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ORIGINAL ARTICLE

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Modulation of the pharmacokinetics of endogenous plasma uridine by 5-(phenylthio)acyclouridine, a uridine phosphorylase inhibitor: implications for chemotherapy

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Abstract *Purpose*: The purpose of this investigation was to evaluate the ability of oral PTAU, 5-(phenylthio)acyclouridine, to increase the concentration of endogenous plasma 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. *Methods*: PTAU was administered to mice orally and parenterally. The plasma levels of PTAU as well as those of uridine and its catabolite uracil were measured by HPLC, and pharmacokinetic analysis was performed. *Results*: PTAU was fully adsorbed after oral administration (over 100% oral bioavailability) and no PTAU metabolites were detected. PTAU administered orally had no apparent toxicity at doses up to 120 mg/kg per day for 5 days. Parenteral administration of PTAU at 30, 45 and 60 mg/kg increased the concentration of endogenous plasma uridine (1.8 ± 0.2 μ*M*) by approximately six-, seven-, and nine-fold, respectively. Plasma uridine concentration remained higher than control values until 8 h after PTAU administration. Similar results were obtained following oral administration of PTAU. The baseline concentrations of endogenous plasma uridine were increased by approximately six-, seven- and ten-fold by oral administration of PTAU at 30, 45 and 60 mg/kg, respectively, and remained higher than the controls until 8 h after PTAU administration. PTAU did not alter the concentration of endogenous plasma uracil. *Conclusion*: The effectiveness of the PTAU in elevating and sustaining high plasma uridine concentrations may be useful in rescuing or protecting the host from toxicities of various chemotherapeutic pyrimidine analogues as well as in the management of medical disorders that respond to the administration of uridine.

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

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Introduction

5-Fluorouracil (FUra) is among the few "standard" drugs effective against solid tumors in humans such as colorectal, breast, and head and neck cancers. However, its anticancer activity in the clinic is limited mainly by myelosuppression. Uridine has been shown to counteract the host toxicity of FUra without impairing its antitumor activity [18, 19, 21, 27, 30, 32]. Nevertheless, because of its rapid degradation [1, 2, 4, 8, 20, 23, 34–37], large doses (10–12 g/m^2) of uridine [34] are required in uridine rescue regimens, and such high doses are associated with toxic side effects. (e.g. phlebitis, pyrogenic reactions, diarrhea, high fever, cellulitis, and superior vena cava syndrome) [5, 28, 29, 34–36]. These side effects are not induced by uridine per se but by the accumulation of uridine catabolites [28, 29]. Therefore, specific inhibitors of uridine catabolism could be used to elevate endogenous plasma uridine concentration and overcome the complications inherent in the use of large doses of exogenous uridine.

The pyrimidine salvage enzyme uridine phosphorylase (UrdPase, EC 2.4.2.3) plays a pivotal role in the catabolism and maintenance of the rigorous homeostasis of plasma uridine. 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 [14, 23, 25]. Activity of hepatic UrdPase is the first step in elimination of plasma uridine delivered to the liver [9, 24]. Indeed, hepatic UrdPase activity has been shown to exhibit a circadian rhythm which is the inverse of that of plasma uridine concentration [12, 26]. Furthermore, several studies have demonstrated that inhibition of UrdPase causes a profound increase of uridine in plasma and different tissues [1–3, 6–11, 22, 24, 28, 31, 33]. This modulation of uridine metabolism by UrdPase inhibitors has been successfully used to mimic the effect of high doses of exogenous uridine without the clinical limitations associated with high doses of uridine [1–3, 6–11, 22, 24, 28, 31, 33].

We have designed and synthesized 5-(phenylthio)acyclouridine (PTAU, Fig. 1) as a highly specific and potent lipophilic inhibitor of UrdPase [13] and as such its access to the liver and intestine, the main organs involved in uridine catabolism [14, 16, 17, 23, 24, 25], would be facilitated. In the present study we investigated the effect of PTAU as a modulator of endogenous plasma uridine in mice. Mice have been successfully used to investigate the in vivo effects of uridine and UrdPase inhibitors on the chemotherapy of various tumors including human tumors [1–3, 6–12, 18, 19, 21, 22, 29, 30]. Hence, the mouse model was used in our investigations.

