Cancer Chemother Pharmacol (2001) 48: 389–397
DOI 10.1007/s002800100353

ORIGINAL ARTICLE

**Omar N. Al Safarjalani · Xiao-Jian Zhou
Fardos N.M. Naguib · Junxing Shi
Raymond F. Schinazi · Mahmoud H. el Kouni**

# Enhancement of the bioavailability of oral uridine by coadministration of 5-(phenylthio)acyclouridine, a uridine phosphorylase inhibitor: implications for uridine rescue regimens in chemotherapy

Received: 26 January 2001 / Accepted: 1 June 2001 / Published online: 23 August 2001
© Springer-Verlag 2001

**Abstract** *Purpose*: The purpose of this investigation was to evaluate the effectiveness of oral 5-(phenylthio)acyclouridine (PTAU) in improving the oral bioavailability of uridine. PTAU is a new 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 not toxic to mice and is fully absorbed after oral administration (100% oral bioavailability). *Methods*: PTAU was administered orally to mice alone or with uridine. The plasma levels of PTAU as well as those of uridine and its catabolite uracil were measured using HPLC, and pharmacokinetic analysis was performed. *Results*: Coadministration of PTAU with uridine elevated the concentration of plasma uridine in a dose-dependent manner over that resulting from the administration of the same dose of uridine alone, and reduced the clearance of uridine as well as the peak plasma concentration ($C_{max}$) and area under the curve (AUC) of plasma uracil. Coadministration of PTAU at 30, 45 and 60 mg/kg improved the low oral bioavailability (7.7%) of uridine administered at 1320 mg/kg by 4.3-, 5.9- and 9.9-fold, respectively, and reduced the AUC of plasma uracil (1227.8 μmol·h/l) by 5.7-, 6.8- and 8.2-fold, respectively. Similar results were observed when PTAU was coadministered with lower doses of uridine. Oral PTAU at 30, 45 and 60 mg/kg improved the oral bioavailability of 330 mg/kg uridine by 1.8-, 2.6- and 2.8-fold, and that of 660 mg/kg uridine by 2.2-, 2.6- and 3.2-fold, respectively. *Conclusion*: The effectiveness of PTAU in improving the oral bioavailability of uridine could be useful in the rescue or protection from host toxicities of various chemotherapeutic pyrimidine analogues as well as in the management of medical disorders that are remedied by administration of uridine.

**Keywords** 5-(Phenylthio)acyclouridine · Uridine · Phosphorylase inhibitor · Chemotherapy

**Abbreviations** *AUC:* area under the curve · *AUMC:* area under the first moment curve · *BAU:* 5-(benzyl)acyclouridine · *Cl:* apparent total plasma clearance · $C_{max}$: peak plasma concentration · $C_0$: plasma concentration at time zero · *HPLC:* high performance liquid chromatography · *HPMC:* hydroxypropylmethylcellulose · *MRT:* mean residence time · *PSAU:* 5-(phenylselenenyl)acyclouridine · *PTAU:* 5-(phenylthio)acyclouridine · $t_{1/2}$: elimination half-life · $T_{max}$: time to peak plasma concentration · *UrdPase:* uridine phosphorylase (EC 2.4.2.3) · $V_d$: apparent total volume of distribution

O.N. Al Safarjalani · X.-J. Zhou · F.N.M. Naguib
M.H. el Kouni (✉)
Department of Pharmacology and Toxicology,
Comprehensive Cancer Center, Center for AIDS Research,
University of Alabama at Birmingham,
Birmingham, AL 35294, USA
E-mail: m.elkouni@ccc.uab.edu
Tel.: +1-205-9341132
Fax: +1-205-9348240

J. Shi
Pharmasset, Inc., Tucker, GA 30084, USA

R.F. Schinazi
Veterans Affairs Medical Center (Atlanta),
Decatur, GA 30033, USA

R.F. Schinazi
Laboratory of Biochemical Pharmacology,
Department of Pediatrics,
Emory University School of Medicine,
Atlanta, GA 30322, USA

## Introduction

Uridine has been used successfully to counteract host toxicity of various anticancer (e.g. 5-fluorouracil) [4, 39, 40, 46, 61, 66] and anti-HIV (e.g. 3′-azido-3′-deoxythymidine and 2′,3′-dideoxycytidine) [37, 68, 69] drugs with-out interfering with their chemotherapeutic

potency. It has also been shown that uridine protects from the toxicity of different anti-inflammatory and immunosuppressive agents used in the treatment of various autoimmune diseases and transplant rejection [14, 30, 56, 72, 77], and potentiates the antipsychotic action of traditional neuroleptics [53, 54]. Furthermore, uridine has been used as a therapeutic agent in the treatment of several other medical disorders including CNS disorders such as cerebrovascular disorders and convulsions [9, 10, 22, 29, 31, 32, 36, 51, 57, 63, 64, 65, 67], sleep promotion [35], muscle performance [42, 43], liver diseases [12, 25, 71], diabetic neuropathy [27], cardiac damage [6, 7, 8, 11, 44, 48] and hereditary orotic aciduria [38]. However, the use of oral uridine in patients is limited by its poor oral bioavailability (7–8%) and short plasma elimination half-life [2, 3, 13, 41, 45, 49, 70, 73, 74, 75, 76].

Therefore, substantial doses of uridine (10–12 g/m$^2$) [73] are required to attain and sustain the high plasma uridine concentrations (70 μ*M*) essential to achieve its therapeutic effect [47]. Unfortunately, such large doses of uridine have toxic side effects including phlebitis, pyrogenic reactions, and diarrhea [15, 26, 58, 59, 73, 74, 75]. Prolonged intravenous infusions of uridine as an alternative to the administration of oral uridine are also limited by high fever, cellulitis, and superior vena cava syndrome [73, 74]. Most of these side effects are not induced by uridine per se but by the accumulation of uridine catabolites [59, 60]. Therefore, coadministration of inhibitors of uridine catabolism could improve the oral bioavailability and plasma concentration of administered uridine as well as prevent the toxic side effects resulting from uridine catabolism.

