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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

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ABSTRACT. Administration of 200 mg/kg of 5-fluorouracil (FUra) to mice bearing human colon carcinoma DLD-1 xenografts resulted in 100% mortality. Oral administration of 2000 mg/kg of 2′,3′,5′-tri-*O*-acetyluridine (TAU), a prodrug of uridine, in combination with 120 mg/kg of 5-(benzyloxybenzyl)barbituric acid acyclonucleoside (BBBA), the most potent known inhibitor of uridine phosphorylase (UrdPase, EC 2.4.2.3), 2 hr after the administration of the same dose of FUra completely protected the mice (100% survival) from the toxicity of FUra. This combination also reduced tumor weight by 67% compared with 46% achieved by the maximum tolerated dose (50 mg/kg) of FUra alone. Similarly, administration of BBBA plus TAU 1 hr before or 4 hr after the administration of FUra reduced the tumor weight by 53 and 37%, respectively. However, these schedules were less effective in protecting the host from the toxicity of FUra than when the treatment was carried out at 2 hr after FUra administration. TAU alone did not protect from FUra host toxicity. The efficiency of the BBBA plus TAU combination in rescuing from FUra host toxicities is attributed to the exceptional effectiveness of this combination in raising and maintaining higher plasma uridine concentrations than those achieved by TAU alone or by equimolar doses of uridine (Ashour *et al.*, *Biochem Pharmacol* 51: 1601–1612, 1996). The present results suggest that the BBBA plus TAU combination can provide a better substitute for the massive doses of uridine required to achieve the high levels of uridine necessary to rescue or protect from FUra host toxicities without the toxic side-effects associated with such doses of uridine. The combination of TAU plus BBBA may also allow the escalation of FUra doses for better chemotherapeutic efficacy. Alternatively, the combination may be used as a rescue regimen in the occasional cases where cancer patients receive a lethal overdose of FUra. BIOCHEM PHARMACOL **60**;3:427–431, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. 5-fluorouracil; toxicity; uridine phosphorylase inhibitor; uridine prodrug; chemotherapy

The pyrimidine nucleoside uridine has been used successfully as a "protective" and/or "rescuing" agent against host toxicity of the anti-cancer drug FUra‖ [1–5] without interfering with its chemotherapeutic efficacy. However, because of its rapid clearance [6–15], it is necessary to administer substantial doses of uridine (10–12 g/m^2) [8] to attain and sustain the high plasma uridine concentrations (70 μM) [16] required to achieve the protective or rescuing effects. Unfortunately, such large doses of uridine also produce dose-limiting side-effects (e.g. phlebitis, pyrogenic reactions and diarrhea, high fever, cellulitis, and superior vena cava syndrome) [8, 10–12, 17–20]. Therefore, alternative approaches to increasing uridine bioavailability to the required concentrations must be sought.

Uridine is maintained in rigorous homeostasis at a concentration of 1–5 μM in the plasma of various species [6, 14, 21, 22]. However, the half-life of plasma uridine is approximately 2 min [21]. Hence, the turnover of plasma uridine must be rapid and efficient. Indeed, more than 90% of the circulating uridine is catabolized in a single pass through the liver by the activity of hepatic UrdPase (EC 2.4.2.3), while constant amounts of uridine are synthesized *de novo* and released into the hepatic vein blood [23, 24]. Less than 2% of the uridine metabolized by the liver is salvaged and recovered in the uracil nucleotide pool in tissues of whole animals [22, 25–27], perfused rat liver [6, 13], or isolated liver cells [26]. The remainder is catabolized rapidly to products beyond uracil in the pyrimidine catabolic pathway [13, 23, 28].

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‖ *Abbreviations*: ATS, rabbit anti-mouse thymocyte serum; BBBA, 5-(benzyloxybenzyl)barbituric acid acyclonucleoside; FUra, 5-fluorouracil; HPMC, hydroxypropylmethylcellulose; TAU, 2′,3′,5′-tri-*O*-acetyluridine; and UrdPase, uridine phosphorylase.

