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Pharmacokinetic Modeling of Species-dependent Enhanced Bioavailability of Trifluorothymidine by Thymidine Phosphorylase Inhibitor

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Summary: TAS-102, a new oral drug, is composed of an antitumor drug, α,α,α-trifluorothymidine (FTD), and its metabolic inhibitor, 5-chloro-6-(2-iminopyrrolidine-1-yl)methyl-2,4(1H,3H)-pyrimidinedione hydrochloride (TPI). It has been reported that the oral administration of TAS-102 increases the AUC of FTD in rodents and monkeys in different manners. In this study, a pharmacokinetic model was developed, in an attempt to evaluate the bioavailability of FTD in these animals after the co-administration of TPI. Since TPI inhibits FTD metabolism competitively, a time-dependent as well as concentration-dependent model for the hepatic intrinsic clearance of FTD was developed including the time courses of both FTD and TPI. Based on this modeling, we were able to quantitatively explain the TPI dose-dependent enhancement of AUC of FTD in monkeys, while little increase was observed in rats. These results are consistent to observations that thymidine phosphorylase (TPase) is predominantly expressed in monkeys; while uridine phosphorylase (UPase) is superior to TPase in rats. Since TPase is also predominantly expressed in humans, the pharmacokinetic model developed in this study can be used to explain the bioavailability of TAS-102 in humans.

Key words: TAS-102; FTD; TPI; simulation; drug-drug interaction

Introduction

α,α,α-trifluorothymidine (FTD) has potent antitumor and antiviral activities in humans,¹–⁴ but it is eliminated from the body with a half life of 12–18 minutes.⁵,⁶ Uridine phosphorylase (UPase) and thymidine phosphorylase (TPase) are enzymes that are responsible for the rapid clearance of FTD in mammals.⁷–¹⁰ It has been reported that TPase is expressed predominantly in monkeys and humans, while rodents have high UPase activities.¹¹–¹⁴

TAS-102, a new antitumor drug, contains a combination of FTD and a specific TPase inhibitor, 5-chloro-6-(2-iminopyrrolidine-1-yl)methyl-2,4(1H,3H)-pyrimidinedione hydrochloride (TPI), in molar ratio of 2:1 to increase the area under the curve (AUC) of FTD following its oral administration in animals.¹⁵ As expected, the AUC for FTD after an oral administration of TAS-102 is increased, to some extent, in mice, while the increase is more dramatic in monkeys due to the differences in the expression of TPase and UPase among animals.¹⁵

In this study, we report on the development of a pharmacokinetic model to evaluate the species specificity of the bioavailability of FTD in rats and monkeys in the presence of TPI. Hepatic clearance appears to play an important role in predicting the bioavailability (F) of FTD, and the inhibitory effect by TPI on FTD metabolism should also be quantified to permit the precise estimation of F in the presence of TPI.

In this report, hepatic intrinsic clearance was evaluated using a Michaelis-Menten type kinetics and a linear method. Simulations of the AUC after the administration of FTD with different doses of TPI revealed a remarkable dose-dependent increase of TPI in monkeys but only a slight increase in rats.
Pharmacokinetic Analysis of TAS-102 in Animals

The results of the present study permit the quantitative evaluation of the species dependent enhancement in the AUC for FTD between rats and monkeys, with the co-administration of TPI.

Materials and Methods

**Chemical:** TPI and 1-methyl-5-chloro-6-(pyrrolidin-1-yl)methyl-2,4(1H,3H)-pyrimidinedione (TAS-1-1543), an internal standard of TPI were synthesized at the Taiho Pharmaceutical Co., Ltd. FTD was purchased from the Yuki Gosai Kogyo Co., Ltd. \(^{14}C\)-FTD labeled at position C-2 of the pyrimidine ring, with a specific activity of 2.072 GBq/mmol was purchased from Moravek Biochemicals Inc. The radiochemical purity was in excess of 97%. 5-iodo-2'-deoxyuridine (IDU), an internal standard of FTD was purchased from Tokyo Chemical Industry Co., Ltd. All other chemicals used were commercially available products.

