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Plasma pharmacokinetics and oral bioavailability of 3,4,5,6-tetrahydrouridine, a cytidine deaminase inhibitor, in mice

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Abstract

Cytidine analogues such as cytosine arabinoside, gemcitabine, decitabine, 5-azacytidine, 5-fluoro-2'-deoxycytidine and 5-chloro-2'-deoxycytidine undergo rapid catabolism by cytidine deaminase (CD). 3,4,5,6-tetrahydrouridine (THU) is a potent CD inhibitor that has been applied preclinically and clinically as a modulator of cytidine analogue metabolism. However, THU pharmacokinetics has not been fully characterized, which has impaired the optimal preclinical evaluation and clinical use of THU. Therefore, we characterized the THU pharmacokinetics and bioavailability in mice. Mice were dosed with THU iv (100 mg/kg) or po (30, 100, or 300 mg/kg). Plasma and urine THU concentrations were quantitated with a validated LC-MS/MS assay. Plasma pharmacokinetic parameters were calculated compartmentally and non-compartmentally. THU, at 100 mg/kg iv had a 73 min terminal half-life and produced plasma THU concentrations >1 µg/ml, the concentration shown to effectively block deamination, for 4 h. Clearance was 9.1 ml/min/kg, and the distribution volume was 0.95 l/kg. Renal excretion accounted for 36–55% of the THU dose. A three-compartment model fit the iv THU data best. THU, at 100 mg/kg po, produced a concentration versus time profile with a plateau of approximately 10 µg/ml from 0.5–3 h, followed by a decline with an 85 min half-life. The oral bioavailability of THU was approximately 20%. The 20% oral bioavailability of THU is sufficient to produce and sustain, for several hours, plasma concentrations that inhibit CD. This suggests the

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feasibility of using THU to decrease elimination and first-pass metabolism of cytidine analogues by CD. THU pharmacokinetics are now being evaluated in humans.

Keywords

Tetrahydrouridine; Bioavailability; Dose-linearity; Metabolism; Mouse; Cytidine deaminase

Introduction

Important anticancer agents, such as cytosine arabinoside (Ara-C), gemcitabine, capecitabine, decitabine and 5-aza-cytidine, are cytidine analogues [23]. Historically, nucleoside analogues were developed as cytotoxic drugs. More recently, several cytidine analogues have been licensed (decitabine and 5-azacytidine), or are in development (5-fluoro-2'-deoxycytidine), based on their ability to inhibit methylation of DNA [2,12]. Cytidine analogues compete with endogenous cytidine and 2'-deoxycytidine and thereby have an impact on important biological processes such as DNA and RNA synthesis. Most cytidine analogue drugs are metabolized by cytidine deaminase (CD; EC 3.5.4.5) [9], and CD-mediated deamination is considered to be the main cause for the short in vivo half-lives of these drugs [8,19,25,36]. Of the anticancer agents, Ara-C, gemcitabine, decitabine, 5-azacytidine, 5-fluoro-2'-deoxycytidine, 5-chloro-2'-deoxycytidine and capecitabine, only deamination of capecitabine is a desired metabolic conversion. The deaminated metabolites of the other agents are either inactive or toxic. With the exception of capecitabine, cytidine analogues are administered parenterally. Oral dosing would lead the drug through the gastrointestinal tract and the liver, which have high levels of CD, thereby limiting the amount of drug that reaches the systemic circulation and resulting in low oral bioavailability. 3,4,5,6-tetrahydrouridine (THU, Fig. 1) is a transition-state analogue of cytidine to uridine [6] that binds tightly to CD and thereby competitively inhibits deamination with a reported K_i of 28–240 nM (7–60 ng/ml) [6,27,42]. By potentially inhibiting CD, THU can prolong the half-life of i.v.-administered cytidine analogues, and, by decreasing first-pass metabolism in the gut and liver, THU can increase the bioavailability of cytidine analogues administered po [2,8,19,25,34]. This latter property may enable convenient, chronic po dosing of cytidine analogues. However, optimal preclinical development and subsequent clinical use of THU in combination with cytidine analogues require characterization of THU pharmacokinetics and oral bioavailability.