Uridine is maintained in rigorous homeostasis of 1–5 μ*M* in the plasma of different species. 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 [28, 49, 52]. 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 [19, 50]. Indeed, hepatic UrdPase activity exhibits a circadian rhythm which is the inverse of that of plasma uridine concentration [23, 55]. Furthermore, several studies have shown that inhibition of UrdPase also inhibits the catabolism of uridine and subsequently causes a profound increase in uridine in plasma and different tissues [1, 2, 3, 5, 16, 17, 18, 19, 20, 21, 47, 50, 60, 62, 70]. Inhibition of uridine catabolism also prevents the toxic side effects associated with high doses of uridine that result from the accumulation of uridine catabolites [60].

This modulation of uridine metabolism by UrdPase inhibitors has been successfully used to attain the effect of high doses of exogenous uridine without the associated clinical complications. However, the effectiveness and bioavailability of the currently available UrdPase inhibitors are limited by metabolism and inadequate pharmacokinetic properties [3, 5, 18, 21, 62, 70]. 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 70 μ*M*) required to potentiate FUra activity [3, 21, 62, 70]. Furthermore, the bioavailability of BAU is severely limited by metabolism [18, 21]. Additionally, the pharmacokinetics of PSAU are altered by coadministration of uridine, indicating that uridine may affect the elimination of PSAU [3, 5].

In attempt to overcome such limitations, we recently synthesized and tested 5-(phenylthio)acyclouridine (PTAU), as a potent, specific inhibitor of UrdPase [24]. PTAU was designed as a lipophilic inhibitor of UrdPase and as such its access to the liver and intestine, the main organs involved in uridine catabolism [28, 33, 34, 49, 50, 52], would be facilitated. PTAU is not toxic in mice or metabolized, has 100% oral bioavailability, and is an enhancer of endogenous plasma uridine concentration [1]. In the present study we investigated the effect of oral PTAU on the bioavailability and plasma pharmacokinetics 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, 2, 3, 4, 5, 16, 17, 18, 19, 20, 21, 26, 39, 40, 41, 46, 47, 60]. Hence, the mouse model was used in our investigations.

## Materials and methods

### Chemicals

Heparinized Natelson pipettes, ammonium acetate, acetonitrile (HPLC grade), trichloroacetic acid, Gelman Acrodisc LC 13 PVDF 0.2-μm filters and ethyl ether (anesthetic grade) were obtained from Fisher Scientific (Pittsburgh, Pa.). Uridine, uracil, tri-*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 [24].

### Animals

Female CD-1 mice (18–20 g) were obtained from Charles River Laboratories (Wilmington, Mass.) 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, 2, 3, 4, 5]. 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 [23, 55], all mice were injected at the same time (between 0830 and 0900 hours).

### Effect of PTAU on the pharmacokinetics of oral uridine

#### *Doses*

PTAU was administered orally at 30, 45 and 60 mg/kg and uridine at 330, 660 and 1320 mg/kg alone or in combination.

#### *Collection of samples*

At various time intervals (5, 10, 15, 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) in 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 –20ºC until analysis by HPLC.

#### *Preparation of samples*

Plasma was allowed to thaw on ice and then was deproteinized with two volumes of 15% trichloroacetic acid. 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 (1,1,2-trichloro-trifluoroethane). The neutralized supernatant was filtered through Gelman Acrodisc LC 13 PVDF 0.2-µm filter, prior to HPLC analysis [1, 2, 3].

#### *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, Colo.) connected in tandem. The mobile phase was composed of two buffers: buffer A was 50 m*M* ammonium acetate and 0.5% acetonitrile (pH 4.8), and buffer B was 50 m*M* ammonium acetate and 75% acetonitrile (pH 4.8) [1, 2, 3, 5]. Typically, 100-µl aliquots of treated plasma samples were analyzed with a multi-step 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 254 and 268 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 and uridine were identified by UV absorption at their $\lambda_{max}$ (259.5 nm and 262 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 and uridine in the samples were calculated by the online computer. Standard curves for uracil, uridine and 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 and PTAU in the samples. Plots of areas under the curve vs uracil, uridine and 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 normalized to the weights of the animals. 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 their 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 zero-time (0830 to 0900 hours).

## Results

The normal baseline concentrations ($C_0$) of plasma uridine and uracil at 0830 to 0900 hours in CD-1 mice were relatively constant averaging $1.8 \pm 0.2$ and $6.9 \pm 0.6$ µ*M*, respectively. In previous studies we investigated the bioavailability and pharmacokinetics of a wide range of oral doses of uridine (330 to 1320 mg/kg) [1, 2, 3, 5]. Therefore, when we studied the effects of PTAU as a modulator of plasma uridine concentration, we used the same doses of uridine. The bioavailability of these oral doses of uridine was 7.7% [2, 3].

The data in Table 1 show that oral administration of uridine at 330, 660, 1320 mg/kg increased the concentration of endogenous plasma uridine ($1.8 \pm 0.2$ µ*M*) by approximately 7.4-, 8.1-, and 9.2-fold, respectively. Coadministration of PTAU with uridine further increased the $C_{max}$ and AUC of plasma uridine. This increase was dose-dependent (Figs. 1 and 2).

