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ORIGINAL ARTICLE

**Omar N. Al Safarjalani · Reem Rais · Junxing Shi
Raymond F. Schinazi · Fardos N. M. Naguib
Mahmoud H. el Kouni**

Modulation of 5-fluorouracil host-toxicity and chemotherapeutic efficacy against human colon tumors by 5-(Phenylthio)acyclouridine, a uridine phosphorylase inhibitor

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Abstract *Purpose*: The purpose of this investigation was to evaluate the effectiveness of oral 5-(phenylthio)acyclouridine (PTAU) in reducing 5-fluorouracil (FUra) host-toxicity and enhancing its chemotherapeutic efficacy against human colon tumors. PTAU is a potent and specific inhibitor of uridine phosphorylase (UrdPase, EC 2.4.2.3), the enzyme responsible for uridine catabolism. *Methods*: SCID mice bearing human colon DLD-1 or HCT-15 tumors were injected intraperitoneally with FUra (50, 200 or 300 mg/kg) on days 17, 24 and 31 after tumor cell inoculation. PTAU (120 mg/kg), uridine (1,320 mg/kg) or their combination was administered orally 2 or 4 h after FUra injection. Another four administrations of PTAU + uridine were given every 8 h after the first treatment with PTAU plus uridine. Survival and body weight were used to evaluate host toxicity. Tumor weight was used to evaluate the efficacy of the drugs on tumor growth. The mice were monitored for 38 days. *Results*: Administration of the maximum tolerated dose (50 mg/kg) of FUra reduced DLD-1 and HCT-15 tumor weights by 48 and 59%, respectively, at day 38 post implantation. Administration of 200 mg/kg FUra resulted in 100% mortality. Oral administration of uridine (1,320 mg/kg) alone, 2 h following the administration of 200 mg/kg FUra, did not alleviate FUra host-toxicity as all the mice died. Administration of 120 mg/kg PTAUresulted in partial rescue from this lethal dose of FUra as 63% of mice survived and tumor weights were reduced by approximately 60%. Coadministration of PTAU plus uridine resulted in complete rescue from the toxicity of FUra as 100% of the mice survived and tumor weights were reduced by 81–82%. Delaying the administration of the combination of PTAU plus uridine to 4 h post FUra treatment was less effective in rescuing from FUra toxicity as only 88% of the mice survived and tumor weights were reduced by only 62%. Administration of PTAU alone, under the same conditions, resulted in a 38% survival rate while the tumor weights were reduced by 47%. Treatment with uridine alone did not protect from FUra toxicity at the dose of 200 mg/kg as all mice died. At the higher dose of 300 mg/kg FUra, neither uridine nor PTAU alone, administered 2 h following the treatment with FUra, had any rescuing effect. On the other hand, the use of the PTAU plus uridine combination reduced the tumor weight by 79%, although this reduction in the tumor weight was accompanied by 37% mortality. There was no significant difference between DLD-1 and HCT-15 in their response to the different regimens employed in this study despite the fact that the tumors have different levels of UrdPase. *Conclusions*: The present results demonstrate that the combination of PTAU plus uridine represents an exceptionally efficient method in increasing FUra chemotherapeutic efficacy while minimizing its host-toxicity. The efficiency of the PTAU plus uridine combination can be attributed to the extraordinary effectiveness of this combinationin raising and maintaining higher levels of uridine in vivo (Al Safarjalani et al., Cancer Chemo Pharmacol 55:541–551, 2005). Therefore, the combination of PTAU plus uridine can

This paper is dedicated to the memory of Daniel S. Martin, a colleague and a dear friend. Daniel S. Martin pioneered the use of combination chemotherapy against solid tumors.

O. N. Al Safarjalani · R. Rais · 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 · R. F. Schinazi
Veterans Affairs Medical Center (Atlanta), Decatur,
GA 30033 and Laboratory of Biochemical Pharmacology,
Department of Pediatrics, Emory University School
of Medicine, Atlanta, GA 30322, USA

provide a better substitute for the large doses of uridine necessary to rescue or protect from FUra host-toxicities, without the toxic side-effects associated with such doses of uridine. This combination may also allow for the escalation of FUra doses for better chemotherapeutic efficacy against human colon carcinoma while avoiding FUra host-toxicities. Alternatively, the combination of PTAU and uridine may be useful as an antidote in the few cases when cancer patients receive a lethal overdose of FUra.