Although THU has been applied as a modifier of cytidine metabolism in several clinical trials [3,25,26,30,36,44], lack of suitable analytical methodology has prevented adequate characterization of THU pharmacokinetics, and the oral bioavailability of THU has never been fully characterized. Because THU has no chromophore, assays to date have inferred THU concentrations from bioassays that measure the reduction in deamination of a substrate or from total radioactivity measured after administration of 2-[^{14}C]-THU (which would count THU metabolites as if they were THU) [14,20,21,26]. Such assays lack specificity and are only acceptable as a method of quantitation if nothing else is available. During the earlier development of THU for clinical use, mass spectrometry had not been adopted as a mainstream detection method in the quantitation of analytes in biological matrices. Now widely available, mass spectrometry is excellently suited to detect an analyte such as THU and, therefore, was utilized in the presented investigation.

We aimed to characterize THU pharmacokinetics and oral bioavailability in mice, using a recently developed specific and sensitive high performance liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) assay [38] that had been validated according to FDA guidelines [43]. Our goal was to generate data that would assist in planning subsequent pharmacokinetic studies of THU in humans.

Materials and methods

Chemicals and reagents

THU (THU:[D_4]-THU ($D_0:D_4$) > 99.9%) was provided by the Developmental Therapeutics Program, National Cancer Institute (Rockville, MD, USA). D_4 -THU internal standard ($D_4:D_0$ > 99.9%) was synthesized by Ash Stevens Inc. (Detroit, MI, USA) and provided by the Developmental Therapeutics Program, National Cancer Institute. All solvents were obtained from Fisher Chemicals (Fair Lawn, NJ, USA). Formic acid was obtained from Sigma-Aldrich (St Louis, MO, USA). All chemicals were of analytical grade. Water was purified using a Q-gard® 1 Gradient Milli-Q system (18.2 M Ω .cm, Millipore, Billerica, MA, USA). Control murine plasma was obtained from Lampire Biological Laboratories (Pipersville, PA, USA).

Animals

Specific-pathogen-free, adult CD₂F₁ male mice were purchased from Taconic (Germantown, NY, USA). The mice were allowed to acclimate to the University of Pittsburgh Cancer Institute Animal Facility for ≥ 1 week before being used. To minimize infection, the mice were maintained in micro-isolator cages in a separate room and handled in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and on a protocol approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Ventilation and airflow were set to 12 changes/h. Room temperatures were regulated at $22 \pm 1^\circ\text{C}$, and the rooms were kept on automatic 12 h light/dark cycles. The mice received Prolab ISOPRO RMH 3000 Irradiated Lab Diet (PMI Nutrition International, St Louis, MO, USA) and water ad libitum, except on the evening before dosing, when all food was removed. The mice were 6–8 weeks old and weighed approximately 20 g at the time of dosing. Sentinel animals were maintained in the rooms housing the study mice and assayed at 3-month intervals for specific murine pathogens by mouse antibody profile testing (Charles River, Boston, MA, USA). The sentinel animals remained free of specific pathogens, indicating that the study mice were pathogen free.

Plasma disposition

To investigate the plasma pharmacokinetics of THU, the mice were dosed with THU, dissolved in 5% dextrose (Baxter, Deerfield, IL, USA), iv by lateral tail vein injection at 100 mg/kg (dosing solution 10 mg/ml; 0.01 ml/g) or po by oral gavage at 30, 100 or 300 mg/kg (dosing solutions of 3, 10 and 30 mg/ml, respectively; 0.01 ml/kg).

Mice (three per time point) were euthanized with CO₂ at 2, 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, 960 and 1,440 min after dosing. Blood was collected by cardiac puncture into heparinized syringes and centrifuged for 5 min at 13,000g to obtain plasma. Plasma samples were stored at -70°C until analysis.

THU concentrations in plasma were quantitated with a previously developed and validated hydrophilic interaction chromatography (HILIC) LC-MS/MS assay [38]. The assay fulfilled the FDA criteria for bioanalytical method validation [43] and was accurate and precise in the concentration range of 0.2–50 $\mu\text{g/ml}$.

Urinary excretion

To assess urinary excretion of THU, the mice scheduled for euthanasia at 16 and 24 h were kept in metabolic cages after dosing. Urine was kept on ice and collected separately at 6 and 24 h after dosing for the 24 h mice, and after 16 h for the 16 h mice. At the end of the collection period, the cages were washed with 15 ml of water. Quantitation of THU in urine and cage wash was accomplished by diluting (at least 10-fold) an aliquot of each sample with control

plasma and analyzing that diluted sample with the HILIC LC-MS/MS assay used for plasma samples [38].