Coadministration of 30, 45 and 60 mg/kg oral PTAU with 330 mg/kg uridine increased the AUC of plasma uridine (47.2 µmol·h/l) resulting from the administration of 330 mg/kg uridine alone by 1.8-, 2.6- and 2.8-fold, respectively, while decreasing the Cl (38.9 l/h per kg) by 2.1-, 3.1- and 3.4-fold, respectively (Table 1 and Fig. 1). Similar results were obtained by increasing the PTAU dose coadministered with 660 mg/kg oral uridine. Coadministration of PTAU at 30, 45 and 60 mg/kg with 660 mg/kg uridine increased the $C_{max}$ (12.9 µ*M*) by 3.3-, 4.2-, 4.3-fold and the AUC (54.9 µmol·h/l) by 2.2-, 2.6- and 3.2-fold, respectively, (Table 1 and Fig. 1) over those achieved by uridine alone. Coadministration of 30, 45 and 60 mg/kg PTAU decreased Cl (74.3 l/h per kg) of 660 mg/kg uridine by 2.9-, 3.5- and 4.3-fold, respectively (Table 1). 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 (69.8 μmol·h/l) resulting from the administration of 1320 mg/kg uridine alone by 4.3-fold, while decreasing the Cl (99 l/h per kg) by 5.2-fold (Fig. 1 and Table 1). Increasing the coadministered dose of PTAU to 45 and 60 mg/kg further improved the pharmacokinetic parameters of plasma uridine. The plasma uridine $C_{max}$ reached 132 and 169 μ*M* while the AUC increased by 5.6- and 9.9-fold, respectively. The Cl further decreased by 7.3- and 12.2-fold, respectively (Table 1).

Plasma uracil pharmacokinetic parameters were also affected by coadministration of PTAU. Coadministration PTAU with uridine decreased plasma uracil $C_{max}$ and AUC (Fig. 1 and Table 1). For example, coadministration of PTAU at 30, 45 and 60 mg/kg with uridine (1320 mg/kg) decreased plasma uracil $C_{max}$ from 275 to 74, 73 and 61 μ*M*, respectively, with a corresponding reduction in the AUC from 1228 to 215, 180 and 149 μmol·h/l, respectively (Table 1). Figure 2 shows the combined effects of coadministration of 30, 45 and 60 mg/kg oral PTAU with different doses of uridine on the $C_{max}$ and AUC of plasma uridine and uracil.

We have previously shown that PTAU is not metabolized and has 100% oral bioavailability in mice [1]. Table 2 and Fig. 3 show the effect of coadministration of different doses of uridine on the pharmacokinetic parameters of various doses of PTAU. Coadministration of uridine with PTAU did not have a significant effect on the pharmacokinetic parameters of plasma PTAU at any of the concentrations tested.

## Discussion

The present results demonstrate that PTAU is an efficient inhibitor of UrdPase in vivo as evidenced by the fact that coadministration of PTAU with uridine increased in the AUC, $C_{max}$ and $C_{max}/C_0$ of plasma uridine while decreasing those of plasma uracil (Table 1 and Fig 2) when compared to the values observed with uridine alone. This effect of PTAU resulted in an enhancement of the oral bioavailability of uridine (7.7%) by as much as 4-fold.

Our present (Tables 1 and 2, Figs. 1, 2, and 3) and previous [1, 24] studies demonstrate that, as an inhibi-

**Table 1** Effect of administration of different doses of 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 ($C_{max}$ peak plasma concentration, $C_0$ zero time plasma concentration, $T_{max}$ time to peak plasma concentration, *AUC* area under the curve, *Cl* total plasma clearance, $t_{1/2}$ elimination half-life, $V_d$ apparent total volume of distribution, *MRT* mean residence time)

| Uridine (mg/kg) | PTAU | $C_{max}$ (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[a] | 30 | 8.7 ± 0.3 | 5.7 ± 0.8 | 2.00 ± 0.00 | 34.6 ± 0.8 | | | | |
| | 45 | 11.4 ± 0.7 | 6.5 ± 1.4 | 2.00 ± 0.00 | 41.5 ± 1.4 | | | | |
| | 60 | 14.8 ± 1.1 | 10.4 ± 2.5 | 2.00 ± 0.00 | 49.5 ± 2.5 | | | | |
| 330 | 0 | 10.7 ± 0.6 | 7.4 ± 1.9 | 0.88 ± 0.25 | 47.2 ± 1.6 | 305 ± 206 | 2.9 ± 0.1 | 38.9 ± 4.5 | 5.3 ± 3.3 |
| | 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 |
| 660 | 0 | 12.9 ± 2.1 | 8.1 ± 4.7 | 1.38 ± 1.11 | 54.9 ± 4.7 | 313 ± 174.1 | 3.1 ± 0.1 | 74.3 ± 22.8 | 2.7 ± 0.8 |
| | 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 |
| 1320 | 0 | 15.3 ± 0.9 | 9.2 ± 4.1 | 0.88 ± 0.25 | 69.8 ± 5.2 | 341 ± 157 | 3.1 ± 0.2 | 98.9 ± 11.5 | 2.3 ± 0.8 |
| | 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 ± 25.7 | 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 |
| | | | | | Uracil | | | | |
| 330 | 0 | 59.5 ± 2.8 | 9.5 ± 1.2 | 1.50 ± 0.58 | 307 ± 9.7 | | | | |
| | 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 | 113 ± 4.6 | | | | |
| 660 | 0 | 136 ± 9.9 | 21.1 ± 1.5 | 1.25 ± 0.50 | 679 ± 37.5 | | | | |
| | 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 | | | | |
| 1320 | 0 | 275 ± 27.2 | 41.4 ± 8.4 | 3.00 ± 0.00 | 1228 ± 102 | | | | |
| | 30 | 74.1 ± 9.8 | 12.1 ± 2.3 | 1.00 ± 0.00 | 215 ± 8.3 | | | | |
| | 45 | 73.4 ± 2.5 | 17.3 ± 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 | | | | |