Keywords 5-(Phenylthio)acyclouridine · 5-Fluorouracil · Chemotherapy · Toxicity · Uridine phosphorylase inhibitor · Uridine

Abbreviations Fura: 5-Fluorouracil · HPMC: Hydroxypropylmethylcellulose · MTD: Maximum tolerated dose · PTAU: 5-(Phenylthio)acyclouridine · UrdPase: Uridine phosphorylase [EC 2.4.2.3]

Introduction

5-Fluorouracil (FUra) was developed in 1957. Nevertheless, it still remains among the few "standard" drugs effective against several human solid tumors including colorectal, breast, and head and neck cancers [1–3]. In fact, it is the second most utilized drug for the treatment of cancer. The longevity and endurance of FUra in cancer chemotherapy is nothing less than spectacular when considering the long history of extensive research to develop alternative or better drugs. FUra is also unique in that it is the only anti-cancer drug on the market that shows synergism with most other anti-cancer agents in combination therapy. In addition, the drug is effective against cancers refractory to other treatments. Over the past 40 years, an increased understanding of the mechanism of action of FUra has led to the development of strategies that improved its anticancer activity. Despite these advances, one of the major limitations of using FUra has been the inability to increase its dose, for better efficacy against the tumors due to host-toxicity [1–3]. Nonetheless, the chemotherapeutic merits of FUra have prompted an effort to develop biochemical modulators of FUra toxicity to enhance its therapeutic index rather than searching for alternatives to FUra. There is also interest in, and a need for, modulators which would protect the intestinal mucosa and allow the oral administration of FUra rather than the usual i.v. route used in the clinic. Biochemical modulation involves different formulations of FUra or combinations of FUra with another drug and/or a natural compound that may modify FUra toxicity.

The natural nucleoside uridine is one of the most promising biochemical modulators of FUra efficacy and host toxicity. It was demonstrated that the administration of uridine rescued and/or protected from FUra-host-toxicity without impairment of FUra antitumor activity [4–9]. In preclinical studies, it was shown that by using delayed uridine treatment, the LD_{50} of a single dose of FUra in mice can be made completely tolerable and that the maximum tolerated dose of FUra can be doubled or tripled [8]. Furthermore, animals treated with FUra plus uridine showed less depression of WBC and hematologic toxicity, as well as a faster recovery than animals receiving FUra alone [4–10]. The combination resulted in a superior antitumor effect and allowed for the increase of the FUra dose from 100 to 250–300 mg/kg [9]. In patients, infusion of uridine allowed the increase of FUra doses from 500 to 750 mg/m^2 [10] and alleviated bone marrow toxicity associated with FUra [11]. Similarly, delayed oral uridine administration to patients treated with high-dose 5-FU in combination with methotrexate and leucovorin, permitted a 33 and 45% increase in the MTD of FUra in the presence or absence, of doxorubicin, respectively. This treatment also rescued from FUra-induced hematologic toxicity without adverse impact on tumor response [12]. Nonetheless, the application of these regimens in patients is still limited [10, 11, 13–15]. To attain and sustain the high uridine concentration (75 μM) required to safely increase the chemotherapeutic doses of FUra, it is necessary to administer uridine in high doses (10–12 g/m^2) due to its rapid degradation and short half life. In addition, such high doses of uridine produced dose-limiting effects including phlebitis, pyrogenic reaction, diarrhea, cellulitis, and vena cava syndrome [13–19]. These side effects of high doses of uridine are not induced by uridine per se but by the accumulation of its catabolites [17, 18]. Therefore, alternative approaches to maintain uridine concentrations at the levels required to enhance the FUra chemotherapeutic effect must be sought.