**Animals:** Male rats (5–7 weeks of age, Sprague-Dawley(Cj:CD) strain IGS) were purchased from Charles River Japan. Male Cynomolgus monkeys (weighed between 2.2 and 6.4 kg) were purchased from Kasei Kogyo Co., Ltd. Other chemicals used were commercially available products.

**Pharmacokinetic studies in rats:** The doses of TAS-102 are expressed in terms of FTD. We observed that the plasma concentrations of FTD and TPI in rats were not affected by their condition (fasted or non-fasted). Thus, we estimated the pharmacokinetic parameters for FTD and TPI from the time-courses without taking these conditions into account.

1) TAS-102, p.o. administration

To determine FTD and TPI concentrations in plasma, TAS-102 50 mg/kg (50 mg/kg FTD + 24 mg/kg TPI) was orally administered to non-fasted male rats (n = 3). Blood samples were withdrawn from the abdominal aorta under ether anesthesia at 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hr post-dose. To determine the amount of FTD (% of dose) in the urine, \(^{14}C\)-FTD 50 mg/kg (3.7 MBq/kg) was orally administered to non-fasted male rats (n = 4) that were immediately housed in metabolic cages. Urine samples were collected for periods up to 24 hr.

2) TAS-102, i.v. administration

\(^{14}C\)-FTD/TAS-102 50 mg/kg (50 mg/kg FTD + 24 mg/kg TPI) (3.7 MBq/kg) was administered intravenously as a bolus via the tail vein to non-fasted male rats (n = 4). To determine FTD concentrations in plasma, blood samples were withdrawn from the abdominal aorta under ether anesthesia at 5, 15, 30 min, 1, 2 and 4 hr post-dose.

3) FTD, p.o. administration

To determine FTD concentrations in plasma, FTD 50 mg/kg was orally administered to fasted male rats (n = 2). Blood samples were withdrawn from the jugular vein at 3, 6, 15, 30, 45, 60, 90 and 120 min post-dose. To determine the amount of FTD (% of dose) in urine, \(^{14}C\)-FTD 50 mg/kg (3.7 MBq/kg) was orally administered to non-fasted male rats (n = 4) that were immediately housed in metabolic cages. Urine samples were collected for periods of up to 24 hr.

4) FTD, i.v. administration

To determine FTD concentrations in plasma, FTD 50 mg/kg was administered intravenously as a bolus via the tail vein of fasted male rats (n = 2). Blood samples were withdrawn from the jugular vein at 3, 6, 15, 30, 45, 60, 90 and 120 min post-dose.

**Pharmacokinetic studies in monkeys:**

1) TAS-102, p.o. administration

To determine FTD and TPI concentrations in plasma, TAS-102 30 mg/kg (TAS-102: 30 mg/kg FTD + 14 mg/kg TPI) was administered orally to non-fasted male monkeys (n = 3). Blood samples were withdrawn from the femoral vein at 0, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hr post-dose.

2) TAS-102, i.v. administration

TAS-102 7.5 mg/kg (7.5 mg/kg FTD + 3.5 mg/kg TPI) or 30 mg/kg (30 mg/kg FTD + 14 mg/kg TPI) was administered intravenously as a bolus to fasted male monkeys (n = 2) via the femoral vein. Blood samples were withdrawn at 3, 10, 30 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6 hr post-dose. The animals were housed in metabolic cages to permit the collection of urine samples at intervals of 0–4, 4–6, 6–8, and 8–24 hours post-dose.

3) FTD, i.v. administration

To determine FTD concentrations in plasma and the amount of FTD (% of dose) in urine, FTD 30 mg/kg was administered intravenously as a bolus via the femoral vein to fasted male monkeys (n = 3). Blood samples were withdrawn at 3, 10, 30 min and 1, 1.5, 2 hr post-dose. The animals were housed in metabolic cages to permit the collection of urine samples at intervals of 0–2, 2–4, 4–6, 6–8, and 8–24 hr post-dose.