[a]Data from reference 1

tor of UrdPase, PTAU has more favorable overall characteristics than its other two known analogues, BAU and PSAU. First, kinetic studies have demonstrated that PTAU inhibits human liver UrdPase at least three times more potently than BAU [24]. Second, PTAU has 100% oral bioavailability denoting efficient absorption and is not metabolized in the liver or the intestine [1]. This contrasts sharply with BAU which is extensively metabolized and hence has a much lower oral bioavailability (40%) [18, 21, 62]. The efficient absorption of PTAU would allow better inhibition of the unusually high activity of intestinal UrdPase [2, 47] leading to increased uridine availability (Table 1, Figs. 1 and 2). Third, when coadministered with oral uridine, PTAU increased plasma uridine levels in a dose-dependent manner (Table 1, Figs. 1 and 2), unlike BAU and PSAU which at high doses fail to improve the relative bioavailability of administered uridine above that achieved with lower doses in mice [3], monkeys [70] and humans [62]. In this regard, the differences between PTAU and BAU may be attributed to the differences in their potencies in inhibiting UrdPase [24] and/or the lower bioavailability of BAU [18, 21, 62]. However, the results with PSAU [3] are rather curious, particularly when considering that PSAU like PTAU has a 100% oral bioavailability and is as efficient in inhibiting UrdPase [1, 3]. This may be explained by the fact coadministration of uridine increases the elimination of high doses of PSAU from the small intestine [3, 5]. On the other hand, coadministration of uridine does not alter the pharmacokinetics of PTAU (Table 2, Fig. 3). These results suggest that PTAU would be better than PSAU as a modulator of uridine concentrations in vivo. Moreover, in spite of the observed chemical stability of PSAU in vivo [3, 5], 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 investigation demonstrated that combining PTAU with uridine for oral administration, secured and sustained higher levels of plasma uridine than either alone (Table 1, Figs. 1 and 2). The high potency, excellent bioavailability (100%), lack of toxicity, and lack of an effect of uridine on the

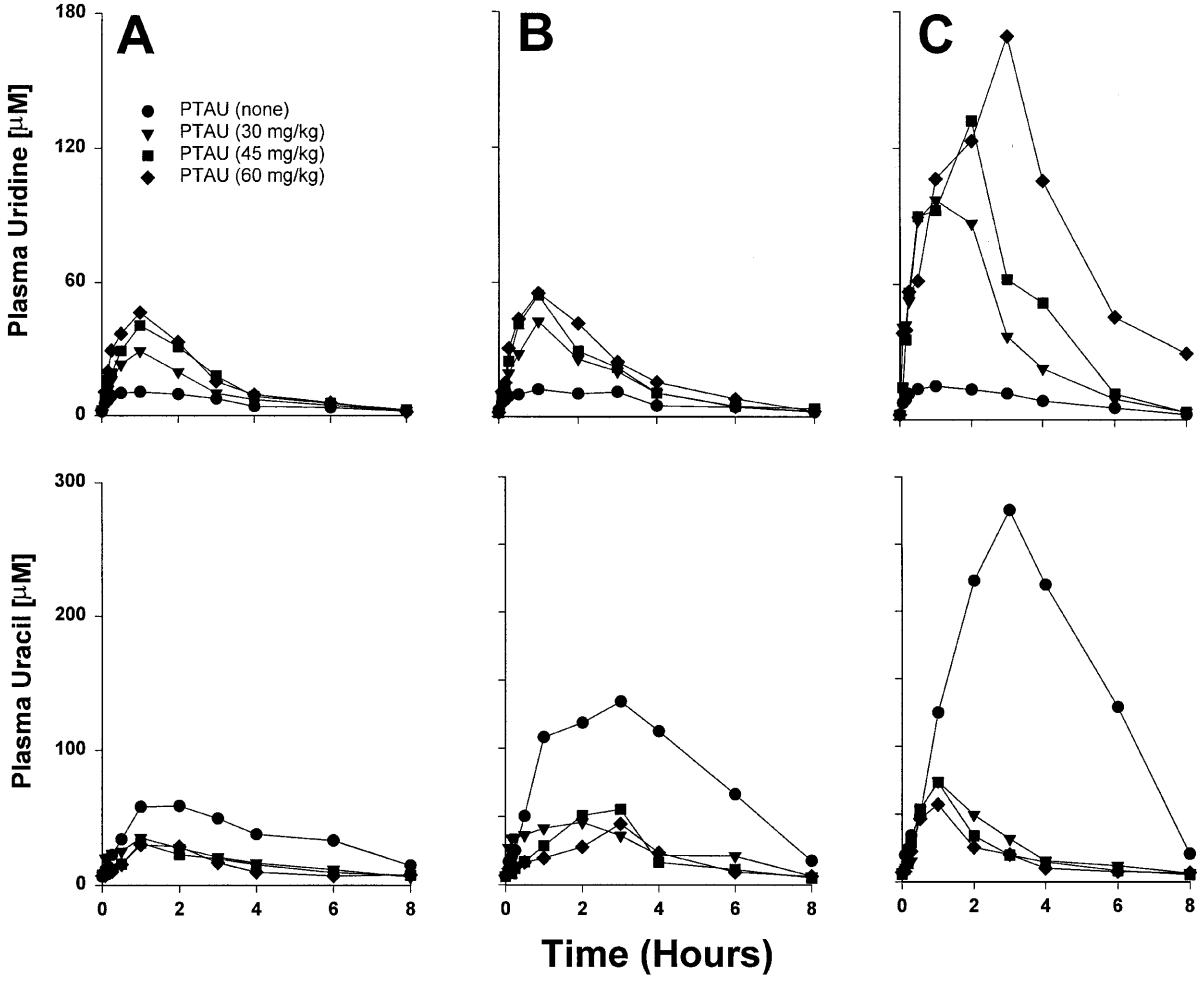


**Fig. 1** Plasma concentration versus time curves of uridine and uracil in CD-1 mice after oral administration of different doses of uridine (**A** 330 mg/kg, **B** 660 mg/kg, **C** 1320 mg/kg), and the effect of coadministration of different doses of PTAU. Each point represents the mean concentration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