Uridine is maintained in rigorous homeostasis at a concentration of 1–5 μM in the plasma of various species. However, the turnover of plasma uridine is rapid and efficient with a half life of approximately 2 min [20, 21]. This rapid and efficient turnover of plasma uridine results from the fact that more than 90% of the circulating uridine is catabolized in a single pass through the liver by the activity of hepatic uridine phosphorylase (UrdPase EC 2.4.2.3), while constant amounts of uridine are synthesized de novo and released into the hepatic vein blood [21–23]. Less than 2% of the uridine metabolized by the liver is salvaged and recovered in the uracil nucleotide pool in tissues of whole animals [23], in perfused rat liver [21, 22, 24], or isolated liver cells [25]. The remainder is rapidly catabolized to products beyond uracil in the pyrimidine catabolic pathway [25, 26].

One approach to maintain a high uridine concentration over a prolonged period is the use of prodrugs of uridine (e.g. 2′,3′,4′-triacetyluridine or PN401 [27, 28]) which are somewhat resistant to catabolism by UrdPase. Although they are better than uridine, such prodrugs also suffer from rapid degradation [27–29]. Hence, high doses must still be administered [4, 27–31] making it impractical to use alone in the clinic. Another approach to increase uridine bioavailability is the use of UrdPase

inhibitors to block the rapid catabolism of uridine. Several studies have shown that UrdPase inhibitors prevent the catabolism of uridine and subsequently increase plasma uridine concentration [4, 18, 26, 32–41] and the salvage of uridine by various tissues [20, 24]. Inhibition of uridine catabolism also prevents the toxic side effects of uridine catabolites associated with high doses of uridine [18]. This modulation of uridine metabolism by UrdPase inhibitors was successfully used to mimic the effect of high doses of exogenous uridine in enhancing the antitumor efficacy of FUra [4, 33, 36, 39, 42], without the clinical limitations associated with high doses of uridine [13–19]. However, available UrdPase inhibitors lack the required potencies, kinetic properties, and metabolic stability needed to elevate uridine concentration to the required levels to antagonize FUra host-toxicity [43].

We have designed and developed 5-(Phenylthio)acyclouridine (PTAU) as a highly specific and potent inhibitor of UrdPase [43–46]. PTAU has 100% oral bioavailability and a prolonged plasma half life of 2.1 h which ensures the inhibition of uridine catabolism. Indeed, when combined with uridine for oral administration, PTAU increased uridine plasma levels by 55-fold (from 1.8 to 105 μM) which is far above the 75 μM required to protect from FUra toxicity. Furthermore, PTAU has no apparent toxicity when given to animals at much higher doses than those needed to increase uridine levels [43–46]. Finally, the relatively higher lipophilicity of PTAU could improve its access to the liver and intestine. The liver and intestine are the main organs involved in uridine catabolism [21–25, 40]. This property could be significant in the likelihood of the design of an oral formulation of PTAU in combination with FUra. These excellent properties of PTAU make it a more convenient modulator of plasma uridine than the other available UrdPase inhibitors or the toxic doses of exogenous uridine required in cancer chemotherapy. However, PTAU was never tested as a modulator of FUra efficacy. Therefore, in this report we tested the effect and time of administration of oral PTAU alone or in combination with uridine on the host toxicity and chemotherapeutic efficacy of FUra in SCID mice bearing DLD-1 or HCT-15 human colon tumor xenografts. These two lines were chosen because DLD-1 has normal levels of UrdPase activity (561 ± 22 pmol/min/mg protein) [47]. HCT-15 has a lower UrdPase activity (371 ± 26 pmol/min/mg protein) [47] and to ensure that the results are not particular to one type of tumor. Furthermore, these two cell lines have been used extensively for antitumor drug screening in vitro and in vivo.

Materials and methods

Chemicals

Hydroxypropylmethylcellulose (HPMC) and other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA). PTAU was synthesized as previously described [46].

Mice

Male SCID mice (18–22 g) were obtained from Fredrick Cancer Research (Fredrick, MD) and housed 5/cage with water and food ad libitum under a normal light cycle (light, 0600–1800 hour; dark, 1800–0600 hour) according to an institutionally approved animal protocol.