Plasma protein binding

THU was added to mouse plasma to produce concentrations of 0.3 and 3 µg/ml. Aliquots of 1 ml of each test solution were added to Centrifree micropartition devices (Millipore, Bedford, MA, USA) with a 30,000 D molecular weight cut-off (typically $N = 3$), which were centrifuged for 20 min at 1500g and room temperature. A 100 µl aliquot of each protein-free ultrafiltrate was stored at -20°C until analysis for THU concentration. In addition, aliquots of the solutions before filtration were taken for quantitative determination of the initial THU concentration. To assess non-specific adsorption of THU to the micropartition device, the same experiments were performed in phosphate-buffered saline (PBS) instead of plasma. THU in ultrafiltrate and PBS were quantitated relative to a plasma calibration curve.

Pharmacokinetic analysis

The maximum THU plasma concentration (C_{max}) and the time to reach it (T_{max}) were determined by visual inspection of the data. Other pharmacokinetic parameters for THU after iv or po dosing were calculated non-compartmentally using WinNonlin Professional, version 4.1 (Pharsight Corporation, Mountain View, CA, USA). The area under the plasma concentration versus time curve of THU was calculated with the linear trapezoidal rule. The half-life was derived from the slope of at least the last two points of the log concentration versus time curve. This half-life was also used to extrapolate the area under the THU plasma concentration versus time curve (AUC) to infinity.

THU concentration versus time data were also analyzed compartmentally using the ADAPT II software for pharmacokinetic/pharmacodynamic systems analysis [7]. First, the most suitable model was selected using the THU plasma concentrations after iv administration. In addition, one overall model was fit to all the data (iv and the three po dosages) under the assumption that these doses were administered to a single individual. The approach included the use of 2- and 3-compartment models with both linear and non-linear absorption and estimation of the bioavailability. The maximum likelihood option in ADAPT II was used for all estimations (intercept 0.00154; slope 0.06006; both allowed to float upon modeling), and model selection was based on Akaike's Information Criterion (AIC) [1].

Results

The concentration versus time profiles of THU in plasma after administration of THU iv or po at various doses are shown in Fig. 2. Pharmacokinetic parameters resulting from non-compartmental analysis are shown in Table 1. After iv administration, peak THU concentrations were observed at the first time point (2 min). Thereafter, plasma concentrations declined multi-exponentially with a terminal half-life of approximately 73 min. THU could be detected until 240 min after dosing (the iv study did not include mice sampled at 360 min). After iv delivery, THU clearance was 9.1 ml/min/kg, and the volume of distribution was 0.95 l/kg.

After po dosing, THU plasma concentrations rose rapidly during the first 30 min, remained relatively constant for approximately 2.5 h, and then decreased (Fig. 2). Plasma THU concentrations increased with the dose of po THU, but the rate of decline in plasma THU concentration was similar in all three studies of po THU. Non-compartmental pharmacokinetic parameters and oral bioavailability (relative to the iv dose of 100 mg/kg, based on $\text{AUC}_{0-\text{inf}}$) for each po dose of THU are displayed in Table 1. The T_{max} after po dosing was between 45 and 105 min but could not be determined more accurately because of the flat THU concentration

versus time profile. The estimated THU half-lives varied from 62 to 92 min, and bioavailabilities after po dosing varied from 15 to 23%.

We also performed compartmental analyses on the plasma concentration versus time data. Initially, the earliest sampling time point after iv dosing of THU was 5 min. Fitting either a two- or three-compartment model to the iv data yielded nearly identical results. The two- and three-compartment fits resulted in AIC values of 423 and 418, respectively, indicating a slight preference for the three-compartment model. However, the parameters obtained for the two-compartment model were precisely estimated with coefficients of variation (CV) <6%, while those of the three-compartment model had CVs ranging from 6.7 to 59%. The addition of the third compartment resulted in slightly higher estimations for the plasma THU concentrations from 0 to 5 min after dosing. To determine which model was correct, we dosed additional mice and euthanized them at 2 min, along with extra 5 min mice to justify using these data with the previously generated iv data. The plasma THU concentrations of the additional mice euthanized at 5 min agreed with those of the mice euthanized at 5 min in the earlier study, which provided confidence in combining the data from the two separate experiments. Compartmental modeling of this expanded data set showed a preference for the three-compartment model over the two-compartment model, with AIC values of 484 and 1004, and R^2 values of 0.978 and 0.791, respectively. However, the data could still not inform the three-compartment model enough to obtain reliable estimates of the parameters, which had CVs ranging from 11 to 796%. Subsequently, a single model was fit simultaneously to the data from the iv and the three po studies. The structure of that model, a three-compartment model that allows for different bioavailabilities (F) for each oral dose, is shown in Fig. 3a. The individual F values can be calculated from the dose and the amount of THU that was eliminated via k_{40} and therefore did not reach the plasma compartment. The best model fit to the data and the associated parameters are shown in Fig. 3b–e and Table 2, respectively. In the final model, the transfer from compartment 4 to the central compartment is non-linear, and the elimination function for THU that never reaches the central compartment is driven by compartment 4. We tested the models where the non-linearity was put between compartment 5 and 4, or the elimination rate was driven by compartment 5. These models resulted in worse model fits to the data (not shown).