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One approach to maintaining a high uridine concentration over a prolonged period is the use of UrdPase inhibitors to block the rapid catabolism of uridine to uracil. Inhibition of uridine catabolism by UrdPase inhibitors would lead to increased plasma uridine concentrations as a result of the continuous *de novo* biosynthesis of uridine in the liver. Indeed, UrdPase inhibitors have been used to increase the concentration and half-life of plasma uridine [13–16, 20, 23, 29–31] and the salvage of uridine by various tissues [16, 20, 24, 32].

Another approach to increasing uridine bioavailability is to use prodrugs of uridine that are resistant to catabolism by UrdPase [14]. It has been shown previously that oral administration of TAU, a prodrug of uridine resistant to catabolism by UrdPase [14], greatly increases and sustains high plasma uridine concentrations in mice [14] and in humans [33]. The high efficiency of TAU in modulating plasma uridine concentration is enhanced further (3.9-fold) by co-administration of BBBA [14], the most potent known inhibitor of UrdPase [34, 35].

Therefore, in the present study, we tested the effect and time of administration of TAU alone or in combination with BBBA on the host toxicity and chemotherapeutic efficacy of FUra in immunosuppressed mice bearing human colon carcinoma DLD-1 xenografts.

MATERIALS AND METHODS

Chemicals

ATS was obtained from Intercell Technologies, Inc. Bovine γ-globulin and dye reagent for protein assays were from Bio-Rad Laboratories. TAU, HPMC, and other chemicals were purchased from the Sigma Chemical Co. BBBA was synthesized as described previously [34, 35].

Mice

Female CD-1 mice (18–20 g) were obtained from Charles River Laboratories and housed five/cage with water and food *ad lib.* under a normal light cycle (light, 6 a.m. to 6 p.m.; dark, 6 p.m. to 6 a.m.) according to an institutionally approved animal protocol.

Cell Line

DLD-1, a human colon adenocarcinoma cell line, was obtained from the American Type Culture Collection. This cell line is epithelial, heterogeneous, and produces moderately to poorly differentiated adenocarcinomas when injected into nude mice. DLD-1 cells were maintained at 37° in growth medium (RPMI 1640 medium containing 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.

Administration of Drugs

TAU alone or combined with BBBA was mixed well with HPMC powder in hot water (80°) and homogenized thoroughly using a Polytron homogenizer (Brinkmann Instruments). 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° to hydrate completely [14, 15]. Drugs were administered orally (0.1 mL/10 g) using 18 gauge intubation needles (Popper & Sons, Inc.). 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)] [36, 37], all mice were treated at the same time between 1:00 and 1:30 p.m.

Host Toxicity and Chemotherapeutic Studies

In a previous study we found that oral administration of TAU alone at 2000 mg/kg or in combination with BBBA at 120 mg/kg was exceptionally effective in elevating and sustaining high plasma uridine concentrations [14]. Therefore, these doses of TAU alone or in combination with BBBA were used in the present study to evaluate their effect on the host toxicity of FUra in the ATS-immunosuppressed mouse xenograft model as previously described [38, 39]. Furthermore, TAU alone or in combination with BBBA was administered prior to or after FUra administration to evaluate the significance of time of administration of the rescue regimen.

Female CD-1 mice (18–20 g) were divided into groups (eight mice/group) and immunosuppressed with intraperitoneal injections (0.3 mL/mouse/day) of ATS (5 mg/kg/day) on days −1, 0, 1, 3, 5, 7, and then twice weekly as described previously [38, 39]. On day 0, DLD-1 cells were harvested from the monolayer cultures, suspended in RPMI 1640 medium (containing no serum), and injected subcutaneously (5×10^6 cells/0.3 mL/mouse) into ATS-treated mice in an area just above the right inguinal region. Mice were injected intraperitoneally with FUra (200 mg/kg) on days 12, 19, and 26 after tumor cell inoculation. TAU alone (2000 mg/kg), or in combination with BBBA (120 mg/kg), was administered orally 1 hr before, 2 hr after, or 4 hr after FUra injection, and thereafter every 8 hr for five doses. ATS was not administered on the days when drugs were to be administered. 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 [long diameter (mm)] × [short diameter (mm)]^2/2. The mice were monitored for 33 days.

