Towards a test to predict 5-fluorouracil toxicity: Pharmacokinetic data for thymine and two sequential metabolites following oral thymine administration to healthy adult males

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Abstract

The fluoropyrimidine drugs 5-fluorouracil and its oral prodrug capecitabine remain first line therapy for solid tumours of the neck, breast and colon. However, significant and unpredictable toxicity affects about 10-25% of patients depending upon the mode of 5-fluorouracil delivery. The pharmacokinetics of thymine (5-methyluracil) may provide an approach for screening for 5-fluorouracil toxicity, based on the rationale that thymine is a close structural analogue of 5-fluorouracil and is catabolized by the same enzymatic pathway. Oral thymine loading tests were performed on 12 healthy volunteers. Each subject was given a single oral dose of 250 mg thymine in capsule form. Blood, urine and saliva samples were collected pre-dose and up to 5 h post-dose. Concentrations of thymine, and its catabolites dihydrothymine and β -ureidoisobutyrate were analysed by HPLC-tandem mass spectrometry in plasma, urine and saliva. The pharmacokinetic data of healthy volunteers were analysed assuming a non-compartmental model. Thymine peaked quickly (30-45 min) in plasma to a maximum concentration of 170 ± 185 $\mu\text{g/L}$ (mean $\pm$ SD). Clearance was high (mean 57.9 L/h/kg) exceeding normal human liver blood flow, suggesting low systemic bioavailability; urinary recovery of the thymine dose was low (<1%). Apparent formation rate-limited kinetics were observed for dihydrothymine, and the plasma concentration of dihydrothymine was consistently 10-fold higher than that of thymine. Plasma β -ureidoisobutyrate concentrations, on the other hand, were similar to that of thymine. Genotyping confirmed that pathological mutations of the *DPYD* gene were absent. The urinary excretion ratio of thymine/dihydrothymine was informative of the maximum concentration. Saliva thymine was highly variable. These data are potentially useful as a basis for developing of a screening procedure to prospectively identify patients who are at risk of toxicity from fluoropyrimidine drugs.

1. Introduction

The fluoropyrimidine drugs remain an important class of anticancer drugs and are an essential component of chemotherapy for solid tumours. 5-fluorouracil (5FU) is used as first line therapy for tumours of the head and neck, colon and rectum (Longley et al., 2003) and for adjuvant treatment in breast cancer (i.e. FAC, FEC). Capecitabine, an oral prodrug of 5FU, is utilised as single-agent therapy for breast and bowel cancer (Walko & Lindley., 2005). Within the normal population there is variation in the response to 5FU and capecitabine, with most patients experiencing some diarrhoea, mild stomatitis and nausea. More recent constant infusion modalities have lowered the rate of severe toxicity, but injurious or life-threatening mucositis or bone marrow suppression still occurs with standard doses of 5FU in approximately 11% of patients on infusion therapy and 25% with bolus therapy (Andre et al., 2003; Twelves et al., 2005).

Part of the variability of treatment response to fluoropyrimidines, in particular toxicity, can be attributed to pharmacogenetic factors. However, despite several decades of effort, a routine method for predicting which individuals will be susceptible to significant grade 3 and 4 toxicity from 5FU or capecitabine is not established. The basic pharmacological assumption for 5FU toxicity here is that it arises from increased systemic exposure to 5FU as a result of reduced clearance of 5FU and/or low catabolism via the endogenous pyrimidine pathway. Fig. 1 illustrates how pyrimidine – and 5FU – catabolism involves a multi-step pathway. Efforts to redress the issue of 5FU toxicity have focussed on a genetic deficiency of the first catabolic step dihydropyrimidine dehydrogenase (DPD), which in humans is encoded by the *DPYD* gene. However, partial deficiency of DPD activity explained retrospectively only about one-third to one-half of recorded cases of severe 5FU toxicity, limiting the sensitivity and utility of any prospective DPD screening test (van Staveren et al., 2013). Therapeutic drug monitoring (TDM) of 5FU is inconsistently utilised because administration of a test dose of 5FU is almost universally avoided due to the perceived risk of inducing tumour tolerance, while administration of a full dose for TDM does not avoid the usual clinical risk of toxicity, which can be life-threatening.