Urinary excretion of THU during the first 16 h after dosing was 55% of the dose after iv administration and varied from 9.7 to 15.8% after po dosing (Table 3). This corresponded to a THU renal clearance of 5.0–6.2 ml/min/kg. Urinary excretion of THU during the first 24 h was slightly more variable and translated into a THU renal clearance of 3.2–10.4 ml/min/kg.

There was negligible mouse plasma protein binding of THU as the bound percentages of 0.3 and 3 μ g/ml solutions of THU in mouse plasma [12.3% ($\pm$ 8.3) and 13.9% ($\pm$ 6.4), respectively] were no different from those of 0.3 and 3 μ g/ml THU solutions in PBS [13.8% ($\pm$ 2.7) and 13.4% ($\pm$ 8.2), respectively].

Discussion

THU, in combination with cytidine analogues, has been studied preclinically and clinically [3,8,18,19,25,26,30,36,44]. However, lack of suitable analytical methodology has prevented adequate characterization of THU pharmacokinetics and oral bioavailability. This has impeded the exploration of its full potential as a modifier of cytidine metabolism. Using a recently developed specific and sensitive analytical method for the quantitation of THU [38], we characterized THU plasma pharmacokinetics and oral bioavailability in mice.

With a terminal half-life of more than 1 h, plasma elimination of THU is slow relative to other pyrimidines like 5-fluorouracil (7 min [28]), gemcitabine (17 min [41]) or its closest analogue,

uridine (approximately 5 min [39]). The plasma half-life of THU determined in the current study agrees with earlier reports of plasma terminal half-lives of [^{14}C]-THU administered to mice (40 min), rats (approximately 60 min), dogs (65 min), monkeys (70 min) and humans (approximately 1 h) [10,26]. This is consistent with THU not being a substrate for thymidine phosphorylase (EC 2.4.2.4) or uridine phosphorylase (EC 2.4.2.3), the enzymes that might be expected to cleave the ribose from the tetrahydrouracil moiety as they do with uridine [9]. Consistent with earlier reports, THU exhibited a multi-exponential decay pattern in plasma [10,21,26]. The THU plasma concentrations were achieved after the three different po doses increased with dose. Based on the model fitted to the observed data, the absorption kinetics of THU appears to be slightly non-linear in the dose range studied. However, the relatively close values of 22.9–16.8% with percent CV > 15% of only three dose levels do not allow a firm conclusion, and the relevance of a saturation effect within the dose range would be limited.

After absorption, THU appears to be distributed uniformly in plasma and tissues, as indicated by the distribution volume of approximately 1 l/kg. Volume V_1 of 42 ml/kg, calculated compartmentally, corresponds with the blood volume of mice. The distribution volume of 1 l/kg suggests little, if any, tissue binding of THU. Similarly, THU had negligible binding to mouse plasma proteins, which agrees with the reported absence of human plasma protein binding of [^{14}C]-THU total radioactivity [26]. These data and the extensive urinary excretion of un-metabolized THU suggest that plasma THU concentrations are reasonably indicative of THU tissue concentrations. This is consistent with similar levels of total radioactivity in serum, liver, intestine and spleen, previously described in mice dosed i.p. with 50 mg/kg [^{14}C]-THU [10]. It is also comparable with levels of CD inhibition reported in various mouse tissues 30 min after an i.p. dose of 75 mg/kg THU [31]. Specifically, CD inhibition was 98% in serum, 84% in spleen, 98% in liver, 84% in bone marrow, 68% in intestine and 93% in 10-day-old s.c. Lewis lung carcinoma. The tissue concentration of THU and its inhibition of CD are relevant because without THU, cancer stem cells may find sanctuary from cytidine analogues in organs such as liver, spleen and intestinal epithelium, which have high CD levels [27]. Moreover, increased levels of CD in leukemic cells were observed at the time of relapse following Ara-C or decitabine therapy [27]. THU may reverse this resistance and has been shown to increase Ara-C triphosphate formation in a leukemic cell line that contains high levels of CD [17]. Complete inhibition of CD in vitro has been reported to require THU concentrations of $\geq 1 \mu\text{g/ml}$ [10,11]. Such THU plasma concentrations were reached and maintained for at least 4 h with all doses used in the present investigation. In monkeys, CD was shown to be completely inhibited for up to 2 h after iv THU doses of 12.5–100 mg/kg [26,32], which are equivalent to 50–400 mg/kg in mice and 4–33 mg/kg in humans [13]. The THU dose of 100 mg/kg used in mice in the current investigation corresponds to a human dose of approximately 8.3 mg/kg or 300 mg/m² [13], which is exactly the dose currently applied in clinical trials utilizing THU [3,36].