Cell line

DLD-1 and HCT-15 were obtained from American Type Culture Collection, Rockville, MD, USA. DLD-1 and HCT-15 cells are human colon adenocarcinomas. These are two epithelial cell lines that are heterogeneous and produce moderately to poorly differentiated adenocarcinomas when inoculated subcutaneously into nude mice. The two cell lines were maintained at 37°C in growth medium (RPMI 1640 medium with 2 mM L-glutamine modified to contain 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/l glucose, 1.5 g/l bicarbonate and supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin) in plastic tissue culture flasks (75 cm^2) in a humidified incubator under 5% CO_2 and 95% air. As mentioned above these two cell lines were chosen because they have different levels of UrdPase activity [47] and to ensure that the results are not particular to one type of tumor. Furthermore, these two cell lines have been used extensively for antitumor drug screening in vitro and in vivo.

Administration of drugs

5-(Phenylthio)acyclouridine alone or combined with uridine was mixed well with HPMC powder in hot water (80°C) and homogenized thoroughly using a polytron homogenizer (Brinkmann Instruments, Westbury, NY, New York). 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 completely [4, 27, 43]. Drugs were administered orally (0.1 ml/10 g) using 18G intubation needles (Popper and Sons, Inc., New Hyde Park, NY, USA). FUra was dissolved in normal saline solution (0.9% NaCl) and injected intraperitoneally at 0.1 ml/10 g. Control mice received the carrier solution (0.9% saline or 0.75% HPMC). To avoid a possible circadian variation in the activities of the major enzymes involved in FUra metabolism (UrdPase, Orotate phosphoribosyltransferase [EC 2.4.2.10] and dihydrouracil dehydrogenase [EC 1.3.1.2])

[48, 49], all mice were treated at the same time starting at 1:00 P.M.

Host toxicity and chemotherapeutic studies

In a previous study we found that oral administration of PTAU at 120 mg/kg alone or in combination with uridine at 1,320 mg/kg was exceptionally effective in elevating and sustaining high plasma uridine concentrations [43–45]. Therefore, these doses of PTAU, uridine or their combination were used in the present study to evaluate their effect on the host-toxicity of FUra in the SCID mouse xenograft model. Furthermore, PTAU alone or in combination with uridine was administered after FUra administration to evaluate the significance of time of administration of the rescue regimen.

Mice were divided into groups (8 mice/group). On day 0, DLD-1 or HCT-15 cells were harvested from the monolayer cultures, suspended in RPMI 1640 medium (containing no serum) and inoculated subcutaneously (10×10^6 cells/0.2 ml/mouse) into mice into an area just at the upper right back. Mice were injected intraperitoneally with FUra (200 mg/kg) on days 17, 24 and 31 after tumor cell inoculation. PTAU (120 mg/kg), uridine (1,320 mg/kg) or their combination was administered orally 2 or 4 h after FUra injection. Another 4 administrations of PTAU plus uridine were given every 8 h after the first treatment with PTAU plus uridine. Survival and body weight were used to evaluate host toxicity. Tumor weight was used to evaluate the efficacy of the drugs on tumor growth. Tumor weight was calculated as [the long diameter (mm)] × [short diameter (mm)]^2/2. The mice were monitored for 38 days.

Results and discussion

Previous studies have established that the administration of UrdPase inhibitors and/or uridine should be delayed until after the administration of FUra [4, 6, 39]. Therefore, we tested the effect of PTAU alone, uridine alone, or their combination, 2 and 4 h following FUra administration. Tables 1 and 2 show the effects of PTAU (120 mg/kg), uridine (1,320 mg/kg) and their combination, on host toxicity and chemotherapeutic efficacy of FUra at 200 and 300 mg/kg. The maximum tolerated dose of FUra (50 mg/kg) in SCID mice [50] was included as a reference for the chemotherapeutic efficacy of FUra.

