Effect of dimethylnitrosamine-induced liver dysfunction on the pharmacokinetics of 5-fluorouracil after administration of S-1, an antitumour drug, to rats

Kunihiro Yoshisue\textsuperscript{a}, Shohei Kanie\textsuperscript{b}, Takako Nishimura\textsuperscript{a}, Junko Chikamoto\textsuperscript{c} and Sekio Nagayama\textsuperscript{a}

\textsuperscript{a}Pharmacokinetics Research Laboratory, \textsuperscript{b}Drug Safety Research Laboratory and \textsuperscript{c}Personal Medicine Research Laboratory, Tokushima Research Center, Taiho Pharmaceutical Co., Ltd, Tokushima, Japan

Abstract

Objectives The anti-tumour agent S-1 comprises tegafur (a prodrug of 5-fluorouracil; 5-FU), gimeracil (2-chloro-2,4-dihydroxypyridine (CDHP); a competitive inhibitor of 5-FU metabolism) and oteracil potassium. The effect of hepatic dysfunction induced by dimethylnitrosamine (DMN) on the pharmacokinetics of 5-FU after administration of S-1 to rats was investigated.

Methods S-1 (5 mg/kg) was administered intravenously and orally to rats with DMN-induced liver dysfunction. Plasma concentrations of S-1 components and 5-FU were measured by HPLC and LC/MS–MS. Blood tests and in-vitro enzymatic investigations were also conducted.

Key findings DMN treatment induced hepatic dysfunction and decreased the conversion of tegafur to 5-FU in the liver without altering renal function or dihydropyrimidine dehydrogenase activity. Following intravenous administration of S-1, the blood concentration–time profiles of CDHP were similar between control rats and rats with hepatic dysfunction, but the half-life of tegafur was significantly prolonged. The maximum plasma concentration (C\textsubscript{max}) of 5-FU was significantly reduced and the area under the blood concentration–time curve (AUC) was reduced by 22%. Following oral administration, the C\textsubscript{max} of tegafur, 5-FU and CDHP were significantly decreased and half-lives significantly increased. Hepatic dysfunction had a less pronounced effect on the AUC of 5-FU (13.6% reduction).

Conclusions The pharmacokinetic profiles of tegafur, 5-FU and CDHP were altered by changes in the elimination rate of tegafur induced by a decrease in the conversion of tegafur to 5-FU. However, hepatic dysfunction had less of an effect on the AUC of 5-FU, which correlates with anti-tumour effect, after the oral administration of S-1.

Keywords 5-FU; dimethylnitrosamine; hepatic dysfunction; pharmacokinetics; S-1

Introduction

The liver plays many pivotal roles in intermediary metabolism as well as in the clearance of drugs and toxins. Normal function of the liver is critical for the activity of hepatic cytochrome \textsubscript{P}450 (CYP) metabolising enzymes. Liver blood flow, binding to plasma proteins and biliary excretion can potentially influence drug pharmacokinetics.\textsuperscript{[1]} Hepatic dysfunction, in particular cirrhosis, can modulate many factors that determine the behaviour of drugs in the body.\textsuperscript{[2]} Impaired liver function can lead to significant alterations in the pharmacokinetics and pharmacodynamics of many drugs, whether or not they are metabolised in the liver. Dimethylnitrosamine (DMN) is a potent hepatotoxin, carcinogen and mutagen. DMN-induced liver injury in rats seems to be a good model for early liver cirrhosis.\textsuperscript{[3]} In addition, a model of cirrhosis induced by discontinuous treatment with a low dose of DMN in the rat has been reported to reproduce several characteristics of this liver disease.\textsuperscript{[4]}