**Bioanalytical method for the determination of FTD and TPI:**

Representative bioanalytical methods are as follows: TAS-1-1543 and IDU were used as internal standards. A 0.5 mL aliquot of plasma was added to 0.1 mL of distilled water and 0.3 mL of 0.1 M hydrochloric acid was added after spiking with 0.1 mL of the internal standard solution. The sample was applied to a Bond Elut PRS column, and eluted with 2 mL of 2% ammonia in methanol after washing with 2 mL of water and 2 mL of methanol. The eluate was reconstituted in 0.2 mL of acetate buffer (pH 4.3) after drying and a 30 μL portion was then injected into the HPLC for the determination of TPI. The pass through and the washing fractions were combined, 0.02 mL of 1 M hydrochloric acid was added, and the resulting
solution applied to an AG 50W-X4 cation exchange column. The column was washed twice with 2 mL of water, and again, the pass through and washing fractions were combined and neutralized with 1 mL of alkaline buffer (50 mM carbonate buffer (pH 10.0)/1 M sodium hydroxide = 3/1) in ice bath. The resulting solution was immediately applied to an AG1-X4, anion exchange column, and eluted three times with 2 mL of 0.3 M acetic acid-methanol solution after washing twice with water and twice with methanol. The eluate was reconstituted with HPLC mobile phase after drying and 50 μL of portion was then injected into the HPLC for the determination of FTD.

The HPLC analysis was performed using a Waters 600E HPLC system with ultraviolet detection. FTD was separated with a TSK gel ODS-80TM column (4.6 mm I.D. × 150 mm L, Tosoh) under the following chromatographic conditions: mobile phase, 10 mM acetate buffer (pH 3.5); flow rate, 1.0 mL/min; monitoring wavelength, 265 nm. TPI was separated on a Mightysil PR-18 column (4.6 mm I.D. × 150 mm L, Kanto Chemicals) under the following chromatographic conditions: mobile phase, 7 mM sodium 1-hexanesulfonate in 10 mM acetate buffer (pH 4.3); flow rate, 1.0 mL/min; monitoring wavelength, 276 nm. The analytical range in concentrations were 10–10000 ng/mL for both FTD and TPI. The accuracies and precisions for concentrations of 20, 400 and 8000 ng/mL were ranged in −0.9–4.0% and 0.6–1.4% for FTD, and 0.5–2.5% and 1.1–2.4% for TPI respectively. Urine samples were analyzed using similar procedures.

The plasma and urine samples after the dosing of 14C-labeled compound were deproteinized with methanol. The extract was reconstituted in a defined volume of mobile phase, and the solution was then subjected to preparative HPLC using an LCSS-905 system (Japan Spectroscopic), equipped with a Chromosorb 300-5C18 (4.6 mm I.D. × 250 mm L) column (Chromo) under the following chromatographic conditions: mobile phase, 20 mM phosphate buffer (pH 7.4)/acetonitrile = 96/4 (vol%); flow rate, 0.5 mL/min; monitoring wavelength, 300 nm. The HPLC eluate was collected in a minivial at 30 sec intervals for liquid scintillation counting. Counting efficiency was corrected by a Channel Ratio method using external standard sources. The background radioactivity was determined using the HPLC eluate before each injection. The radioactivity concentrations were converted into ng equivalent/mL to FTD.

