**a**) C_{max} and (**b**) AUC of plasma uridine and uracil in CD-1 mice. Each point represents the mean value from five mice

inhibitor of UrdPase [26] and has excellent characteristics as an enhancer of the bioavailability of oral uridine. These characteristics include: lack of toxicity (Table 1), exceptionally high oral bioavailability [1], prolonged plasma half-life of 2–3 h (Table 2), and lack of effect of uridine on PTAU pharmacokinetics (Table 2). The effectiveness of PTAU as a powerful and efficient inhibitor of UrdPase in vivo is evidenced by the fact that coadministration of PTAU with uridine increased the relative oral bioavailability of uridine (7.7%) in a dose-dependent fashion to as much as 145-fold, and increased the concentration of plasma uridine by over 100-fold from 1.8 µ*M* to 200 µ*M* (Table 3), which is far above the concentration of plasma uridine (75 µ*M*) required to increase the doses of FUra. Furthermore, administration of PTAU, even at the lowest dose (30 mg/kg), allowed uridine exposure (AUC and C_{max}) to increase with increasing doses of administered uridine, and no plateau was reached (Table 3, Figs. 3 and 4). The effect of PTAU on uridine exposure was the same across all doses of uridine tested and is likely to continue to increase as a function of its dose beyond the 1320 mg/kg dose of uridine.

The effectiveness of PTAU in inhibiting UrdPase in vivo is also indicated by the increase in the AUC, C_{max} and fold change (C_{max}/C_0) of plasma uridine, with the concurrent decrease in the AUC and C_{max} of plasma uracil (Tables 3, Figs. 3 and 4), when compared to the values observed with uridine alone. This observation is not unexpected since administered uridine is subject to the activities of intestinal UrdPase and reflects the inhibition of uridine catabolism by UrdPase in the intestine. In this regard, it should be noted that UrdPase activity in the intestine is the highest of all studied organs of the body. In mice, intestinal UrdPase activity (47.3 ± 1.5 nmol/min/mg protein) is 146-fold higher than that of the liver [3]. Such high activity of uridine catabolism in the intestine and liver is considered among the principal reasons for the rapid disappearance of uridine from plasma following oral administration [1–4, 30, 56, 61, 68, 80]. The effectiveness of PTAU as an enhancer of the oral bioavailability of uridine can also

be ascribed to the lack of PTAU metabolism as well as its lipophilicity [1, 2] which probably enhances its absorption from the gastrointestinal tract and reabsorption from the renal tubules. Therefore, a large proportion of administered PTAU can be transported or diffused into the plasma unchanged acting as a depot and providing PTAU over a long period of time.

The overall favorable properties of PTAU as an enhancer of the bioavailability of oral uridine makes it a more promising and convenient modulator of plasma uridine than its other two known analogues, BAU and PSAU. First, kinetic studies have demonstrated that PTAU is at least a threefold more potent inhibitor of human liver UrdPase than BAU [26]. Secondly, PTAU has 100% oral bioavailability denoting its efficient absorption and the absence of metabolism in the liver or intestine [1]. This contrasts sharply with BAU which is extensively metabolized and, hence, has approximately 60% oral bioavailability [20, 23, 71]. Thirdly, the effect of PTAU on plasma uridine concentration is incrementally elevated by increasing the dose of administered uridine, and no plateau was reached. Such a phenomenon has not been observed with either BAU or PSAU [4, 71, 80]. In this context, the differences between PTAU and BAU may be attributed to the differences in their potencies in inhibiting UrdPase [26] and/or the lower bioavailability and extensive metabolism of BAU [20, 23, 71].

The efficient absorption and high bioavailability of PTAU would allow better inhibition of the unusually high activity of intestinal UrdPase [3, 56] leading to increased uridine availability. However, the results with PSAU [4] are rather curious, particularly when considering that PSAU like PTAU has a 100% oral bioavailability and is as efficient in inhibiting UrdPase [1, 4]. This may be explained by the fact that coadministration of uridine increases the elimination of high doses of PSAU from the small intestine [4, 6] while it does not alter the pharmacokinetics of PTAU (Table 2, Fig. 2). This differential effect of uridine on the pharmacokinetics of PTAU and PSAU would explain the superiority of PTAU over PSAU as a modulator of uridine concentrations in vivo. Moreover, in spite of the observed chemical stability of PSAU in vivo [4, 6], the use of PTAU rather than PSAU would overcome concerns about potential toxicities from the possible release of selenium from PSAU.

In conclusion, the present (Table 3, Figs. 3 and 4) and previous [1, 2] investigations demonstrate that combining PTAU with uridine for oral administration is quite an effective regimen in inhibiting the catabolism of oral uridine and elevating uridine concentrations to levels that would be more than adequate to increase the doses of FUra for better antitumor efficacy. Furthermore, the high potency, excellent bioavailability (100%), lack of toxicity, lack of uridine effect on the pharmacokinetics of PTAU, and the extraordinary ability to elevate uridine levels, render PTAU a very promising and more convenient modulator of plasma uridine and may translate better to the clinical setting for the therapy of cancer with FUra than the previously known UrdPase inhibitors or the toxic massive doses of uridine.

Finally, it should be stressed that the use of uridine as an adjunct in therapy is also not limited to the treatment of cancer. Uridine, has been used successfully as a ''protective'' and/or ''rescuing'' agent against host toxicity and mitochondrial dysfunction caused by various anti-HIV drugs without interfering with their chemotherapeutic efficacy [42, 48, 78, 79, 87, 88]. Uridine has also been shown to protect from the toxicity of different antiinflammatory and immunosuppressive agents used in the treatment of various autoimmune diseases and transplant rejection [15, 32, 65, 82, 89]. It also potentiates the antipsychotic action of traditional neuroleptics [62, 63]. Furthermore, uridine has been used as a therapeutic agent for its effects in sleep promotion [40] and on muscle performance [50, 51], and in the treatment of several other medical disorders including: CNS disorders (e.g. cerebrovascular disorders, convulsions, Huntington's disease, bipolar disorder) [10, 11, 24, 31, 34, 35, 41, 49, 60, 66, 72–75, 77], liver diseases [13, 27, 81], diabetic neuropathy [29], cardiac damage [7–9, 12, 52, 53, 57] and hereditary orotic aciduria [43].

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