The results in Tables 1 and 2 indicate that, PTAU alone did not cause any host toxicity confirming our previous results [43–45]. FUra at the maximum tolerated dose (50 mg/kg) also did not cause any mortality and reduced DLD-1 and HCT-15 tumor weights by 48% (Table 1) and 59% (Table 2) respectively. Increasing the dose of FUra to 200 mg/kg or above was accompanied by a 100% mortality (all mice were dead by Day 38). Administration of PTAU alone 2 h following the administration of 200 mg/kg FUra resulted in partial rescue from this lethal dose of FUra (63% of mice survived) and a reduction in DLD-1 and HCT-15 tumor weights by approximately 60% (Tables 1, 2). Table 1 also shows that delaying the administration of PTAU to 4 h post FUra administration was less effective in protecting from FUra toxicity (38% survival) and in reducing the DLD-1 tumor (47% reduction). Administration of uridine alone under the same conditions did not protect from FUra host-toxicity as all mice died by day 38.

On the other hand, coadministration of PTAU with uridine, 2 h following the administration of 200 mg/kg Fura, resulted in a better outcome than either PTAU or uridine alone (100% survival and no appreciable weight loss at day 38, Tables 1, 2). In addition, administration of uridine plus PTAU, 2 h after FUra treatment, had a greater chemotherapeutic effect than that achieved by the maximum tolerated dose of FUra (50 mg/kg) by reducing tumor weights by almost 80% (Tables 1, 2). Delaying the coadministration of PTAU plus uridine combination to 4 h after FUra administration reduced tumor weights by only 62%. In addition, this regimen was less effective than the 2 h regimen in rescuing the mice from FUra host toxicity as 12% of the mice died (Table 1). Therefore, we used the 2 h delayed regimen to test the efficacy of PTAU, uridine and the combination on rescuing from a higher dose of FUra (300 mg/kg). Table 2 shows that neither uridine nor PTAU alone had any rescuing effect (0% survival) from this high dose of FUra. On the other hand, the use of PTAU plus uridine combination reduced the tumor weight by almost 80%; however, this reduction in the tumor weight was accompanied by 37% mortality (Table 2). The results in Tables 1 and 2 suggests that there is no difference between DLD-1 and HCT-15 in their response to the different regimens employed in this study despite the fact that the tumors have different levels of UrdPase. These results demonstrate that the combination of PTAU plus uridine is quite promising in protecting from FUra host-toxicity. The combination of PTAU plus uridine also allowed the escalation of the maximum tolerated dose of FUra from 50 to 200 mg/kg.

The mechanism by which PTAU plus uridine combination protect from FUra host toxicity while maintaining its efficacy against the tumors, is based on the complexity of FUra's mechanism of action. FUra itself is inactive and must be converted to one of its active nucleotides (FdUMP, FdUTP or FUTP) before its antitumor activity can be realized. FdUMP inhibits the enzyme thymidylate synthase [EC 2.1.1.45] resulting in inhibition of DNA synthesis. FdUTP may be incorporated into DNA while FUTP is incorporated into various classes of RNA. These events lead to the disruption of DNA and RNA synthesis and are responsible for the therapeutic activity and cytotoxicity of FUra [8]. There is a general consensus that the activity of FUra against solid tumors is due mainly to inhibition of thymidylate

Table 1 Effects of 5-(Phenyl)acyclouridine (PTAU) alone or in combination with uridine, and the time of administration on host toxicity and chemotherapeutic efficacy of 5-FUra (200 mg/kg) in SCID mice bearing human colon tumor DLD-1 xenografts

Drug (mg/kg)			Tumor weight (mg)	%T/C[b]	% Survival at day			Day of first death	Body weight (g) at day 38
FUra	PTAU	Uridine			24	31	38		
0	0	0	1,638 ± 100[a]	100	100	100	–		25.4 ± 0.7
0	0	1,320	1,514 ± 179	92	100	100	100	–	24.8 ± 0.8
0	120	0	1,315 ± 157	80[d]	100	100	100	–	24.9 ± 1.5
50[c]	0	0	858 ± 116	52[d]	100	100	100	–	25.1 ± 1.0
Two hours after FUra administration									
200	0	0	–	–	63	25	0	24	–
200	0	1,320	–	–	100	50	0	28	–
200	120	0	683 ± 93	42[d, e]	100	100	63	35	23.5 ± 0.9
200	120	1,320	295 ± 74	18[d, f]	100	100	100	–	23.2 ± 1.0
Four hours after FUra administration									
200	0	1,320	–	–	75	25	0	24	–
200	120	0	870 ± 87	53[d]	100	88	38	31	22.6 ± 1.2
200	120	1,320	624 ± 118	38[d, e]	100	100	88	38	23.8 ± 1.0