**Enzyme kinetics studies of TPI for the inhibition of FTD metabolism:** Enzyme kinetics studies of TPI for the inhibition of FTD metabolism were carried out using cytosols prepared from the commercially available liver S9 of a Sprague-Dawley rat and a Cynomolgus monkey (XenoTech, USA). The cytosols obtained from the rat and monkey were prepared with a protein content of 0.5 mg/mL with 100 mM Tris-HCl buffer (pH 7.4). When the kinetic parameters were determined without inhibition by TPI, 0.14 mL of 143 mM phosphate buffer (pH 7.4) and 0.02 mL of FTD solution were combined and pre-incubated at 37°C for 1 min, and then incubated for 3 min after the addition of 0.04 mL of the cytosol solution. The reaction conditions were as follows, concentration of phosphate buffer; 100 mM, protein content; 0.1 mg/mL, concentration of FTD; 25, 50, 100, 250, 500 and 1000 μM for the rat, 10, 25, 50, 100, 250 and 500 μM for the monkey, and were triplicated for each concentration. Under these conditions, it would be expected that most of the FTD in the reaction mixtures was present in the free form, based on ultrafiltration data (data not shown). The reaction was stopped by the addition of 0.2 mL of 10% perchloric acid, and then vortexed for 5 sec. The supernatant from the incubated solution after centrifugation was directly analyzed by HPLC to determine the concentration of FTY, the main metabolite of FTD, an inactive form degraded by TPase. HPLC analyses were performed using the LC-10AD system (Shimadzu), equipped with a Hydrospere C18 (4.6 mm I.D. × 100 mm, i.d.; 3 μm (YMC) under the following chromatographic conditions: mobile phase, 0.06% trifluoracetic acid/methanol = 76/24 (vol%); flow rate, 1.0 mL/min; monitoring wavelength, 261 nm. When the kinetic parameters were determined with the solution applied to AG1-X4, anion exchange column, and eluted three times with 2 mL of 0.3 M acetic acid-methanol solution after washing twice with water and twice with methanol. The eluate was reconstituted with HPLC mobile phase after drying and 50 μL of portion was then injected into the HPLC for the determination of FTD.

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Fig. 1. Pharmacokinetic models of FTD and TPI for prediction in animals. After an oral administration of TAS-102, each drug was absorbed according to the rate constant ($k_a$) and the bioavailability ($F$). The value of $F$ for FTD was calculated according to Eq. (13) numerically. The $CL_{int,h}$ of FTD was also calculated according to Eq. (12) including the time courses of blood concentrations of both FTD and TPI.

**Determination of Blood/Plasma concentration ratios ($R_b$) of FTD:** For monkeys, blood was obtained from 3 individuals and mixed. A 10 μL aliquot of $^{14}$C-FTD solution was added to 490 μL of the mixed blood to give final concentrations of 0.1, 1, 10 and 100 μg/mL. For rats, blood was obtained from 2 individuals 11 weeks of age and mixed. A 20 μL aliquot of $^{14}$C-FTD solution was added to 980 μL of the mixed blood to give final concentrations of 0.3, 3, 30 and 350 μg/mL. Since it was shown that TPase is identical to platelet-derived endothelial cell growth factor, the same reaction described below was also conducted in the presence of TPI. However, the results obtained were the same as in the absence of TPI, therefore we used these values for simulations. After incubation for 10 min, aliquots of blood samples were centrifuged to obtain plasma samples. The blood samples were then dissolved in 0.3–0.4 mL soluen-350 (Packard)/isopropl alcohol mixture (1/1), decolorized with hydrogen peroxide and 10 mL of liquid scintillator added. The plasma samples were dissolved with 0.2 mL soluen-350/isopropl alcohol mixture (1/1) and 10 mL liquid scintillator added. The radioactivity of these samples was measured with a liquid scintillation counter (2000CA, Packard).

**Model development for FTD and TPI in rats and monkeys:** PK parameters were estimated by fitting the time courses for plasma concentration curves in rats and monkeys after oral administrations of 50 mg/kg and 30 mg/kg of TAS-102, respectively, with the MULTI fixing all weights as 1/C, and the initial conditions were estimated with the Simplex method.

**Estimation of systemic, renal and hepatic clearances ($CL_{tot,b}, CL_{r,b}$ and $CL_{h,b}$) in rats and monkeys:** The area under the curve (AUC), the bioavailability and the systemic clearance were calculated using the equations below.

- **monkeys, FTD, i.v.:** $AUC_{iv} = \frac{A}{k_{el}}$ (3)
- **other conditions, i.v.:** $AUC_{iv} = \frac{A}{\alpha} + B \beta$ (4)
- **monkeys, FTD, p.o.:** $AUC_{po} = A \left( \frac{1}{k_{el}} - \frac{1}{k_a} \right)$ (5)
- **other conditions, p.o.:** $AUC_{po} = A \frac{B + \alpha}{\beta} - A + B k_e$ (6)

\[ F = \frac{AUC_{po}}{AUC_{iv}} \] (7)