Materials and methods

Chemicals

Heparinized Natelson pipettes, ammonium acetate, acetonitrile (HPLC grade), trichloroacetic acid (TCA), Gelman Acrodisc LC 13 PVDF 0.2 μm filters and ethyl ether (anaesthetic 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 [13].

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.

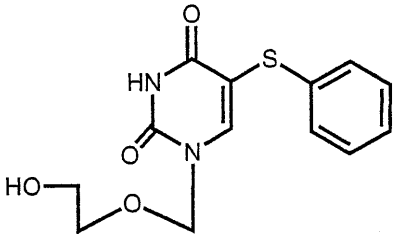

Fig. 1 Chemical structure of 5-(phenylthio)acyclouridine (PTAU)

Administration of drugs

PTAU was administered at 30, 45 and 60 mg/kg. For oral administration, PTAU was 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 completely [1–3]. Drugs were administered (0.1 ml/10 g) using 18G intubation needles (Popper and Sons, New Hyde Park, N.Y.). For intraperitoneal (i.p.) injection, PTAU was dissolved in normal saline solution (0.9% NaCl) containing 10% DMSO and injected at 0.1 ml/10 g. The effect of parenteral uridine on plasma levels of uridine and uracil was considered as the reference effect. Control mice received the carrier solution (0.75% HPMC or 0.9% saline with 10% DMSO). To avoid a possible circadian variation in UrdPase and dihydrouracil dehydrogenase (EC 1.3.1.2) activities [14, 36], all mice were injected at the same time (between 0830 and 0900 hours).

Determination of the toxicity of PTAU

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

Effect of PTAU on plasma uridine and uracil concentrations

Collection of samples

At various times (5, 10, 15 and 30 min, 1, 2, 3, 4, 6 and 8 h) after drug administration, 250 μl of whole blood were 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 at –20°C until analysis by high-performance liquid chromatography (HPLC).

Preparation of the samples

Plasma was allowed to thaw on ice and then deproteinized with two volumes of 15% TCA. After centrifugation (16,000 *g*, 4°C) for 5 min in a Fisher microcentrifuge, the supernatant acid-soluble material was neutralized by extraction with a 1:2 mixture of tri-*n*-octylamine in freon. The neutralized supernatant was filtered through Gelman Acrodisc LC 13 PVDF 0.2 μm filter, prior to HPLC analysis [1, 2, 3, 12].

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 (50 m*M* ammonium acetate, 0.5% acetonitrile, pH 4.8) and buffer B (50 m*M* ammonium acetate, 75% acetonitrile, pH 4.8). Typically, 100 μl of treated plasma sample was analyzed with a multistep elution protocol. A 23-min isocratic elution in buffer A was followed by a 15-min linear gradient to 75% buffer B, then a 27-min isocratic elution in 75% buffer B was followed by a 20-min re-equilibration wash with 100% buffer A. The flow rate was 1 ml/min, except for two 0.5-ml/min segments (8–23 min and 38–55 min). 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. No metabolites of PTAU were detected in the plasma under these conditions.

Uracil, uridine and PTAU were identified by UV absorption at λ_{max} (259.5, 262 and 243 nm, respectively)/254 nm, and coelution with authentic standards. The recovery of uracil and uridine was more than 98% using [6-^{14}C]uracil and [2-^{14}C]uridine. The peak areas for uracil and uridine in the sample were integrated by the online computer. The concentrations of uracil or uridine in the samples were determined using standard curves for uracil and uridine prepared in double-distilled water. Plots of peak area vs uracil, uridine and PTAU concentrations were linear between 1 and 3000 μM.

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) 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. The absolute bioavailability (F) of PTAU was estimated as $dose_{i.p.}/dose_{oral} \times AUC_{i.p.}/AUC_{oral}$. Other pharmacokinetic parameters including maximal plasma concentration (C_{max}) and time to C_{max} (T_{max}), were obtained directly from the plasma data. C_0 was the plasma baseline concentration of endogenous uridine and uracil observed at time zero (0830 to 0900 hours).