At 20%, the oral bioavailability of THU in mice is relatively low, but still higher than the 7% oral bioavailability of uridine in mice [24] and, more importantly, high enough to allow relevant plasma concentrations of $\geq 1 \mu\text{g/ml}$ to be achieved [10]. Human oral bioavailability, inferred from relative urinary excretion of total radioactivity after a po and iv dose of 50 mg/kg [^{14}C]-THU, is reported to be approximately 10–14% [21,26]. However, total radioactivity does not represent only THU. After iv dosing of THU to humans, only one or, depending on the assay technique, two distinct analytes were observed in urine; but after po dosing, three to four analytes were detected in urine [26]. Obviously, before po THU can be used clinically, the disposition and oral bioavailability of THU in humans must be defined at doses that produce pharmacologically relevant plasma concentrations.

Already in 1965, it was suggested “It would appear to be therapeutically wiser to administer arabinoside cytosine (Ara-C) parenterally than orally, ... and, similarly, it might be possible

to prolong Ara-C blood levels ... with an inhibitor of deaminase activity.” [4]. However, co-dosing of THU with cytidine analogues has potential effects on various levels. First, THU can improve the po absorption of a cytidine analogue into the systemic circulation by reducing the first-pass metabolism by intestinal and liver CD [34,35]. Second, THU can increase, and prolong, plasma concentrations of cytidine analogues by reducing systemic metabolism by CD [2,25]. Third, THU can make organs with high CD activity therapeutically accessible to cytidine analogues [17,27]. Finally, THU can affect the intracellular pharmacokinetics and, thereby, ultimately increase the concentrations of the active phosphate anabolites of cytidine analogues [5,15,17,22,37,40].

While THU can prevent cytidine analogues from being metabolized to an inactive compound, in certain cases THU inhibition of CD-mediated metabolism can be relevant in a different way because the products of CD-mediated metabolism may be toxic instead of inactive. Uracil arabinoside is associated with Ara-C toxicity of the central nervous system [29,33], and 5-fluoro-2'-deoxyuridine and 5-fluorouracil are associated with undesirable cytotoxic effects related to 5-fluoro-2'-deoxycytidine [2].

The urinary excretion of approximately 50% of the i.v.-administered THU dose observed in the present investigation is roughly half that reported previously [10] in mice, rats, dogs, monkeys and humans [21,26]. A possible explanation for this discrepancy is the presence of metabolites of THU that were indiscriminately quantitated with the previously available, radioactivity-based assays, but were not quantitated with our specific LC-MS/MS assay. The variability in the percentage of the THU dose recovered most likely reflects a combination of biological variability (e.g., fluid and food intake of the respective mice) and experimental variability (incomplete collection of urine and/or THU adsorbed on the metabolic cages).

THU has a very good safety profile in a variety of animal species. Dogs and monkeys tolerate doses as high as 1,000 mg/kg [8,13,16]. Furthermore, iv and po THU doses up to 50 mg/kg (in some cases for up to 23 days at 50 mg/kg/day iv) have been used clinically without any signs of toxicity [26,44].

The data presented here suggest the feasibility of using THU to decrease elimination and first-pass metabolism of cytidine analogues by CD. The plasma half-life and oral bioavailability of THU appear sufficient to allow po co-administration with active cytidine drugs. THU appears to be relatively safe and has a long history of clinical use, albeit in an investigational setting. Ongoing investigations are evaluating THU pharmacokinetics in humans, and future investigations will focus on evaluating oral co-dosing of THU with various cytidine analogues.