\[ CL_{tot} = \frac{D}{AUC_{iv}} \] (8)

where A, B and alpha are constants, and $k_{el}$, $k_a$ and $k_e$ (1/min), $AUC_{po}$, $AUC_{iv}$ (mg·mL/min), $F$, D (mg/kg) and $CL_{tot}$ (mL/min) represent the elimination rate constant, the absorption rate constant, the area under the curve after oral and intravenous administration, the
AUC and Vf represent the Akaike’s information criterion and the distribution volume, and k10 and k01 are the intercompartmental transfer rate constants. Other parameters are defined in Methods. PK parameters were obtained based on the plasma concentrations. Values represent average ± standard deviation.

a. Time courses of FTD in rats and TPI in rats and monkeys were fitted with the 2-compartment-open model as follows:

\[ C_f = \frac{A}{1+e^{-\beta t}} \]

b. Time course of FTD in monkeys was fitted with the 1-compartment model as follows:

\[ C_f = \frac{Ae^{-\alpha t} + B}{1+e^{-\beta t}} \]

c. AUCf in rats and monkeys were obtained by intravenous administration of 50 and 30 mg/kg of TAS-102, respectively.

d. F in rats and monkeys were calculated using AUCf and AUCpo of each animal after administration of 50 and 30 mg/kg of TAS-102, respectively.

Renal clearance (CLR) was estimated from the FTD excreted into the urine after the oral (rats) or intravenous (monkeys) administration according to the following equation:

\[ CLR = CL_{tot} - CL_{inj} \]
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Fig. 2. Pharmacokinetic analysis of FTD and TPI in rats and monkeys. Curves represent the fitted curves for FTD (A) and TPI (B) in rats after an oral administration of 50 mg/kg of TAS-102 and of FTD (C) and TPI (D) in monkeys after an oral administration of 30 mg/kg of TAS-102.

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tion, the CLint,h was calculated numerically using the time courses for Cb and I, which represent the concentration of FTD and TPI, respectively, in the blood, based on the flow model in Fig. 1. In this analysis, CLint,h was estimated using a scaling factor (α) between in vitro and in vivo, and a correction factor (β), which is a concentration independent component especially in the case of rats:

$$ CL_{int,h}(t) = \alpha \times \frac{V_{max}}{K_m(1 + I(t)/K_i) + C_b(t)/f_u} + \beta $$ (12)

where I(t), Cb(t) and f_u represent the concentration of TPI and FTD in the blood, as functions of time, and the unbound fraction of FTD in the blood, respectively. The term β was required only in rats to represent the contribution of UPase which is predominantly expressed in rodents.15) It was assumed to be a constant since its Km value (57–958 μM)14) was much higher than the concentration of FTD in blood (≤ 8 μM). The α and β terms were estimated by simulating the time course for FTD in rats or monkeys.

Since the liver was assumed to be the only compartment where FTD is metabolized, bioavailability, which is also a function of time, was estimated as follows, assuming f_u = 1 and a well-stirred model:

$$ F(t) = f_u \times f_d(t) = f_d(t) = \frac{Q_h}{Q_h + f_dCL_{int,h}(t)} $$ (13)

where F(t), f_d(t), f_u, and Q_h (mL/min) are the bioavailability, the availability in the liver, the availability in the G.I. tract, and the hepatic blood flow, respectively.

Concerning the other parameters, the values summarized in Tables 1 and 2 were used. The AUC was calculated numerically by the trapezoidal formula from time 0 to 240 (rats) or 720 (monkeys).

$$ AUC = \sum_{i=1}^{n} \frac{(t_i-t_{i-1})(C_i+C_{i-1})}{2} $$ (14)

Simulations of non-linear drug disposition of FTD were performed with STELLA 5.1.1 (High Performance Systems, Inc.).

Effect of the co-administration of TPI on the time course of FTD: For a quantitative evaluation of the effect of TPI on FTD kinetics in rats and monkeys, the time courses for FTD in blood after the oral co-administration of several doses of TPI were simulated based on the model constructed above (Fig. 1).