S-1 is an antitumour agent derived from 5-fluorouracil (5-FU) that is administered orally. It was developed based on the biochemical modification of 5-FU. It consists of 1-(2-tetrahydrofuryl)-5-fluorouracil (tegafur), gimeracil (2-chloro-2,4-dihydroxypyridine; CDHP) and oteracil potassium (monopotassium 1,2,3,4-tetrahydro-2, 4-dioxo-1,3,
5-triazine-6-carboxylate) in a molar ratio of 1:0.4:1 (Figure 1). Tegafur, which is a prodrug of 5-FU, is the effector drug. Gimeracil and oteracil potassium do not have antitumour activities but function as modulators. Gimeracil competitively inhibits dihydropyridine dehydrogenase (DPD; EC 1.3.1.2), which is expressed in the liver and mediates the rate-limiting degradation of 5-FU about 180 times more effectively than does uracil in vitro, prolonging the retention of an effective concentration of 5-FU in the blood. Oteracil potassium competitively inhibits orotate phosphoribosyltransferase (EC 2.4.2.10), which converts 5-FU to fluorouridine monophosphate and relieves gastrointestinal toxicity caused by 5-FU. The anti-tumour effect of S-1 components after dialysis in homogenisation buffer were reconstituted in homogenisation buffer (10 mmol/l Tris-HCl, pH 7.4, containing 0.5 mmol/l dithiothreitol and 1 mol/l EDTA).

**Materials and Methods**

**Chemicals**

Tegafur, CDHP, oteracil potassium and $\left[^{13}C_2\right]$oteracil potassium were synthesised by Taiho Pharmaceutical Co. (Tokushima, Japan) or Sumitomo Chemical Co. (Osaka, Japan). $\left[6-^{14}C\right]$5-FU, $\left[^{13}C_3,^{15}N\right]$CDHP and $\left[^{15}N_2\right]$5-FU were purchased from Moravek Biochemicals (Brea, CA, USA), Taiyo Nippon Sanso (Tokyo, Japan) and Isotec (Westminster, CO, USA), respectively. Mouse monoclonal anti-CYP3A1 antibodies were purchased from Xenotech (Kansas City, KS, USA), goat polyclonal anti-CYP1A antibodies from Sekisui Medical Co. (Tokyo, Japan) and mouse monoclonal anti-thymidine phosphorylase (TPase) antibodies from Abcam (Cambridge, UK). Mouse polyclonal anti-DPD antibodies were prepared as described previously. All reagents and solvents were reagent or HPLC grade.

**Animal studies**

The animal study was approved by the institutional Animal Ethics Committee of Taiho Pharmaceutical Co. Six-week-old male Sprague-Dawley rats purchased from Charles River Japan (Shiga, Japan) were used. Rats had free access to tap water and commercially available chow. Hepatic dysfunction was induced by intraperitoneal administration of 1% DMN (1 ml/kg) on Monday, Wednesday and Friday mornings for 3 weeks. Control rats were given physiological saline.

To evaluate the pharmacokinetics of tegafur, 5-FU, CDHP and oteracil potassium, S-1 was administered orally by gavage or injected intravenously via the tail vein at a dose of 5 mg/kg. (The dose of S-1 is indicated as tegafur dose because the active component is tegafur.) S-1 was dissolved in 0.5% hydroxypropylmethylcellulose solution for oral dosing and in 25 mol/l NaHCO$_3$ for intravenous administration.

Blood samples were collected from the jugular vein at 5, 15 (intravenous only) and 30 min and 1, 2, 4, 6, 8, 10 and 24 h after drug administration. Blood samples for measurement of haematocrit and serum chemical tests were collected under ether anaesthesia after the 24 h pharmacokinetic blood sample. The liver was then excised and perfused with ice-cold physiological saline. Plasma was prepared from each sample by centrifugation.

**Measurement of haematocrit, serum chemical tests and blood-to-plasma concentration ratios**

Serum chemical tests were performed using a Hitachi 7170 autoanalyser (Tokyo, Japan) and an automatic electrophoresis system (CTE-150, Jokoh, Tokyo, Japan). Haematocrit was determined using an integrated haematological analyser (ADVIA120, Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

Blood-to-plasma concentration ratios (Rb) for tegafur, 5-FU, CDHP and oteracil potassium were calculated by dividing the theoretical concentration of the added compound (1 $\mu$g/ml) in blood by the actual plasma concentration of the drug.

**Preparation of liver microsomes and cytosol**

Enzymatic fractions were prepared from the livers by differential centrifugation after homogenising in homogenisation buffer (10 mmol/l Tris-HCl, pH 7.4, containing 0.5 mmol/l dithiothreitol and 1 mol/l EDTA).