Sumi and coworkers (1998) demonstrated the possibility of detecting carriers of DPD and dihydropyrimidinase deficiencies by measuring basal urinary excretion of thymine and dihydrothymine, having found that neither urinary uracil nor dihydrouracil concentration readily detects carriers. Accordingly, we proposed that thymine might provide a better approach for screening for deficiency of DPD and the second catabolic step,

dihydropyrimidinase, based on the following rationale: (i) Thymine (5-methyluracil) is a close structural analogue of 5FU and is catabolized by the same enzymatic pathway (Fig. 1); (ii) Compared to uracil, endogenous thymine concentrations in human urine and plasma are much lower and less variable (Simmonds et al., 1990; Ni et al., 2013), which would result in a lower level of endogenous metabolic ‘noise’, thereby potentially providing increased sensitivity; (iii) Purified thymine is inexpensive, readily available, and is stable at room temperature over long periods; (iv) Sparse blood level data published more than 50 years ago (Spray, 1958) indicated that thymine appears to be rapidly absorbed and eliminated after oral dosing, and has a short elimination half-life which would potentially make a PK-based screening test convenient for cancer patients; (v) thymine is a naturally occurring compound in humans which appears safe in oral doses up to at least 2 g (Spray, 1958); (vi) There is no salvage of thymine into nucleic acids or other synthetic pathways which would otherwise confound any PK data.

Therefore, with the aim of developing a new PK-based screening test for fluoropyrimidine toxicity, we collected preliminary data on the human PK disposition of thymine and its sequential metabolites dihydrothymine and β -ureidoisobutyrate, following administration of an oral dose of thymine to twelve healthy volunteers.

2. Participants and methods

2.1 Participants

Prior ethics approvals were obtained from the Human Research Ethics Committees of Mater Health Services, and The University of Queensland. All subjects had provided signed informed consent. Twelve male Caucasian volunteers, aged 37-58 years (mean $\pm$ SD = 49 $\pm$ 8.4), were recruited. All volunteers were non-smokers, moderate or non-drinkers, and were healthy based on physical/medical assessments: mean height 1.8 $\pm$ 0.036 m, weight 82 $\pm$ 5.8 kg and BMI 25.5 $\pm$ 1.34 kg/m². ECG, serum biochemistry and haematology testing, as well as a physical examination by a physician, were conducted within two days of the study date. None of the subjects had a history of drug hypersensitivity or a clinically significant illness up to at least four weeks before the study nor had any subject been hospitalized during the previous three months.

2.2. Materials

Commercially available powdered thymine (>99% purity, Sigma-Aldrich Ltd, Sydney, NSW, Australia) was verified for its suitability for human oral consumption according to specifications of the British Pharmacopeia 2004. Physical, microbiological and chemical analyses, including by HPLC-linked triple quadrupole mass spectrometry (LC-MS/MS), were undertaken and met the specifications of the Australian Therapeutic Goods Administration such that thymine was acceptable for clinical trial registration. Powdered dihydrothymine material was purchased from Sigma-Aldrich Chemical Co (St Louis, MO, USA). β -ureidoisobutyrate is not commercially available, therefore it was synthesised by the chemical reduction of dihydrothymine using the method of Fink and coworkers (1956), and tested for purity by melting point analysis and LC-MS/MS. All other reagents and standards used for HPLC were of analytical reagent quality.

2.3. Dosing and sampling

On the study day, the subjects had a meat-free, light breakfast approximately 2 h before thymine administration, and were closely observed by medical staff during, and 1 h after, the blood sampling period. Powdered thymine (250 mg) was encapsulated using a capsule-filling machine by hospital pharmacy staff, and involved no excipients. The thymine was administered in a single hard gelatin capsule to avoid oral contamination of saliva. Each subject swallowed the capsule with 100 mL of tap water at approximately 09:00. Blood was drawn via an indwelling venous catheter at 0 h (pre-dose), then nominally at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 and 5 h, post-dose. Mixed unstimulated saliva samples were obtained via passive drool into a plastic vial when blood samples were collected. Urine was collected nominally at 0, 1, 2, 3 and 5 h, post-dose. Water was allowed *ad libitum* during the sampling period and a light lunch was provided 3 h post-dose.