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Fig. 1.
Chemical structure of THU and its inhibitory action on cytidine deaminase

Fig. 2.

Mean plasma concentration ($\pm$ SD, $N = 3$ /time point) versus time curves of THU after iv administration at 100 mg/kg (*filled triangle*) or po administration at 30 mg/kg (*open diamond*), 100 mg/kg (*open triangle*) or 300 mg/kg (*open square*) to male, CD₂F₁ mice

Fig. 3.

A compartmental model (a) was constructed and fit to THU mouse plasma concentration data (filled circle, mean ($\pm$ SD)) after administration of 100 mg/kg iv (b), 300 mg/kg po (c), 100 mg/kg po (d), or 30 mg/kg po (e). The entire po THU dose is delivered to compartment 5, is transferred to compartment 4 (k_{54}), and from there is either eliminated (k_{40} , never absorbed or pre-systemically metabolized) or taken up into the central compartment V_1 via a non-linear transfer rate (V_{max} , K_m). Distribution takes place to compartments 2 and 3 by their respective rate constants (k_{12} , k_{21} , k_{13} , k_{31}), and elimination from V_1 is determined by k_{10} .

Table 1Non-compartmental pharmacokinetic parameters of THU after iv or po administration to male CD₂F₁ mice

Parameter	Route (dose, mg/kg)			
	iv (100) ^c	po (30)	po (100)	po (300)
$C_{\max}$ (µg/ml)	814	3.1	13.7	20.8
$T_{\max}$ (min)	2	105	45	45
Half-life (min) ^a	73	92	85	62
AUC _{0–240} (mg/ml min)	10.8	0.470	2.01	3.93
AUC _{0–inf} (mg/ml min) ^b	11.0	0.633	2.57	4.82
Vd/F (l/kg)	0.953	6.28	4.78	5.53
Cl/F (ml/min/kg)	9.06	47.4	38.9	62.2
F (%)	—	19.1	23.3	14.6

Vd volume of distribution, F oral bioavailability, i.e., the fraction/percentage of the administered dose that reaches the systemic circulation or plasma compartment, relative to when the drug is administered iv (for iv administration F is assumed to be unity/100%), Cl total body clearance

^aThe half-lives were derived from the slope of the last points in the log concentration versus time curve. The last three time points were used, except for the 100 mg/kg po study where the last two points were used

^bThe percentage of the AUC_{0–inf} that was extrapolated from the observed AUC ranged from 2.1–22%

^cThe parameters calculated for the 100 mg/kg iv administration included the 2 min time point that was obtained later (see text)

Table 2

Estimated values for the parameters of the compartmental model shown in Fig. 3a

Parameter/model	Estimate (%CV)
V_1 (l/kg)	0.0422 (27)
k_{10} (1/min)	0.232 (21)
k_{12} (1/min)	0.389 (19)
k_{21} (1/min)	0.144 (20)
k_{13} (1/min)	0.0552 (19)
k_{31} (1/min)	0.0140 (19)
k_{54} (1/min)	0.00954 (27)
k_{40} (1/min)	0.0435 (18)
$V_{\max}$ (µg/ml/min)	12.6 (19)
K_m (µg/ml)	930.6 (22)
F (%)	
30 mg/kg po	22.9 (15.3)
100 mg/kg po	20.9 (15.4)
300 mg/kg po	16.8 (17.3)
R^2	0.976
AIC	742

See legend of Fig. 3 for explanation of the parameters

Table 3
Urinary excretion of THU at 0–16 h and 0–24 h after iv or po administration to male CD₂F₁ mice

Time (h)	Route (dose, mg/kg)	iv (100)	po (30)	po (100)	po (300)
0–16	Dose (mg/mouse)	2.17	0.555	2.22	6.26
	Excreted (mg/mouse)	1.20	0.0726	0.351	0.606
	Excreted (% dose)	55.4	13.1	15.8	9.69
	<i>Cl</i> _{renal} (ml/min/kg)	5.02	6.20	6.15	6.03
0–24	Dose (mg/mouse)	2.18	0.575	2.18	6.36
	Excreted (mg/mouse)	0.778	0.0971	0.233	1.06
	Excreted (% dose)	35.6	16.9	10.7	16.6
	<i>Cl</i> _{renal} (ml/min/kg)	3.23	8.01	4.16	10.4