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

Dose (mg/kg/day) ×5 days	Body weight (g)		Mortality
	Day 1	Day 28	
0	19.1 ± 0.3	23.6 ± 0.7	0
30	19.7 ± 0.7	22.5 ± 0.8	0
60	19.5 ± 0.8	22.6 ± 0.5	0
120	19.6 ± 1.1	22.8 ± 0.7	0

Results and discussion

Toxicity of PTAU

Table 1 shows that oral administration of PTAU at 120 mg/kg per day for five consecutive days did not cause any mortality, and did not significantly affect the mean body weight of the mice at 4 weeks posttreatment. These results demonstrate the safety of PTAU administration at least at the doses tested.

Effects of PTAU on plasma uridine concentration

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 ± 0.2 and 6.9 ± 0.6 μM, respectively. Treatment of mice with PTAU produced a dose-dependent increase in the AUC and C_{max} of endogenous plasma uridine (Fig. 2 and Table 2). The pharmacokinetic parameters of plasma uridine after PTAU administration are shown in Table 2.

Parenteral administration of PTAU at 30, 45 and 60 mg/kg resulted in a plasma uridine C_{max} of 10, 14 and 17 μM, and increased the baseline concentration of plasma uridine (C_0) by six-, seven- and ninefold, respectively (Table 2). The AUC of plasma uridine after i.p. PTAU administration at 30, 45 and 60 mg/kg were 47, 62 and 63 μmol·h/l, respectively. Plasma uridine concentration remained higher than control values until 8 h after PTAU administration (Fig. 2).

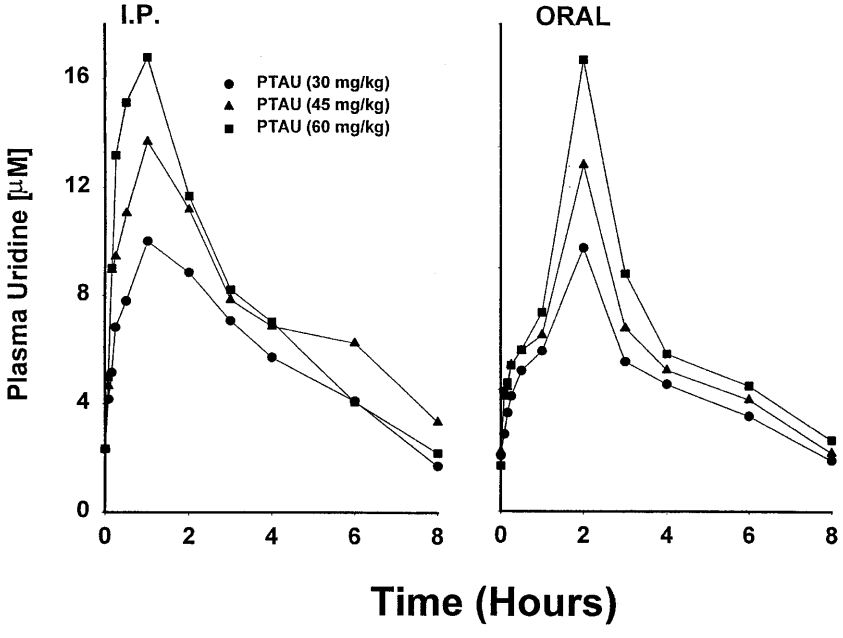

Fig. 2 Effect of i.p. and oral administration of different doses of PTAU on endogenous plasma uridine concentration in CD-1 mice. Each point represents the mean concentration from five mice

Table 2 Effect of administration of different doses of PTAU on the pharmacokinetic parameters of endogenous plasma uridine in CD-1 mice. Values are means ± S.D. from at least 5 mice at each time point (C_{max} peak plasma concentration, C_0 baseline plasma concentration at time zero, T_{max} time to peak plasma concentration, AUC area under the curve)

Administration route	PTAU dose (mg/kg)	C_{max} (μM)	Fold change (C_{max}/C_0)	T_{max} (h)	AUC (μmol·h/l)
Intraperitoneal	30	10.0 ± 0.7	6.0 ± 2.3	1.00 ± 0.00	46.8 ± 2.7
	45	13.7 ± 1.2	6.8 ± 1.1	1.00 ± 0.00	62.5 ± 2.6
	60	17.0 ± 1.5	9.1 ± 2.8	0.75 ± 0.29	62.5 ± 2.9
	Mean ± SD	13.6 ± 3.5		0.92 ± 0.14	57.2 ± 9.1
Oral	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
	Mean ± SD	11.6 ± 3.1		2.00 ± 0.00	41.9 ± 7.5