[a]Mean tumor weight ± SD from 5 to 8 tumors
[b]%T/C, Percentage of tumor weight in treated mice/tumor weight in untreated control mice
[c]Maximum tolerated dose of FUra in SCID mice [50]
[d]Significantly different ($P < 0.01$) from that obtained by the untreated controls
[e]Significantly different ($P < 0.05$) from that obtained by the maximum tolerated dose of FUra (50 mg/kg)
[f]Significantly different ($P < 0.01$) from that obtained by the maximum tolerated dose of FUra (50 mg/kg)

Table 2 Effect of 5-(Phenyl)acyclouridine (PTAU) alone or in combination with uridine on the chemotherapeutic efficacy and host toxicity of 5-FUra (200 or 300 mg/kg) in SCID mice bearing human colon tumor HCT-15 xenografts

Drug (mg/kg)			Tumor weight (mg)	%T/C[b]	% Survival at day			Day of first death	Body weight (g) at day 38
Fura	PTAU	Uridine			24	31	38		
0	0	0	1,651 ± 172[a]	100	100	100	100	–	24.9 ± 1.4
0	0	1,320	1,482 ± 176	90	100	100	100	–	27.3 ± 1.3
0	120	0	1,351 ± 148	82[d]	100	100	100	–	26.8 ± 1.4
50[c]	0	0	680 ± 90	41[d]	100	100	100	–	25.8 ± 2.3
200	0	0	–	–	63	0	0	24	–
200	0	1,320	–	–	100	50	0	28	–
200	120	0	660 ± 65	40[d]	100	100	63	35	26.6 ± 1.6
200	120	1,320	307 ± 59	19[d, e]	100	100	100	–	25.5 ± 2.0
300	0	0	–	–	13	0	0	24	–
300	0	1,320	–	–	100	0	0	28	–
300	120	0	–	–	100	0	0	28	–
300	120	1,320	351 ± 70	21[d, e]	100	100	63	38	22.3 ± 1.1

PTAU, uridine or their combination was administered 2 h after FUra administration
[a]Mean tumor weight ± SD from 5 to 8 tumors
[b]%T/C, Percentage of tumor weight in treated mice/tumor weight in untreated control mice
[c]Maximum tolerated dose of FUra in SCID mice [50]
[d]Significantly different ($P < 0.05$) from that obtained by the untreated controls
[e]Significantly different ($P < 0.01$) from that obtained by the maximum tolerated dose of FUra (50 mg/kg)

synthase; whereas, the toxic side effect results from incorporation of FUra into RNA. Indeed, it was shown that the efficacy of FUra against colon carcinoma was reversed by thymidine, but not by uridine [1], indicating that the efficacy of FUra in colon carcinoma is a DNA-based mechanism. Therefore, administration of the PTAU plus uridine combination after FUra would not affect the inhibition of thymidylate synthase by FdUMP. In addition, the increased availability of uridine, by administration as well as by the inhibition of its catabolism by PTAU [43–45], reduces FUra host-toxicity by increasing UTP concentration in host cells, which will compete with FUTP for incorporation into RNA [8]. This would allow the use of higher doses of FUra which would increase its antitumor activity. However, this delayed rescue from host toxicity was time dependent and appeared to be optimum at 2 h post FUra administration. The efficiency of the PTAU plus uridine combination in rescuing from FUra host-toxicities can be attributed to the exceptional effectiveness of this combination in elevating and sustaining high uridine concentrations in vivo [43–45]. The combination of PTAU and uridine is more effective in raising and maintaining high plasma uridine concentrations than

those achieved by either uridine or PTAU alone [43–45] or other previously known UrdPase inhibitors [43]. Further adjustments of the PTAU plus uridine regimen may yield even better results.