TABLE 1. Effect of TAU alone or in combination with BBBA administrations and time of administration on host toxicity and chemotherapeutic efficacy of 5-FUra (200 mg/kg) in ATS-immunosuppressed female CD-1 mice bearing human colon tumor DLD-1 xenografts

Drug (mg/kg)			Tumor		% Survival at day			Day of first	Body weight
FUra	TAU	BBBA	weight* (mg)	%T/C†	19	26	33	death	at day 33‡ (g)
0	0	0	1940 ± 415	100	100	100	100		22.3 ± 2.1
0	2000	0	1679 ± 229	87	100	100	100		22.4 ± 2.1
0	2000	120	1448 ± 439	75	100	100	100		23.3 ± 1.7
50§	0	0	1024 ± 156	54	100	100	100		22.7 ± 3.0
Two hours after FUra administration									
0	0	0	1207 ± 177	100	100	100	100		22.3 ± 2.0
200	0	0			100	0	0	22	
200	2000	0			100	75	0	26	
200	2000	120	492 ± 113	33‖	100	100	100		21.5 ± 1.6
Four hours after FUra administration									
0	0	0	987 ± 206	100	100	100	100		20.5 ± 1.9
200	0	0			100	13	0	24	
200	2000	0			100	75	0	27	
200	2000	120	625 ± 177	63	100	63	13	22	21.2 ± 0.7
One hour before FUra administration									
0	0	0	1080 ± 233	100	100	100	100		20.6 ± 2.2
200	0	0			100	0	0	21	
200	2000	0			100	75	0	24	
200	2000	120	506 ± 189	47	100	75	13	21	20.6 ± 1.1

Tumor weight averaged 131 mg on day 12 before treatment.

*Mean tumor weight ± SD from 5–8 tumors.

†%T/C: percentage of tumor weight in treated mice/tumor weight in untreated control mice.

‡Values are means ± SD from at least two mice.

§Maximun tolerated dose of FUra in ATS-immunosuppressed mice [38].

‖Significantly different ($P < 0.05$) from that obtained by the maximun tolerated dose of FUra (50 mg/kg).

RESULTS AND DISCUSSION

Table 1 shows that TAU alone or in combination with BBBA at the doses used did not cause any host toxicity, confirming our previous results [14, 39], and reduced the tumor weight slightly.

Table 1 also shows the effect of TAU alone and in combination with BBBA on host toxicity and chemotherapeutic efficacy of 200 mg/kg of FUra. FUra at 50 mg/kg, the maximum tolerated dose in immunosuppressed mice [38], was included as a reference for the chemotherapeutic efficacy of FUra. FUra at 50 mg/kg reduced tumor weight by 46% compared with the control group. Administration of FUra at 200 mg/kg was accompanied by 100% mortality (all mice were dead by day 26). Administration of TAU alone, 2 hr following FUra administration, protected the mice for a limited period, as only 75% of the mice survived until day 26, but at day 33 all mice receiving FUra plus TAU alone died. Co-administration of BBBA with TAU resulted in complete protection from FUra-induced host toxicity (100% survival and no significant weight loss at day 33) and reduced tumor weight by 67% (Table 1). Similarly, administration of BBBA plus TAU 1 hr before or 4 hr after the administration of FUra reduced the tumor weight by 53 and 37%, respectively. However, these schedules were less effective in protecting the host from the toxicity of FUra (13% survival at day 33) than when the treatment was carried out at 2 hr after FUra administration.

These results demonstrate that the combination of TAU plus BBBA is quite promising in rescuing from FUra host toxicity. The combination of BBBA and TAU also allowed the escalation of the maximum tolerated dose of FUra from 50 to 200 mg/kg. This increase in the administered dose of FUra was accompanied by a greater reduction in tumor weight (67%) than that achieved by the maximum tolerated dose of FUra alone (46%). Further adjustments of the BBBA plus TAU regimen may yield even better results. The efficiency of the BBBA plus TAU combination in rescuing from FUra host toxicities can be attributed to the exceptional effectiveness of this combination in elevating and sustaining high plasma uridine concentrations. The combination of BBBA and TAU is more effective in raising and maintaining higher plasma uridine concentrations than those achieved by TAU alone or by equimolar doses of uridine [14]. These results suggest that the combination of BBBA plus TAU 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 TAU

and BBBA may be useful as an antidote in the few cases when cancer patients receive a lethal overdose of FUra.

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