Supernatant components after dialysis in homogenisation buffer were used as the cytosol. Pellets reconstituted in homogenisation buffer were used as the microsomal fraction.
buffer after washing and preparation by repeated centrifugation were used as microsomes. The protein content was determined according to the Bradford method using a Bio-Rad protein assay kit and bovine serum albumin as the standard.

Enzyme assays
For determination of DPD activity, an incubation mixture containing 40 μmol/l [6-14C]5-FU, 5 mmol/l MgCl2, 0.5 mmol/l NADPH, 1 mmol/l dithiothreitol and cytosol (0.25 mg protein) in 70 mmol/l phosphate buffer (pH 7.5) was incubated for 3 min at 37°C. The reaction was terminated by the addition of methanol. After centrifugation the supernatant was evaporated to dryness under nitrogen and then reconstituted in water for HPLC analysis. Analytical separation was accomplished using a Daisopak column (250 × 4.6 mm, 5 μm; Osaka, Japan); the mobile phase was 10 mmol/l phosphate buffer (pH 3.0) at a flow rate of 1.0 ml/min. Radioactivity in the column effluent was detected using a flow scintillation counter (A-500; Packard Bioscience, Meriden, CT, USA) by mixing with scintillation cocktail (Ultima-Flo M, Packard Bioscience). DPD activity was determined from the fraction of eluted radioactivity excluding [6-14C]5-FU.

Testosterone hydroxylase activity and 7-ethoxyresorufin-O-deethylase activity were determined as reported previously.

Immunoblot analysis
Immunoblot analysis using anti-CYP, anti-DPD and anti-TPase antibodies was conducted as reported previously. Microsomes or cytosol loaded at 50 μg protein per well were subjected to SDS-PAGE on 10% acrylamide gels and transferred onto the PVDF membrane. Immunoglobulin G was followed by peroxidase-conjugated antibody. Staining of antigen–antibody complexes was achieved using an enhanced chemiluminescence system (GE Healthcare, Buckinghamshire, UK). The optical density of each stained band was determined using an image analyser (LAS-3000 mini; Fuji, Tokyo, Japan).

Measurement of tegafur, 5-FU and CDHP concentrations
Concentrations of tegafur in plasma were determined by HPLC using a Waters 2695 separation module and an ultraviolet spectrophotometer operated at 270 nm; the internal standard was 7-(1-hydroxyethyl)theophylline. Separations were achieved using an L-column ODS (150 × 4.6 mm, 5 μm; Ceri Tokyo, Tokyo, Japan). The mobile phase consisted of 10 mmol/l potassium dihydrogenphosphate/acetate buffer (9 : 1 v/v) at a flow rate of 1.0 ml/min.

Levels of 5-FU, CDHP and oteracil potassium were measured using liquid chromatography/tandem mass spectrometry (LC/MS–MS) analysis. The LC/MS–MS system consisted of an Agilent 1100 series LC system (Santa Clara, CA, USA) and API 4000 mass spectrometer (Applied Biosystems; Foster City, CA, USA) operated in negative-ion electrospray ionisation mode. The internal standards were [15N3]5-FU, [15C3]5-FU and [15C3]5-FU/DHP and [15C3]5-FU oteracil potassium.

The LC/MS–MS analysis of 5-FU and CDHP was performed using a Develosil C30-UG-5 (150 × 2.0 mm, 5 μm; Nomura Chemical, Aichi, Japan). The mobile phase used for gradient elution consisted of 0.1% acetic acid/acetonitrile. HPLC separations for oteracil potassium were achieved on a TSK-gel amide-80 column (150 × 2.0 mm, 5 μm; Tosoh, Tokyo, Japan); the mobile phase consisted of 10 mmol/l ammonium acetate/acetonitrile (15 : 85 v/v) at a flow rate of 0.2 ml/min. The m/z values observed for 5-FU, CDHP and oteracil potassium were 128.9, 143.8 and 112, respectively.

Pharmacokinetic analysis
Blood concentration was calculated by multiplying the plasma concentration by the Rb value.