2.4. Analyses of thymine, dihydrothymine and β -ureidoisobutyrate

Plasma and urine concentrations of thymine, dihydrothymine and β -ureidoisobutyrate were measured using a validated rapid method based on LC-MS/MS, using an atmospheric pressure chemical ionisation (APCI) interface, as described previously by some of us (Ni et al., 2013). This method also was applied with minor modifications to analyse thymine and dihydrothymine in saliva samples. Briefly, saliva was ultrafiltered to remove particulate matter and mucin by centrifuging in 0.5 mL, 50 kDa cutoff Amicon ultrafilters (Merck Pty. Ltd., Australia) at 9500 g for 10 min at room temperature. The ultrafiltrate was treated similarly to plasma and urine, by mixing 200 μ L of sample with 1 mL of an internal standard

(d4-thymine in acetonitrile), followed by vortex-mixing and centrifuging to remove any precipitate. The supernatant was evaporated to dryness under nitrogen gas, then redissolved in 150 μ L of 0.1% formic acid in deionised water. Salivary β -ureidoisobutyrate was not analysed, as its measurement had unacceptably high variability by this LC-MS/MS method (Ni et al 2013).

2.5. Pharmacokinetics

The pre-dose (natural baseline) plasma concentration (C_0), maximum plasma concentration (C_{max}), and time to reach the maximum plasma concentration (T_{max}) were recorded directly from the observed data. The area under the curve (AUC) was calculated by trapezoidal integration with linear interpolation between concentration-time data points; apparent systemic clearance (CL/F) and apparent volume of distribution (V/F) were calculated by non-compartmental analysis; the first-order elimination rate constant (λ) was estimated as the slope of the line of best fit of the terminal phase using linear regression of logarithm of concentration on time (T); terminal half-life ($T_{0.5}$) was calculated by the expression, $T_{0.5} = 0.693/\lambda$. The amount of thymine excreted in urine (X_{0-T}) was estimated by the product of the concentration multiplied by urine volume obtained in a given collection period summed over all collection periods to provide the cumulative amount excreted from the dosing time up to sampling time, T.

2.6. Genotyping of DPYD

DPYD exons and approximately 10 bases of each flanking intronic sequence were amplified by PCR. Subsequently, purified amplicons were sequenced and analysed for mutations essentially as described previously (van Kuilenburg et al., 2000). This covered the presence of known functional mutations; the deep intronic mutant c.1129-5923C>G/hapB3 (Amstutz et al., 2009) was not sequenced.

3. Results

There was marked inter-subject variability in the individual concentration-time profiles. The number of observed log-linear ‘compartments’ varied from one to three, which together with the limited number of data points precluded the use of non-linear regression. However, using non-compartment analysis the CL/F of thymine could be readily calculated from the $AUC_{0 \rightarrow T}$

data, corrected for the pre-dose plasma concentrations, since the respective values of the pre-dose and the post-dose concentrations at the final sampling time differed insignificantly ($P>0.05$).

Fig. 2 (panel A) shows mean ($\pm$ SD) plasma concentration data for thymine, dihydrothymine and β -ureidoisobutyrate at baseline (pre-dose) and after thymine administration. The short delay lag of approximately 15-30 min in the appearance of thymine in plasma and the absence of multiple peaks in most profiles suggested that there were few, if any, disintegration/dissolution limitations for encapsulated thymine, or ‘dose-dumping’ from the stomach into the intestinal tract.

Orally-administered thymine was well tolerated with no apparent adverse effects reported by the subjects or observed by the investigators. All subjects were genotyped for *DPYD* and no pharmacogenetic mutations (excluding the B3 haplotype) were found. Thymine was rapidly absorbed and attained a mean $\pm$ SD peak concentration (C_{max}) of 170 ± 185 $\mu\text{g/L}$ at 1.06 ± 0.415 h. The disappearance of thymine, dihydrothymine and β -ureidoisobutyrate from plasma was rapid; the terminal elimination phases of thymine and dihydrothymine were approximately parallel, with similar $T_{0.5}$ values (0.972 ± 0.470 h and 0.922 ± 0.373 h, respectively) indicating likely formation rate-limited kinetics for dihydrothymine from thymine.