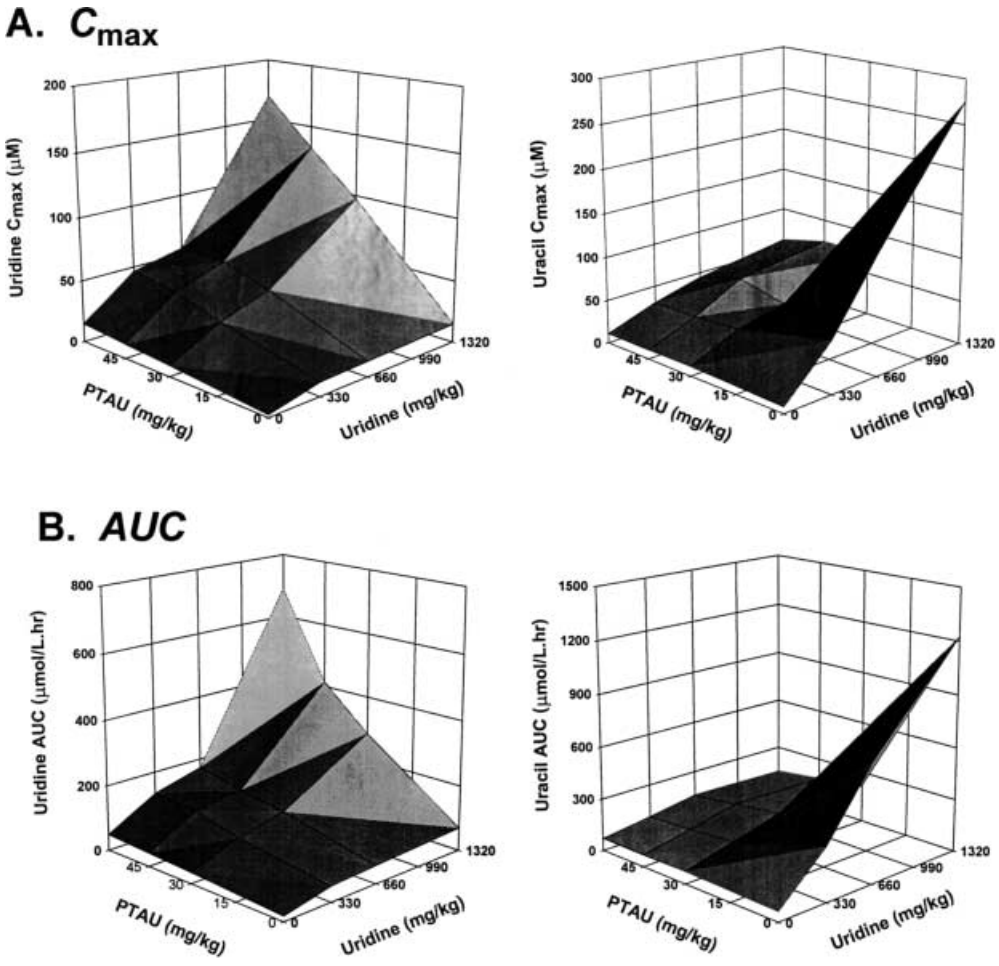


**Fig. 2** The effect of coadministration of different doses of oral PTAU with different doses of uridine on the (**A**) $C_{max}$ and (**B**) AUC of plasma uridine and uracil in CD-1 mice. Each point represents the mean value from five mice

**Table 2** Pharmacokinetic parameters of plasma PTAU in CD-1 mice and the effect of coadministration of different concentrations of uridine. Values are means ± SD from at least five mice at each time point ($C_{max}$ peak plasma concentration, $T_{max}$ time to peak plasma concentration, *AUC* area under the curve, *Cl* total plasma clearance, $t_{1/2}$ elimination half-life, $V_d$ apparent total volume of distribution, *MRT* mean residence time)

| 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 |

pharmacokinetics of PTAU, make PTAU a promising and more convenient modulator of plasma uridine than the previously known UrdPase inhibitors or the toxic massive doses of exogenous uridine. The favorable pharmacological properties of PTAU indicate its potential for the therapy of cancer and AIDS, as well as other pathological and physiological disorders which can be remedied by the administration of uridine.

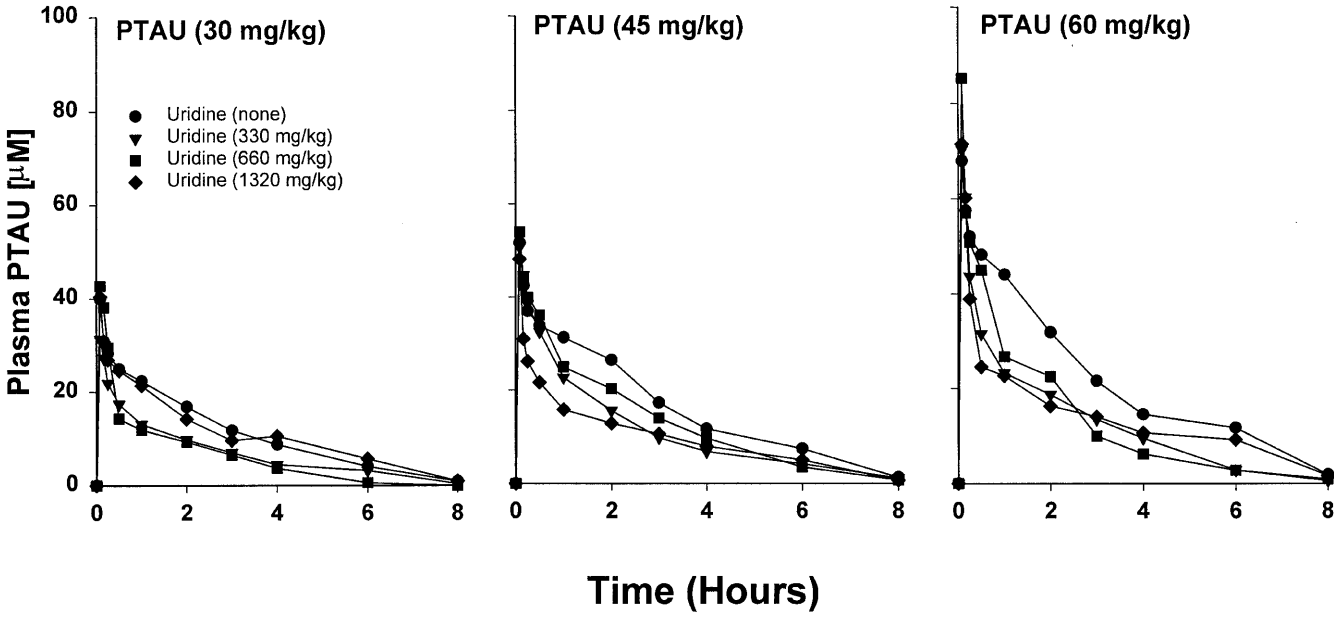


**Fig. 3** Plasma concentration-time curves of PTAU in CD-1 mice after oral administration of PTAU and the effect coadministration of different doses of oral uridine. Each point represents the mean concentration from five mice