In summary, the combination of PTAU plus uridine provides a potentially powerful, practical and less invasive modulation regimen which may meet all the desired criteria to increase the chemotherapeutic doses of FUra without encountering its renowned host-toxicity. The combination of PTAU plus uridine allowed the escalation of the maximum tolerated dose of FUra by fourfolds (from 50 to 200 mg/kg). This increase in the administered dose of FUra was accompanied by a greater reduction in tumor weight (81–82%) than that achieved by the maximum tolerated dose of FUra alone (48–59%) without any apparent host-toxicity. In addition, it appears that it is likely that tumors with different levels of UrdPase would respond in the same manner to this rescue regimens. Therefore, the combination of PTAU plus uridine can provide a better substitute for the massive doses of uridine necessary to rescue or protect from FUra host-toxicities, without the toxic side-effects associated with such doses of uridine. The combination may also allow the escalation of FUra doses for better chemotherapeutic efficacy. Alternatively, the combination of PTAU and uridine may be useful as an antidote in the few cases when cancer patients receive a lethal overdose of FUra.

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References

1. Grem JL (1990) Fluorinated Pyrimidines, In: Chabner BA, Collins JM (eds) Cancer chemotherapy; principles and practice. JB Lippincott Co, Philadelphia, pp 180
2. Peters GJ, van Groeningen CJ (1991) Clinical relevance of biochemical modulation of 5-fluorouracil. Ann Oncol 2:469
3. van Groeningen CJ, Peters GJ, Pinedo HM (1992) Modulation of fluorouracil toxicity with uridine. Semin Oncol 19:148
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. Bagrij T, Kralovanszky J Gyergyay F, Kiss E, Peters GJ (1993) Influence of uridine treatment in mice on the protection of gastrointestinal toxicity caused by 5-fluorouracil. Anticancer Res 13:789
6. Klubes P, Cerna I, Meldon MA (1982) Uridine rescue from the lethal toxicity of 5-fluorouracil in mice. Cancer Chemother Pharmacol 8:17
7. Klubes P, Cerna I (1983) Use of uridine rescue to enhance the antitumor selectivity of 5-fluorouracil. Cancer Res 43:3182
8. 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
9. 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
10. 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
11. 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
12. Schwartz GK, Christman K, Saltz L, Casper E, Quan V, Bertino J, Martin DS, Colofiore J, Kelsen D (1996) A phase I trial of a modified, dose intensive FAMTX regimen (high dose 5-fluorouracil + doxorubicin + high dose methotrexate + leucovorin) with oral uridine rescue. Cancer 78:1988
13. 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
14. 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
15. 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 Report 70:745
16. Cradock JC, Vishnuvajjala BR, Chin TF, Hochstein HD, Ackerman SK (1986) Uridine-induced hyperthermia in the rabbit. J Pharm Pharmacol 38:226
17. 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. Pharmaceut Res 4:113
18. 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
19. van Groeningen CJ, Peters GJ, Nadal JC, Leyva A, Laurensse E, Pinedo HM (1991) Clinical and pharmacologic study of orally administration uridine. J Natl Cancer Inst 83:437
20. Chan TCK, Markman M, Pfeifle CE, Taetle R, Abramson I, Howell SB (1988) Uridine pharmacokinetics in cancer patients. Cancer Chemother Pharmacol 22:83
21. Gasser T, Moyer JD, Handschumacher RE (1981) Novel single-pass exchange of circulating uridine in rat liver. Science 213:777
22. Monks A, Cysyk RL (1982) Uridine regulation by the isolated rat liver: perfusion with an artificial oxygen carrier. Am J Physiol 242:R465
23. Moyer JD, Oliver JT, Handschumacher RE (1981) Salvage of circulating pyrimidine nucleosides in the rat. Cancer Res 41:3010
24. >Holstege A, Gengenbacher H-M, Jehle L, Gerok W (1992) Uridine catabolism by the isolated perfused rat liver. J Hepatology 14:335
25. Holstege A, Leser H-G, Pausch J, Gerok W (1985) Uridine catabolism in Kupffer cells, endothelial cells, and hepatocytes. Eur J Biochem 149:169
26. 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
27. 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
28. Ashour OM, Naguib FNM, Goudgaon NM, Schinazi RFand 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