The collective amount of thymine, dihydrothymine and β -ureidoisobutyrate in urine measured over the sampling period amounted to less than 0.5% of the dose. The PK parameters for thymine, dihydrothymine and β -ureidoisobutyrate in plasma and urine are summarised in Table 1 (Subjects 1 and 2 provided plasma and urine samples only to 3 h). The CL/F for thymine was 57.9 ± 49.4 L/h/kg, and V/F was 66.6 ± 59.4 L/kg.

Salivary concentration-time data, as shown in Fig. 2 panel B, were available only for thymine and dihydrothymine because of assay limitations for β -ureidoisobutyrate. Although the mean salivary profiles tended to track the corresponding plasma concentration-time profiles, there was very high inter-subject variability, including in the pre-dose concentrations. The salivary T_{max} values for thymine and dihydrothymine (1.0 ± 0.32 h and 1.2 ± 0.36 h respectively) were comparable to the corresponding plasma values (Table 1), while the mean salivary C_{max} values for thymine (760 ± 994 $\mu\text{g/L}$) and dihydrothymine (1056 ± 661 $\mu\text{g/L}$) were markedly higher than the respective plasma concentrations (Table 1). Fig. 3 shows a linear relationship between the ratio of total urinary thymine/dihydrothymine excretion over 3-5 h *versus* the

logarithm of thymine $C_{\max}$ (line-of-best-fit, $r = 0.87$); the relationship between urinary thymine versus log thymine $C_{\max}$ was also linear, demonstrating the effect of plasma thymine concentration on its excretion in urine but the linear correlation coefficient of the line-of-best-fit ($r = 0.81$) was slightly weaker.

Basal plasma dihydrothymine concentrations ranged from 2-fold to 9-fold higher than thymine suggesting that, since the half-lives of thymine and dihydrothymine were similar, dihydrothymine may have a smaller V/F than thymine. To test whether there were any differences in the distribution of thymine caused by exclusion of dihydrothymine from erythrocytes, the concentrations of thymine and dihydrothymine in both plasma and whole blood were measured in a sample from a subject taken at 0.75 h post-dose. Thymine concentrations were 230 $\mu\text{g/L}$ in plasma and 470 $\mu\text{g/L}$ in whole blood, while the dihydrothymine concentrations were 490 $\mu\text{g/L}$ and 1070 $\mu\text{g/L}$, respectively; therefore, the dihydrothymine/thymine ratios were similar in plasma (2.1) and in blood (2.3).

4. Discussion

Predicting 5FU toxicity has eluded researchers for decades, despite a strong clinical need for a simple test. Studies have focused on polymorphism of the DPD gene, but screening of *DYPD* has been complicated by the large size of the gene (23 exons) and that mutant alleles are diverse. The most commonly described loss-of-function polymorphism (*DYPD**2A: c.1905+1G>A) accounts for ~40% of cases of low *DYPD* activity and about a quarter (24-28%) of all cases of 5FU toxicity among most European Caucasians (van Kuilenburg et al., 2002; Yen and McLeod, 2007). A large number of other mutations have a much lower incidence (Offer et al., 2014). The *DPYD* c.1129-5923C>G/hapB3 deep intronic mutation has been associated with half of severe toxicity and a relatively high carrier rate of 4.6% in a Swiss population (Froehlich et al., 2015) but its applicability to other, non-Swiss populations requires confirmation. The low sensitivity of *DPYD* genotyping presently makes prospective genetic testing of patients unattractive, with genotyping not generally adopted for prospective testing (van Staveren et al 2013).

The need to seek alternative screening approaches to predict fluoropyrimidine toxicity has spawned various pharmacokinetic (PK) methods that have centred largely upon uracil as an endogenous marker, typically determining the plasma concentration ratio of uracil to its catabolite dihydrouracil (or vice versa) as a surrogate for DPD activity (Garg et al., 2002;

Jiang et al., 2004; Remaud et al., 2005; Ciccolini et al., 2006; Sistonen et al., 2014). More recently, a retrospective study administering oral uracil dose-loading to known carriers of DPD deficiency has provided encouraging results, identifying DPD-deficient patients from plasma uracil concentrations 60 min post-loading (van Staveren et al., 2011).