## References


1. Al Safarjalani ON, Zhou X-J, Naguib FNM, Goudgaon NM, Schinazi RF, el Kouni MH (2001) Modulation of the pharmacokinetics of endogenous plasma uridine by 5-(phenylthio)acyclouridine a uridine phosphorylase inhibitor: implications for chemotherapy. Cancer Chemother Pharmacol 48:145
2. Ashour OM, Naguib FNM, el Kouni MH (1996) 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, Implication for chemotherapy. Biochem Pharmacol 51:1601
3. Ashour OM, Al Safarjalani ON, Naguib FNM, Goudgaon NM, Schinazi RF, el Kouni MH (2000) Modulation of plasma uridine concentration by 5-(phenylselenenyl)acyclouridine an inhibitor of uridine phosphorylase: relevance to chemotherapy. Cancer Chemother Pharmacol 45:351
4. Ashour OM, Naguib FNM, Panzica RP, Al Safarjalani ON, el Kouni MH (2000) Modulation of 5-fluorouracil host-toxicity by 5-(benzyloxybenzyl)barbituric acid acyclonucleoside, a uridine phosphorylase inhibitor, and 2′,3′,5′-tri-*O*-acetyluridine, a prodrug of uridine. Biochem Pharmacol 60:427
5. Ashour OM, Naguib FNM, Goudgaon NM, Schinazi RF, el Kouni MH (2000) Effect of 5-(phenylselenenyl)acyclouridine, an inhibitor of uridine phosphorylase, on plasma concentration of uridine released from 2′,3′,5′-tri-*O*-acetyluridine, a prodrug of uridine: relevance to uridine rescue in chemotherapy. Cancer Chemother Pharmacol 46:235
6. Aussedat J (1983) Effect of uridine supply on glycogen resynthesis after ischaemia in the isolated perfused rat heart. Cardiovasc Res 17:145
7. Aussedat J (1984) Uridine incorporation in normal and ischaemic perfused rat heart. Mol Physiol 6:247
8. Aussedat J, Verdetti J, Grably S, Rossi A (1982) Nucleotides uridyliques et glycogene cardiaque: effet de l'administration d'uridine et de ribose chez le rat. J Physiol Paris 78:331
9. Bonavita B, Zito M (1968) Analisi dell'azione di nucleosidi pirimidinici sull'elettroencefallogramma umano attivato con megimide. Riv Neurol 38:317
10. Bonavita V, Monaco P, Tripi E (1964) Analisi degli effetti centrali di nucleotidi purinici e pirinmidinici e loro derivati III. Esperimenti farmacologici. Acta Neurol (Napoli) 19:215
11. Buckley NM, Tsuboi KK, Zeic NJ (1959) Effects of nucleosides on acute left ventricular failure in isolated dog heart. Circ Res 7:847
12. Bushma MI, Parkhovchenko EI, Peschanskii VS (1980) Effects of cytidine and uridine on the regeneration of the liver in rats poisoned with carbon tetrachloride. Bull Exp Biol Med 88:722
13. Chan TCK, Markman M, Pfeifle CE, Taetle R, Abramson I, Howell SB (1988) Uridine pharmacokinetics in cancer patients. Cancer Chemother Pharmacol 22:83
14. Cherwinski HM, Byars N, Ballaron SJ, Nakano GM, Young JM, Ransom JT (1995) Leflunomide interferes with pyrimidine nucleotide biosynthesis. Inflamm Res 44:317
15. Cradock JC, Vishnuvajjala BR, Chin TF, Hochstein HD, Ackerman SK (1986) Uridine-induced hyperthermia in the rabbit. J Pharm Pharmacol 38:226
16. Darnowski JW, Handschumacher RE (1985) Tissue-specific enhancement of uridine utilization and 5-fluorouracil therapy in mice by benzylacyclouridine. Cancer Res 45:5364
17. Darnowski JW, Handschumacher RE (1986) Tissue uridine pools: evidence in vivo of a concentrative mechanism for uridine uptake. Cancer Res 46:3490
18. Darnowski JW, Handschumacher RE (1988) Benzylacyclouridine: pharmacokinetics, metabolism and biochemical effects in mice. Biochem Pharmacol 37:2613
19. Darnowski JW, Handschumacher RE (1989) Enhancement of fluorouracil therapy by the manipulation of tissue uridine pools. Pharmacol Ther 41:381
20. Darnowski JW, Handschumacher RE, Wiegand RA, Goulette FA, Calabresi P (1991) Tissue-specific expansion of uridine pools in mice. Effects of benzylacyclouridine, dipyridamole and exogenous uridine. Biochem Pharmacol 41:2031
21. Davis ST, Joyner SS, Chandrasurin P, Baccanari DP (1993) Species-dependent differences in the biochemical effects and metabolism of 5-benzylacyclouridine. Biochem Pharmacol 45:173
22. Dwivedi C, Harbison RD (1975) Anticonvulsant activities of Δ-8 and Δ-9 tetrahydrocannabinol and uridine. Toxicol Appl Pharmacol 31:452