Table 3 Pharmacokinetic parameters of plasma PTAU in CD-1 mice. Values are means ± SD. from at least 5 mice at each time point (C_{max} peak plasma concentration, T_{max} time to peak plasma concentration, AUC area under the curve, V apparent total volume of distribution, MRT mean residence time, Cl apparent total plasma clearance, F oral bioavailability, $t_{1/2}$ elimination half-life)

Administration route	PTAU dose (mg/kg)	C_{max} (μM)	T_{max} (h)	AUC (μmol·h/l)	V_d (l/kg)	Cl (l/h/kg)	F (%)	MRT (h)	$t_{1/2}$ (h)
Intraperitoneal	30	70.5 ± 6.0	0.12 ± 0.07	71.6 ± 6.4	1.2 ± 0.5	1.4 ± 0.1	–	0.80 ± 0.04	0.57 ± 0.11
	45	110.0 ± 6.4	0.12 ± 0.05	99.1 ± 11.5	0.8 ± 0.6	1.0 ± 0.1	–	0.83 ± 0.05	0.57 ± 0.10
	60	129.3 ± 6.7	0.13 ± 0.07	140.0 ± 6.6	0.6 ± 0.4	0.7 ± 0.0	–	0.84 ± 0.68	0.60 ± 0.04
	Mean ± SD	103.3 ± 30.1	0.12 ± 0.01	103.6 ± 34.4	1.0 ± 0.4	1.0 ± 0.4	–	0.82 ± 0.02	0.58 ± 0.02
Oral	30	40.1 ± 1.6 0	08 ± 0.00	87.4 ± 0.1	–	–	122.1	2.3 ± 0.1	2.1 ± 0.4
	45	51.7 ± 2.1	0.08 ± 0.00	127.8 ± 0.1	–	–	129.0	2.4 ± 0.1	1.5 ± 0.0
	60	68.1 ± 3.0 0	08 ± 0.00	170.9 ± 0.0	–	–	122.1	2.4 ± 0.0	1.7 ± 0.2
	Mean ± SD	74.1 ± 7.6	0.09 ± 0.01	121.7 ± 33.0	–	–	124.4 ± 4.0	2.2 ± 0.4	1.6 ± 0.2

The pharmacokinetic parameters of plasma uridine following oral administration of PTAU were essentially the same as those obtained after administration of identical doses of PTAU parenterally (Table 2). Oral administration of PTAU 30, 45 and 60 mg/kg resulted in a plasma uridine C_{max} of 9, 11 and 15 μM, respectively, which increased endogenous plasma uridine baseline concentration (C_0) by six-, seven- and tenfold, respectively. Similarly, as with parenteral administration of PTAU, oral administration of PTAU maintained plasma uridine concentrations higher than control values for 8 h after administration (Fig. 2). The AUC values were 35, 42 and 50 μmol·h/l after oral PTAU administration at 30, 45 and 60 mg/kg, respectively (Table 2). No significant changes in plasma uracil concentrations were observed following PTAU administration by either the oral or parenteral route (data not shown).

These results demonstrate that PTAU is a powerful modulator of the concentration of endogenous plasma uridine in mice as it increased plasma concentration of uridine by five- to ten-fold depending on the dose of PTAU used. The sustained increase in plasma uridine concentration as a result of PTAU treatment demonstrates that PTAU is a potent inhibitor of UrdPase in vivo as well as in vitro [13]. However, the doses of PTAU used in the present study did not increase plasma uridine levels to those required to counteract FUra host toxicity (75 μM) [22]. This could have been due to the low doses of PTAU administered. Therefore, higher concentrations of PTAU may be required, alone or in combination with exogenous uridine, to increase the plasma uridine to the levels required for rescue or protection of the host from FUra toxicity. It is encouraging that higher doses of PTAU showed no host toxicity (Table 1).

The lack of significant changes in the pharmacokinetic parameters of plasma uridine following oral and i.p. administration of PTAU indicates the efficiency of PTAU absorption and the absence of a first-pass effect (metabolism) in the liver or the intestine. Indeed, the pharmacokinetics of PTAU demonstrated that oral PTAU was fully absorbed at doses up to 60 mg/kg and no PTAU metabolites were detected in the plasma.