Our study reports the first human pharmacokinetic data of thymine together with its catabolites dihydrothymine and β -ureidoisobutyrate in healthy adult subjects. We found the basal concentration of thymine in plasma to be low, with a relatively small amount of variability (11 ± 3 $\mu\text{g/L}$), thus providing good sensitivity for the loading assay at low doses. This was in contrast to the wide discrepancies reported for basal plasma uracil concentrations, from 10 $\mu\text{g/L}$ (Remaud et al., 2005) to 5 mg/L (Jiang et al., 2002).

Thymine was rapidly absorbed following an oral 250 mg dose and reached peak plasma concentrations which averaged 16-fold higher than the baseline (pre-dose) concentration. Plasma concentrations then declined to baseline levels within 3-5 h, in a log-linear profile comprising up to three identifiable compartments. The actual CL and V could not be estimated since intravenous dosing of thymine was not possible, thus, the values of these parameters are predicated on the fraction (F) of the oral dose which is absorbed systemically from the gut. Likewise, the actual values CL and V of the sequential catabolites dihydrothymine and β -ureidoisobutyrate also depend on F, and also on the fraction of substrate enzymatically converted, respectively, from thymine to dihydrothymine, and from dihydrothymine to β -ureidoisobutyrate. The high estimated CL/F of 57.9 L/h/kg for thymine far exceeded normal human liver blood flow, suggesting low systemic bioavailability perhaps arising from either high first-pass clearance (Gu et al., 1998) and/or capacity-limited pyrimidine transport (active or facilitated) from the gut (Bronk and Hastewell, 1987; Wohlhueter et al., 1980; Li et al., 2002).

Interestingly, the mean CL/F of uracil following oral administration of a 500 mg/m^2 suspension to 11 healthy volunteers was 1.05 L/h/kg (van Staveren et al., 2011), which is 50-fold lower than we report here for thymine, despite elimination half-lives of uracil and thymine being comparable.

Human data for intravenous thymine has not been reported, however the average CL of thymine reported in healthy dogs following an intravenous bolus was 2.6 L/h/kg (Covey and Straw, 1983). It is reasonable to assume that since that CL per kg of 5FU in dogs and humans is comparable (Maring et al., 2002; Covey and Straw, 1983), then this comparison should

also hold for thymine, which is a close structural analogue of 5FU. If so, the systemic bioavailability of thymine in our subjects is estimated to be less than 5%.

DPD is widely distributed in the human body although the relative contributions of these tissues to metabolism is unknown, so thymine catabolism to dihydrothymine may also occur in tissues other than the liver (Naguib et al., 1985; Gonzalez et al., 1995; van Kuilenburg et al., 2006). We found that the baseline plasma concentrations of dihydrothymine were markedly higher than those for thymine (with a mean plasma dihydrothymine/thymine ratio of 8.1). Plasma dihydrothymine remained higher than thymine after dosing, which suggested rapid conversion of thymine to dihydrothymine, then dihydrothymine to β -ureidoisobutyrate, soon after thymine reaches the systemic circulation. Moreover, the parallel terminal elimination phases, ranging from 3-5 h in most subjects for thymine and dihydrothymine, suggested formation rate-limited kinetics for the conversion of thymine to dihydrothymine, and to a lesser extent for dihydrothymine to β -ureidoisobutyrate. In contrast, the ratio of basal plasma dihydrouracil/uracil in healthy adults varies greatly in other reports, from a mean of 0.67 (Ciccolini et al., 2004) to 9.05 (Remaud et al., 2005). An explanation of the wide discrepancies in these ratios may be methodological, as HPLC with UV detection below 210 nm is poorly selective in the assay of biological matrices (Ciccolini et al., 2004). Following uracil oral loading of normal volunteers, plasma dihydrouracil does not track uracil, and was far lower than uracil for up to 120 min (van Staveren et al., 2011), which contrasts with our thymine data.