Standard pharmacokinetic parameters obtained from blood concentration–time profiles of tegafur, 5-FU, CDHP and oteracil potassium were calculated using non-compartmental methods using WinNonlin (version 5.2; Pharsight, Mountain View, CA, USA). Peak blood concentration (Cmax), time to Cmax (tmax) and the distribution volume of the central compartment (V1) were obtained directly from blood concentration data. The half-life (t1/2) of each compound was determined by linear regression of the log-linear portion of the blood concentration–time profile. The area under the blood concentration–time curve (AUC) was calculated using the trapezoidal rule from time 0 to the last concentration time point, followed by extrapolation to infinity. V1 for tegafur, CDHP and oteracil potassium was calculated by dividing the dose by the concentration of each compound at 0 min, estimated from the blood concentration–time profiles after intravenous administration. The apparent blood clearance (CL) and the oral bioavailability (F) of tegafur, CDHP and oteracil potassium were determined according to the equations CL = dose/AUCiv and F = AUCoral/AUCiv × 100.

Statistical analysis
Data storage and statistical analyses were carried out using SAS (version 8.02; Cary, NC, USA). Differences between groups were tested using Student’s t-test, and Wilcoxon’s test for tmax.

Results
Effects of hepatic dysfunction
Body weight, liver weight, haematocrit, serum chemistry findings and Rb values of tegafur, 5-FU, CDHP and oteracil potassium are shown in Table 1. Body weight and liver weight were significantly lower in HD rats than control rats (12.0% and 19.1% reductions, respectively) but the liver weight as a percentage of body weight was not significantly different between the two groups. Levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and γ-glutamyl transferase were significantly higher in the HD rats (increases of 87.2%, 115%, 78.1% and 155%, respectively, vs control rats) and serum levels of total proteins and haematocrit were significantly lower (reductions of 7.0% and 12.7%, respectively). Serum levels of albumin, total bilirubin and creatinine were not significantly different.
between the two groups. Rb values for tegafur, 5-FU and CDHP were not significantly different between control rats and HD rats whereas values for oteracil potassium were increased significantly in the HD rats.

Liver enzyme activities

Table 2 shows the activities of 7-ethoxyresorufin O-deethylase and testosterone hydroxylase. The activities of 7-ethoxyresorufin O-deethylase and testosterone 2α-, 6α-, 16β- and 6β-hydroxylase were all significantly reduced in HD rats compared with controls.

Pharmacokinetics of tegafur, 5-FU, CDHP and oteracil potassium after intravenous administration of S-1

Mean blood concentration–time profiles of tegafur, 5-FU, CDHP and oteracil potassium after intravenous administration of S-1 are shown in Figure 3. The pharmacokinetic parameters are listed in Table 4. After intravenous administration of S-1, mean blood concentrations of tegafur were higher in the HD rats. The t1/2 of tegafur was significantly prolonged in the HD rats, which resulted in a greater AUC and t1/2 significantly prolonged (34.8% increase) and reduced CL (21.4% reduction), although there was no significant difference in the V1 of tegafur between control and HD rats. Blood concentration–time profiles and pharmacokinetic parameters of CDHP and oteracil potassium in HD rats were similar to those in control rats. The Cmax of 5-FU was decreased significant (41.8% reduction) in HD rats and t1/2 significantly prolonged (34.8% increase), resulting in a 22% reduction in the AUC of 5-FU.

Pharmacokinetics of tegafur, 5-FU, CDHP, and oteracil potassium after oral administration of S-1

Mean blood concentration–time profiles of tegafur, 5-FU, CDHP and oteracil potassium after oral administration of S-1 are shown in Figure 4 and pharmacokinetic parameters are
Liver dysfunction and S-1 pharmacokinetics

Kunihiro Yoshisue et al. 1647

In this study, we investigated the effect of DMN-induced hepatic dysfunction on the pharmacokinetics of tegafur, 5-FU, CDHP and oteracil potassium after administration of S-1. To clarify alterations in the elimination of tegafur, CDHP and oteracil potassium in HD rats, we initially administered S-1 intravenously. Blood concentration–time profiles of CDHP and oteracil potassium in HD rats were similar to those in control rats, and there was no significant difference in t1/2 and V1. This indicates that hepatic dysfunction had no effect on the pharmacokinetics of CDHP and oteracil potassium after intravenous administration, which is as expected because both agents are eliminated primarily by renal clearance. The t1/2 of tegafur was prolonged and AUC increased in HD rats, although V1 was not altered. Cmax of 5-FU was reduced by hepatic dysfunction and t1/2 prolonged, leading to a 22% decrease in the AUC of 5-FU. Elimination of tegafur is mainly mediated by hepatic dysfunction (13.6% reduction).