Pharmacokinetics of PTAU

The pharmacokinetic parameters of plasma PTAU are shown in Table 3. Parenteral administration of PTAU at 30, 45 and 60 mg/kg resulted in a peak plasma PTAU concentration (C_{max}) of 71, 110 and 129 μM at 0.12 h with AUC values of 72, 99 and 140 μmol·h/l, respectively (Table 3 and Fig. 3). Oral administration of PTAU at 30, 45 and 60 mg/kg showed essentially similar pharmacokinetic parameters to those obtained by administration of identical doses parenterally. The mean estimated oral bioavailability of PTAU (F) was 124 ± 4% across the dose range studied. The higher than 100% oral bioavailability of PTAU and the increase in

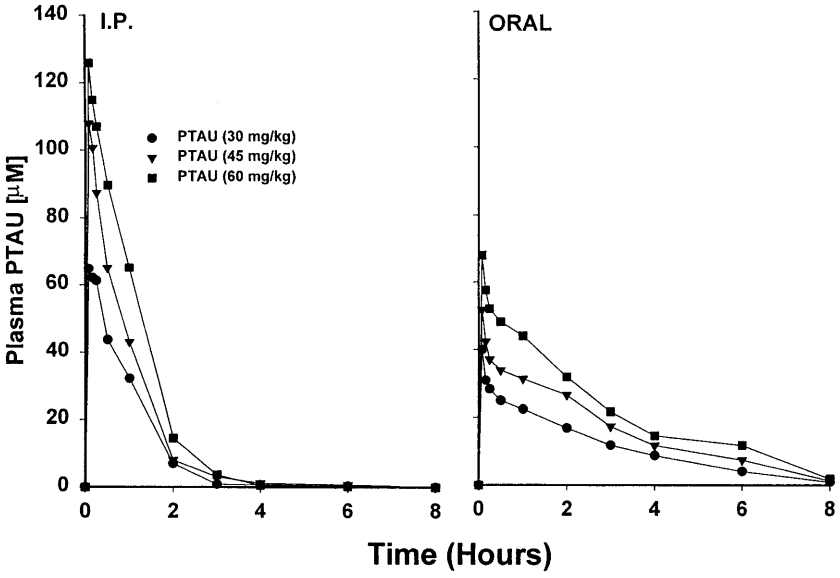

Fig. 3 Plasma concentration-time curves of PTAU after i.p. and oral administration of different doses of PTAU in CD-1 mice. Each point represents the mean concentration from five mice

MRT seem to be a function of elimination rather than absorption or metabolism. However, no PTAU metabolites were detected in the plasma following oral or i.p. administration of PTAU. Furthermore, Fig. 3 shows that oral PTAU is absorbed very quickly but its elimination was slower than that of parenteral PTAU. These results suggest that oral PTAU may be temporarily retained by the intestine and/or liver before it enters the circulation. Therefore, the intestine and/or the liver may act as a depot releasing PTAU into the circulation. This would account for the slower elimination and higher MRT of oral PTAU, hence the high oral bioavailability. Further studies are required to verify this proposition.

In conclusion, PTAU efficiently increased the concentration of endogenous plasma uridine in a dose-dependent manner. The high potency and excellent bioavailability make PTAU a promising and more convenient modulator of plasma uridine than previously known UrdPase inhibitors, e.g. 5-benzylacyclouridine (BAU) [6–11, 22, 24, 29, 31, 33] and 5-(phenylselenenyl)acyclouridine (PSAU) [2, 3]. For example, the efficacy of BAU [31, 33] and PSAU [2, 3] in increasing plasma uridine is restricted only to lower doses of the inhibitors. Furthermore, the bioavailability of BAU is limited by metabolism [8, 11] and its administration has been shown to enhance the host toxicity of FUra [15]. Additionally, the pharmacokinetics of PSAU are altered by the coadministration of uridine, indicating that uridine may affect the elimination of PSAU [2, 3].

PTAU as modulator of plasma uridine could also be used as a substitute for the toxic massive doses of exogenous uridine required in the therapy of cancer and AIDS as well as other pathological and physiological disorders that respond to the administration of uridine (see references 1 and 2 and references therein). Further adjustments of the PTAU doses may yield even better results. In this context, it is encouraging that higher doses of PTAU showed no host toxicity (Table 1).

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