Assuming that all dihydrothymine in plasma was formed only from thymine metabolism, one explanation for our observation is that the volume of distribution of dihydrothymine is much less than for thymine, as has been rationalised for some other drugs exhibiting similar profiles, e.g. propranolol (Walle et al., 1979). By contrast, the plasma concentrations of β -ureidoisobutyrate were only slightly less than the corresponding orally-administered thymine concentrations. Since plasma and not whole blood was used as the analytical matrix, there was a possibility that these observations were caused by a differential distribution between formed elements (e.g. erythrocytes) and plasma, since purine nucleobases (e.g. adenine, guanine) may be actively transported into erythrocytes via high-affinity facilitated diffusion (Domin et al., 1988). However, data from one of our subjects showed that the concentrations of both thymine and dihydrothymine following dosing were approximately 2-fold higher in whole blood than plasma, demonstrating that there did not appear to be a selective exclusion of dihydrothymine from erythrocytes. In support, uracil, which has a similar molecular

structure to thymine, has been shown by others to have a low affinity ($K_m \sim 5,800 \mu M$) for erythrocyte permeation (Domin et al., 1993).

Alternately, the high plasma dihydrothymine/thymine ratio may be based on differences in the affinities of the enzymes that break down thymine and dihydrothymine, i.e. DPD and dihydropyrimidinase, respectively. Lu and coworkers (1992) reported V_{max}/K_m values (i.e. as a measure of enzyme efficiency) of purified human liver DPD for thymine and uracil of 122 and 146 L/min/mg, respectively, which are comparable with the values reported by them for purified DPD from rat liver (333 and 240 L/min/mg), and bovine liver (179 and 130 L/min/mg). Although human liver dihydropyrimidinase has not been purified, much lower V_{max}/K_m values for dihydrouracil and dihydrothymine were reported by Kikugawa and coworkers (1994) for purified rat liver, and by Brooks and coworkers (1983) for bovine liver dihydropyrimidinase (65 and 36 L/min/mg, respectively). Given the constant bodily flux of these pyrimidines through the catabolic pathway, it can be predicted that the dihydropyrimidine form will be constantly elevated because dihydropyrimidinase, rather than DPD, acts as the rate-limiting enzyme due to its lower substrate affinity and efficiency; this conclusion has been supported by others (Traut and Loechel, 1984; Naguib et al., 1985).

Less than 1% of the administered thymine dose was excreted unchanged in urine. In contrast, Spray (1956) administered a dose which was 8-fold higher than used here, and reported that approximately 9% of the dose was excreted over 3 h, although those data were obtained using a microbiological assay that had disadvantages, particularly lower sensitivity, compared to state-of-the-art chromatography. Nonetheless, this raised the possibility of dose-dependent saturation of the catabolism of thymine (and hence 5FU) at higher doses, as reported in dogs (Covey and Straw, 1983). A dose-ranging PK study to test this hypothesis in humans was not possible during our investigations. However, van Staveren and coworkers (2011) reported two doses for a uracil loading test: 500 mg/m² (i.e. approximately 900-1000 mg, or 4-fold higher than our dose) and 1000 mg/m² (approximately 8-fold higher than used here) and found linear PK, thereby providing evidence that there was no saturation of the catabolic path by uracil at these higher doses.

Urinary excretion of thymine factored by dihydrothymine excretion (i.e. urine thymine/dihydrothymine) was predictive of the C_{max} for thymine. It would be expected that urinary clearance of thymine would be a predictor of its C_{max} , because renal clearance is partially a function of plasma concentration, but the correlation of C_{max} was lower with

urinary thymine alone. Further study of the urinary thymine/dihydrothymine ratio is warranted, as this may provide a simpler method of predicting fluoropyrimidine toxicity, in combination with a thymine load, compared to blood sampling. In one study, the mean basal urinary ratio of uracil/dihydrouracil in 167 healthy subjects was 63.3/23.8, i.e. 2.66 (Hayashi et al., 1996); this value is within our reported range of 0.46-2.79 for the basal urinary ratio of thymine/dihydrothymine (Fig. 3).

Saliva collection was included in the present study protocol because it provides a potentially more convenient and safer alternative to venepuncture, especially in any prospective test for pyrimidine toxicity. Although there was a general trend for the mean salivary concentration-time data of thymine and dihydrothymine to track the mean plasma profiles, there was high inter-subject variability in the saliva concentrations. In particular, salivary thymine concentrations were mostly higher than dihydrothymine, which contrasted with our plasma data where basal dihydrothymine levels were consistently much higher than thymine, demonstrating that saliva is not simply a filtrate of plasma with respect to pyrimidine bases. Therefore, further investigations using saliva as the biological sample matrix were discontinued.