23. el Kouni MH, Naguib FNM, Park KS, Cha S, Darnowski JW, Soong S-J (1990) Circadian rhythm of hepatic uridine phosphorylase activity and plasma concentration of uridine in mice. Biochem Pharmacol 40:2479
24. el Kouni MH, Goudgaon NM, Rafeeq M, Al Safarjalani ON, Schinazi RF, Naguib FNM (2000) 5-Phenylthioacyclouridine: a potent and specific inhibitor of uridine phosphorylase. Biochem Pharmacol 60:851
25. Elrick H, Hlad CJ Jr, Arai Y (1962) Influence of certain nucleosides on glucose metabolism in man. Metabolism 11:46
26. Falcone A, Darnowski JW, Ruprecht RM, Chu SH, Burnetti I, Calabresi P (1990) Differential effect of benzylacyclouridine on the toxic and therapeutic effects of azidothymidine in mice. Blood 76:2216
27. Gallai V, Mazzotta G, Montesi S, Sarchielli P, Del Gatto F (1992) Effects of uridine in the treatment of diabetic neuropathy: an electrophysiological study. Acta Neurol Scand 86:3
28. Gasser T, Moyer JD, Handschumacher RE (1981) Novel single-pass exchange of circulating uridine in rat liver. Science 213:777
29. Geiger A, Yamasaki S (1956) Cytidine and uridine requirement of the brain. J Neurochem 1:93
30. Greene S, Watanabe K, Braatz-Trulson J, Lou L (1995) Inhibition of dihydroorotate dehydrogenase by the immunosuppressive agent, leflunomide. Biochem Pharmacol 50:861
31. Guarneri P, Guarner R, Mocciaro C, Piccoli F (1983) Interaction of uridine with GABA binding sites in cerebellar membranes of the rat. Neurochem Res 8:1537
32. Guarneri P, Guarner R, Mocciaro C, Piccoli F (1985) Interaction of uridine with GABA-mediated inhibitory transmission: studies in vivo and in vitro. Epilepsia 26:666
33. Holstege A, Leser H-G, Pausch J, Gerok W (1985) Uridine catabolism in Kupffer cells, endothelial cells, and hepatocytes, Eur J Biochem 149:169
34. Holstege A, Pausch J, Gerok W (1986) Effect of 5-diazouracil on the catabolism of circulating pyrimidines in rat liver and kidneys. Cancer Res 46:5576
35. Inoué S (1993) Sleep-promoting substance (SPS) and physiological sleep regulation. Zool Sci 10:557
36. Jann G, Delzanno GB (1969) Azione della citidina ed uridina nel trattamento delle indromi neuropsichiche da encefalopatie di varia origine. Minerva Med 60:2092
37. Keilbauch SA, Hobbs GA, Simpson MV (1993) Anti-human immunodeficiency virus type 1 therapy and peripheral neuropathy: prevention of 2′,3′-dideoxycytidine toxicity in PC12 cells, a neuronal model by uridine and pyruvate. Mol Pharmacol 44:702
38. Kelly WN, Smith LH Jr (1978) Hereditary orotic aciduria. In: Stanbury JB, Wyngaarden JB, Fredrickson DS (eds) The metabolic basis of inherited disease, 4th edn. McGraw-Hill, New York, p 1045
39. Klubes P, Cerna I (1983) Use of uridine rescue to enhance the antitumor selectivity of 5-fluorouracil. Cancer Res 43:3182
40. Klubes P, Cerna I, Meldon MA (1982) Uridine rescue from the lethal toxicity of 5-fluorouracil in mice. Cancer Chemother Pharmacol 8:17
41. Klubes P, Geffen DB, Cysyk RL (1986) Comparison of the bioavailability of uridine in mice after either oral or parenteral administration. Cancer Chemother Pharmacol 17:236
42. Kypson J, Hait G (1976) Effects of uridine and inosine on glucose metabolism in skeletal muscle and activated lipolysis in adipose tissue. J Pharmacol Exp Ther 199:565
43. Kypson J, Hait G (1977). Metabolic effects of inosine and uridine in rabbit hearts and rat skeletal muscles. Biochem Pharmacol 26:1585
44. Kypson J, Hait G, Mathew R (1978) Effects of uridine on the performance and the metabolism of oxygenated and hypoxic rabbit hearts. J Mol Cell Cardiol 10:545
45. Leyva A, van Groeningen CJ, Kraal I, Peters GJ, Lankelma J, Pinedo HM (1984) Phase I and pharmacokinetic studies of high-dose uridine intended for rescue from 5-fluorouracil toxicity. Cancer Res 44:5928
46. Martin DS, Stolfi RL, Sawyer RC, Spiegelman S, Young CW (1982) High-dose 5-fluorouracil with delayed uridine "rescue" in mice. Cancer Res 42:3964
47. Martin DS, Stolfi RL, Sawyer RC (1989) Use of oral uridine as a substitute for parental uridine rescue of 5-fluorouracil therapy, with and without the uridine phosphorylase inhibitor 5-benzylacyclouridine. Cancer Chemother Pharmacol 24:9
48. Meerson FZ, Dosmagabetova RS (1985) The use of glucose and uridine to control contractility and extensibility disturbances in the nonischemized compartments of the heart in myocardial infarction. Kardiologiya 25:91
49. Monks A, Cysyk RL (1982) Uridine regulation by the isolated rat liver: perfusion with an artificial oxygen carrier. Am J Physiol 242:R465
50. Monks A, Ayers O, Cysyk RL (1983) Effect of 5-benzylacyclouridine, a potent inhibitor of uridine phosphorylase, on the metabolism of circulating uridine by the isolated rat liver. Biochem Pharmacol 32:2003
51. Monticone GF, Bergamasco B, Congnazzo A (1966) Sull'impiego terapeutico dei nucleosidi citidina ed uridina in alcune affezioni neurologiche. Minerva Med 57:4348
52. Moyer JD, Oliver JT, Handschumacher RE (1981) Salvage of circulating pyrimidine nucleosides in the rat. Cancer Res 41:3010
53. Myers CS, Napolitano M, Fisher H, Wagner GC (1993) Uridine and stimulant-induced motor activity. Proc Soc Exp Biol Med 204:49
54. Myers CS, Fisher H, Wagner GC (1994) Uridine potentiates haloperidol's disruption of conditioned avoidance responding. Brain Res 651:194
55. Naguib FNM, Soong S-j, el Kouni MH (1993) Circadian rhythm of orotate phosphoribosyltransferase, pyrimidine nucleoside phosphorylases and dihydrouracil dehydrogenase in mouse liver: possible relevance to chemotherapy with 5-fluoropyrimidines. Biochem Pharmacol 45:667
56. Nair RV, Cao W, Morris RE (1995) Inhibition of smooth muscle cell proliferation in vitro by leflunomide, a new immunosuppressant, is antagonized by uridine. Immunol Lett 48:77
57. Page T, Yu A, Fontanesi J, Nyhan WL (1997) Developmental disorder associated with increased cellular nucleotidase activity. Proc Natl Acad Sci U S A 94:11601
58. Peters GJ, van Groeningen CJ (1991) Clinical relevance of biochemical modulation of 5-fluorouracil. Ann Oncol 2:469
59. Peters GJ, van Groeningen CJ, Laurensse EJ, Lankelma J, Kraal I, Leyva A, Lankelma J, Pinedo HM (1987) Effect of pyrimidine nucleosides on body temperatures of man and rabbit in relation to pharmacokinetic data. Pharm Res 4:113
60. Peters GJ, van Groeningen CJ, Laurensse E, Levya A, Pinedo HM (1987) Uridine-induced hypothermia in mice and rats in relation to plasma and tissue levels of uridine and its metabolites. Cancer Chemother Pharmacol 20:101
61. Peters GJ, van Dijk J, Laurensse E, van Groeningen CJ, Lankelman J, Levya A. Nadal JC, Pinedo HM (1988) In vitro biochemical and in vivo biological studies of the uridine "rescue" of 5-fluorouracil. Br J Cancer 57:259
62. Pizzorno G, Yee L, Burtness BA, Marsh JC, Darnowski JW, Chu MYW, Chu HS, Chu E, Leffert JJ, Handschumacher RE, Calabresi P (1998). Phase I clinical and pharmacological studies of benzylacyclouridine, a uridine phosphorylase inhibitor. Clin Cancer Res 4:1165
63. Roberts CA (1973) Anticonvulsant effects of uridine: comparative analysis of metrazol and penicillin induced foci. Brain Res 55:291
64. Roberts CA, Kreisman NR, Waltman M (1974) Uridine anticonvulsant effects: selective control of nucleoside incorporation in experimental epilepsy. Epilepsia 15:479