Results

PK parameters of rats and monkeys: PK parameters for FTD and TPI were estimated in rats or monkeys following the oral administration of TAS-102 using MULTI.17) and the results are shown in Fig. 2 and the resulting parameters are summarized in Table 1.

The kinetic parameters, K_m and V_max, for hepatic metabolism were obtained from the in vitro experiments on the inhibition of FTD metabolism by TPI in the hepatic cytosol in rats and monkeys (Table 2).

To determine the scaling factor in Eq. (12), α was
Fig. 3. Relationship between the dose of TPI and \( CL_{r,b} \). The values were calculated as described in the Methods section and are summarized in Table 3. [●] and [○] represent renal clearance, hepatic clearance and systemic clearance, respectively.

A) In rats, the equation representing the relationship between the renal clearance and the dose of TPI was obtained as follows:

\[
CL_{r,b} = 0.168 \times TPI + 6.64 \quad (g^{2} = 1.000)
\]

B) In monkeys, the equation was as follows:

\[
CL_{r,b} = 0.873 \times TPI + 6.21 \quad (g^{2} = 0.981)
\]

These relationships were used in the simulations.

Table 3. Effect of TPI on the clearance of FTD in rats and monkeys

<table>
<thead>
<tr>
<th></th>
<th>Rat-p.o.</th>
<th>Monkey-i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTD (mg/kg)</td>
<td>50</td>
<td>30</td>
</tr>
<tr>
<td>TPI (mg/kg)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>( X_{u}/D )</td>
<td>0.059</td>
<td>0.0445</td>
</tr>
<tr>
<td>( CL_{r,b} ) (mL/min) ( \beta )</td>
<td>6.64</td>
<td>0.333**</td>
</tr>
<tr>
<td>( CL_{h,b} ) (mL/min) ( \beta )</td>
<td>14.8</td>
<td>7.27</td>
</tr>
<tr>
<td>( CL_{tot,b} ) (mL/min) ( \beta )</td>
<td>21.4</td>
<td>156</td>
</tr>
</tbody>
</table>

a. Parameters were calculated based on the oral administration of FTD in the presence or absence of TPI.
b. Parameters were calculated based on the intravenous administration of FTD in the presence or absence of TPI.
c. Value was obtained from AUCiv and AUCpo after the administration of FTD alone.
d. Renal clearances based on blood concentration were calculated according to Eq. (9) for rats and Eq. (10) for monkeys.
e. Hepatic clearances based on blood concentration were calculated according to Eq. (11).
f. Systemic clearances based on blood concentration were calculated according to Eq. (8).
g. Significant differences were observed against FTD alone. (*; \( P < 0.05 \), **; \( P < 0.01 \), ***; \( P < 0.001 \))

Simulation of FTD disposition: The parameters used in simulation (Tables 1 and 2) were fundamentally based on the PK parameters for each animal obtained from the PK analysis of plasma drug concentrations following the oral administration of TAS-102. \( CL_{int,h} \) and \( F \) are non-linear as well as time dependent, and are functions of both FTD and TPI blood concentrations according to Eq. (12) and (13), respectively. The other parameters were constant.

The simulated AUCs in both rats and monkeys were close to the observed values (AUC simulated / AUC observed were 0.9 and 0.8 in rats and monkeys, respectively).

Effect of TPI on the FTD availability: The effect of TPI co-administration with FTD on the bioavailability of FTD after oral administration was simulated based on the parameters obtained. The time courses for FTD after the oral administration of different doses of TPI were simulated (Fig. 4). A remarkable TPI dose-dependent enhancement (up to 80 fold) on the AUC of FTD in monkeys was found, while the increase was minimal in rats (Fig. 5).

Discussion

The efficacy of FTD as an antitumor and antivirus drug has been clearly demonstrated in clinical studies, however, its rapid elimination from the human body...
Fig. 4. Simulations of FTD blood concentration in rats and monkeys after the co-administration of different doses of TPI. FTD blood concentrations were simulated based on the PK-model in Fig. 2 with parameters shown in Tables 1 and 2. The dose of TPI was normalized by the value of TAS-102 and altered from 0 to 2 in rats (A) and monkeys (B). and represent the simulated time courses after co-administration of TPI at 0, 0.125, 0.25, 0.5, 1, and 2-fold of TPI in TAS-102, respectively. Filled circles show the observed concentration of FTD at indicated time.