5. Conclusion

The PK for thymine and its catabolites dihydrothymine and β -ureidoisobutyrate were studied following administration of a 250 mg oral dose of thymine to healthy adult volunteers, and provisional ranges for PK parameters were established. Baseline plasma dihydrothymine was approximately an order of magnitude higher than thymine, an interesting phenomenon that may arise from enzymatic flux through the catabolic pathway rather than volume of distribution influence. Urine recovery was low but the ratio of urinary thymine/dihydrothymine was informative. Importantly, these preliminary data will inform the development of a PK-based screen for fluoropyrimidine toxicity, which we are presently studying in cancer patients.

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6. Conflict of interest

The authors declare no financial interest.

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Footnotes:

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LEGENDS FOR FIGURES

Fig. 1. Catabolic pathway for 5-Fluorouracil (5FU) and Thymine (THY).

Fig. 2(A). Plasma concentration versus time. Plots show thymine (THY), dihydrothymine (DHT) and β -ureidoisobutyrate (UIB) after oral administration of 250 mg thymine to 12 healthy adult volunteers. Note the logarithmic scale of the concentration axis (data points are shown as the mean $\pm$ SD).

Fig. 2(B). Salivary concentration versus time. Plots show thymine (THY) and dihydrothymine (DHT) after oral administration of 250 mg thymine to 12 healthy adult volunteers (data points are shown as the mean $\pm$ SD).

Fig. 3. $C_{\max}$ values for plasma thymine (THY) against the ratio of urinary thymine/dihydrothymine (THY/DHT). The line of best fit is described by: $y = 0.882 \cdot \ln(X) - 2.76$; Pearson correlation coefficient, $r = 0.872$.

Figure 1

Figure 2A

Figure 2b

Figure 3

Table 1.

Plasma PK parameters of THY, DHT and UIB for 12 healthy male subjects

Parameter	THY		DHT		UIB	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
C_0 ($\mu\text{g/L}$)	10.6	(2.95)	85.5	(17.9)	8.91	(3.48)
$C_{\max}$ ($\mu\text{g/L}$)	170	(185)	550	(224)	27.5	(8.43)
$T_{\max}$ (h)	1.06	(0.415)	1.21	(0.334)	1.38	(0.42)
AUC ($\mu\text{g.h/L}$)	107	(97.5)	586	(310)	34.5	(12.7)
CL/F (L/h/kg)	57.9 ^a	(49.3)	7.16 ^b	(4.39)	107 ^c	(58.4)
V/F (L/kg)	66.6 ^a	(59.4)	8.21 ^b	(4.67)	422 ^c	(429)
$T_{0.5}$ (h)	0.972	(0.470)	0.922	(0.373)	2.48	(1.04)
X_{0-T} (mg)	0.587	(0.561)	0.380	(0.177)	0.228	(0.107)
λ (/h)	1.22	(1.68)	1.10	(1.25)	0.313	(0.0964)

THY: thymine; DHT: dihydrothymine; UIB: ureidoisobutyrate; C_0 : Pre-dose (baseline) plasma concentration; $C_{\max}$: Maximum plasma concentration; $T_{\max}$: Time to reach $C_{\max}$; AUC: Area-under-the-curve; CL/F: Clearance; V/F: Volume of distribution; $T_{0.5}$: Terminal half-life; X_{0-T} : Amount excreted in urine to final sample time T; λ : Elimination rate constant; F: Oral bioavailability of THY. ^aApparent CL and V predicated on F; ^bApparent CL and V predicated on F, and the fraction of thymine dose catabolized to DHT; ^cApparent CL and V predicated on F, the fraction of thymine dose catabolized to DHT, and the fraction of DHT catabolized to UIB.

Graphical abstract

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Towards a test to predict 5-fluorouracil toxicity: Pharmacokinetic data for thymine and two sequential metabolites following oral thymine administration to healthy adult males

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