65. Sanguineti I, Zerbi D (1965) L'impiego di alcuni nucleosidi non vasoattivi (citidina e uridina) nella terapia delle vasculopatie cerebrali. Minerva Med 56:3352
66. Seiter K, Kemeny N, Martin D, Schneider A, Williams L, Colofiore J, Sawyer R (1993) Uridine allows dose escalation of 5-fluorouracil when given with *N*-phosphoancetyl-L-aspartate, methotrexate, and leucovorin. Cancer 71:1875
67. Sepe O (1970) Efficacia dell'assoiazione citidina-uridina nelle affezioni vascolari dell'encefalo. Minerva Med 61:5934
68. Sommadossi J-P, Carlisle R, Schinazi RF, Zhou Z (1988) Uridine reverses the toxicity of 3′-azido-3′-deoxythymidine in normal human granulocyte-macrophage progenitor cells in vitro without impairment of antiretroviral activity. Antimicrob Agents Chemother 32:997
69. Sommadossi J-P, Zhu Z, Carlisle R, Xie MY, Weidner DA, el Kouni MH (1990) Novel pharmacologic approaches for the treatment of AIDS and potential use of uridine phosphorylase inhibitors In: Diasio RB, Sommadossi J-P (eds) Advances in chemotherapy of AIDS. Pergamon Press, New York, p 63
70. Sommadossi J-P, Cretton EM, Kidd LB, McClure HM, Anderson DC, el Kouni MH (1995) Effects of 5-benzylacyclouridine, an inhibitor of uridine phosphorylase, on the pharmacokinetics of uridine in rhesus monkeys: Implications for chemotherapy. Cancer Chemother Pharmacol 37:14
71. Songu E, Haugaard ES, Wildley G, Haugaard N (1980) The relationship between uracil nucleotide concentrations and glycogen synthesis in hepatocytes from fed and fasted rats. Metabolism 30:119
72. Tamura K, Woo J, Bakri MT, Thomson AW (1993) Brequinar sodium inhibits interleukin-6-induced differentiation of a human B-cell line into IgM-secreting plasma cells. Immunology 79:587
73. van Groeningen CJ, Leyva A, Kraal I, Peters GJ, Pinedo HM (1986) Clinical and pharmacokinetic studies of prolonged administration of high-dose uridine intended for rescue from 5-FU toxicity. Cancer Treat Rep 70:745
74. van Groeningen CJ, Peters GJ, Leyva A, Laurensse E, Pinedo HM (1989) Reversal of 5-fluorouracil induced myelosuppression by prolonged administration of high-dose uridine. J Natl Cancer Inst 81:157
75. van Groeningen CJ, Peters GJ, Nadal JC, Leyva A, Laurensse E, Pinedo HM (1991) Clinical and pharmacologic study of orally administered uridine. J Natl Cancer Inst 83:437
76. van Groeningen CJ, Peters GJ, Pinedo HM (1992) Modulation of fluorouracil toxicity with uridine. Semin Oncol 19:148
77. Williamson RA, Yea CM, Robson PA, Curnock AP, Gadher S, Hambleton AB, Woodward K, Bruneau J-M, Hambleton P, Moss D, Thomson TA, Spinella-Jaegle S, Morand P, Courtin O, Sautés C, Westwood R, Hercend T, Kuo EA, Ruuth E (1995) Dihydroorotate dehydrogenase is a high affinity binding protein for A77 1726 and mediator of a range of biological effects of the immunomodulatory compound. J Biol Chem 270:22467