29. Kelsen DP, Martin D, O = Neil J, Schwartz G, Saltz L, Sung MT, von Borstel R, Bertino J (1997) Phase I trial of PN401, an oral prodrug of uridine, to prevent toxicity from fluorouracil in patients with avoidance responding. J Clin Oncol 15:1511
30. Hidalgo M, Villalona-Calero MA, Eckhardt SG, Drengler RL, Rodriguez G, Hammond LA, Diab SG, Weiss G, Garner AM, Campbell E, Davidson K, Louie A, O'Neil JD, von Borstel R, Von Hoff DD, Rowinsky EK (2000) Phase I and pharmacologic study of PN401 and fluorouracil in patients with advanced solid malignancies. J Clin Oncol 18:167
31. Saydoff JA, Liu LS, Garcia RA, Hu Z, Li D, von Borstel RW (2003) Oral uridine pro-drug PN401 decreases neurodegeneration, behavioral impairment, weight loss and mortality in the 3-nitropropionic acid mitochondrial toxin model of Huntington's disease. Brain Res 994:44
32. 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
33. Darnowski JW, Handschumacher RE (1985) Tissue-specific enhancement of uridine utilization and 5-fluorouracil therapy in mice by benzylacyclouridine. Cancer Res 45:5364
34. Darnowski JW, Handschumacher RE (1986) Tissue uridine pools: evidence in vivo of a concentrative mechanism for uridine uptake. Cancer Res 46:3490
35. Darnowski JW, Handschumacher RE (1988) Benzylacyclouridine: pharmacokinetics, metabolism and biochemical effects in mice. Biochem Pharmacol 37:2613
36. Darnowski JW, Handschumacher RE (1989) Enhancement of fluorouracil therapy by the manipulation of tissue uridine pools. Pharmacol Ther 41:381
37. 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
38. 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
39. 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
40. 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
41. 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 Inc, New York, pp 63
42. Yee LK, Chu E, Pan Bc, Chu SH, Chen Tm, Lipsky MH, Chu MYW, Calabresi P (1998) Benzylacyclouridine enhances 5-fluorouracil cytotoxicity against human prostate cancer cell lines. Pharmacology 56:80
43. Al Safarjalani ON, Zhou X-J, Rais RH, Shi J, Schinazi RF Naguib FNM, el Kouni MH (2005) 5-(Phenylthio)acyclouridine, a powerful enhancer of oral uridine bioavailability: relevance to chemotherapy with 5-fluorouracil and other uridine rescue regimens. Cancer Chemother Pharmacol 55:541
44. 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
45. Al Safarjalani ON, Zhou X-J, Naguib FNM, Shi J, Schinazi RF, el Kouni MH (2001) Enhancement of the bioavailability of oral uridine by coadministration of 5-(phenylthio)acyclouridine, a uridine phosphorylase inhibitor: implications for uridine rescue regimens in chemotherapy. Cancer Chemother Pharmacol 48:389
46. 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
47. Ashour OM, Naguib FNM, Khalifa MMA, Abdel-Raheem MH, Panzica RP, el Kouni MH (1995). Enhancement of 5-fluoro-2′-deoxyuridine antitumor efficacy by the uridine phosphorylase inhibitor 5-(benzyloxybenzyl)barbituric acid acyclonucleoside. Cancer Res 55:1092
48. 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
49. 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
50. Naguib FNM, Hao H-N, el Kouni MH (1994) Potentiation of 5-fluorouracil antineoplastic activity by the dihydrouracil dehydrogenase inhibitor 5-benzyloxybenzylurcil. Cancer Res 54:5166