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(half life of 12–18 min) has limited its clinical application. TPI, a specific inhibitor of TPase, plays an important role in FTD metabolism, and, when combined with FTD as TAS-102 it increases the efficacy of FTD. It was observed that the oral administration of TAS-102 in experimental animals resulted in a dramatic increase in the AUC for FTD compared to the administration of FTD alone. In this study, we were successful in predicting the pharmacokinetics, especially the AUC for FTD in rats and monkeys after the oral administration of several doses of TPI by means of a numerical calculation to clarify the species specificity based on the difference in the level of expression of TPase between rats and monkeys. In this prediction, we used in vivo kinetic parameters for the metabolism of FTD as well as TPI and in vitro pharmacokinetic parameters for rats and monkeys.

In our predictions, we adopted a perfusion model (Fig. 1) to describe the disposition of FTD in animals following the oral administration of TAS-102. We assumed that all metabolism occurred only in the liver in our modeling (fg.i. = 1 in Eq. (13)). To describe the metabolic interaction between FTD and TPI, Michaelis-Menten kinetics were introduced (Eq. (12)). Furthermore, CL_{int,h} varies with time due to the different concentrations of FTD and TPI according to Eq. (12). Equation (12) is composed of two terms. The first term represents FTD metabolism by TPase, which is described by the Michaelis-Menten equation including time-dependent variables such as the concentration of FTD (C_b) and TPI (I). K_m, V_{max}, and K_i were obtained from in vitro experiments (Table 2). Since the activity of UPase in rats is relatively high compared to that of TPase, a potent inhibition of FTD metabolism by TPI was not observed in rats, which is not the case in monkeys.

Concerning the renal clearance of FTD, TPI enhanced CL, dose-dependently as shown in Fig. 3. In the presence of TPI (24 and 14 mg/kg in the rat and monkey, respectively), CLr was higher than CLr in both rats and monkeys. Therefore, we included this effect in our model quantitatively.

In a recent report, it was reported that FTD in
addition to some antiviral nucleoside analogs was transported via rOAT1 which is a known primary transporter in the tubular secretion of endogenous and exogenous organic anions. In the report, the authors suggested that pyrimidine and purine rings might participate in the interaction of substrates with rOAT1. This suggestion may well explain our observation of the TPI-dependent increase in urinary excretion rate. One possible explanation is that FTY, which is a major metabolite of TDF, may compete with FTD for binding to the recognition site in rOAT1. Based on this hypothesis, it can be assumed that the decrease in FTY as the result of TPI secretion of FTD. We used the obtained parameters, we simulated time courses for FTD in blood after an oral administration with different doses of TPI in rats and monkeys (Fig. 4). The simulation after the oral administration of TAS-102 successfully demonstrated the appropriate time-courses for FTD in both animals, however, in the case of other doses, quite different responses between rats and monkeys were found, suggesting species specificity in TPase expression levels. Despite the much higher concentrations of TPI than the Ki (the mean blood concentration for FTD in both animals, however, in the case of other doses, quite different responses between rats and monkeys were found, suggesting species specificity in TPase expression levels. Despite the much higher concentration of TPI than the Ki (the mean blood concentrations in rats and monkeys when orally administered 50 and 30 mg/kg of TAS-102 were 429 and 81.2 ng/mL, respectively), systemic clearance in rats was largely unaffected while the clearance in monkeys was totally suppressed. Therefore, we introduced the term, β, to represent the UPase activities only in case of rats. As shown in Fig. 5, a remarkable TPI-dependent increase in the AUC for FTD was found in monkeys, while the effect of TPI in rats was minimal. In this study, we successfully explained the species differences for FTD disposition after co-administration with TPI using a quantitative evaluation of the TPI inhibitory effect in hepatic metabolism as well as the enhanced effect of renal clearance in rats and monkeys. Our pharmacokinetic model will provide useful information in developing a combination of TPI for enhanced bioavailability in humans.

References
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