The F values of CDHP in control and HD rats were comparable (approximately 0.3) and F values of tegafur in control and HD rats were 0.90 and 0.97, respectively. The F value for oteracil potassium was small in both groups (0.04 and 0.08 in control and HD rats, respectively).

Discussion

S-1 is a combination of tegafur, CDHP (gimeracil) and oteracil potassium. Tegafur is a prodrug of 5-FU. CDHP is a competitive inhibitor of DPD and inhibits degradation of 5-FU. Oteracil potassium inhibits the phosphorylation of 5-FU in the small intestine and thereby reduces its gastrointestinal toxicity. The pharmacokinetic properties of tegafur, CDHP and oteracil potassium are entirely different. Elimination of CDHP and oteracil potassium is mainly by renal excretion whereas that of tegafur is by non-renal clearance. Oteracil potassium shows very low bioavailability because of a very low absorption ratio. 5-FU is rapidly decomposed by DPD in the liver after intravenous administration of 5-FU alone, but combination with a DPD inhibitor in S-1 prolongs the retention of 5-FU concentrations in the blood. The blood concentration–time profile of 5-FU after S-1 administration is controlled by tegafur and CDHP. Because of these complicated pharmacokinetic profiles of 5-FU in S-1, it is not easy to predict the alteration in pharmacokinetics of 5-FU in S-1 caused by hepatic dysfunction. In a clinical situation, cancer patients often also have hepatic dysfunction.

Rats treated with 1% DMN three times weekly for 3 weeks showed a significant increase in the markers of hepatic dysfunction (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and γ-glutamyl transferase) but did not show any differences in markers of renal impairment (serum creatinine concentration) compared with control rats. These results indicate that rats treated with DMN developed hepatic dysfunction without renal dysfunction; a previous report also suggested that liver dysfunction induced by discontinuous DMN treatment is a good animal model for early liver cirrhosis.

Figure 2 Effect of dimethylnitrosamine-induced hepatic dysfunction on the protein concentrations of dihydropyridine dehydrogenase, cytochrome P450s CYP1A and CYP3A, and thymidine phosphorylase.

(a) Immunoblots of DPD, CYP1A2, CYP3A and TPase proteins; (b) quantitative immunoblot analysis to determine the levels of protein in liver microsomes and cytosol. Data represent means ± SD arbitrary densitometric units (n = 3 three rats). DPD, dihydropyridine dehydrogenase; HD, hepatic dysfunction; TPase, thymidine phosphorylase. **P < 0.01 vs control rats.

listed in Table 5. After oral administration of S-1, the Cmax of tegafur, 5-FU and CDHP were significantly lower in HD rats but blood concentrations of these compounds at and beyond 6 h remained higher than in control rats. Although the Cmax of tegafur was significantly decreased in HD rats (34.0% reduction vs control), the tmax and the t1/2 were significantly prolonged and AUC increased (by 37.0%) compared with control rats. Cmax of CDHP also decreased significantly (42.5% reduction) in HD rats, but its AUC was less affected by hepatic dysfunction because the t1/2 showed significant prolongation and blood concentration remained high from 6 h.

The Cmax of oteracil potassium showed no significant difference between the two groups of rats but tmax was significantly prolonged in those with hepatic dysfunction. The AUC of oteracil potassium increased in the HD rats, but its F value remained very low.

The Cmax of 5-FU showed a significant decrease (58.1% reduction) and tmax and t1/2 were significantly prolonged in HD rats. Because the t1/2 of 5-FU was significantly prolonged and blood concentration remained high from 6 h (as for CDHP), the AUC of 5-FU was less affected by hepatic dysfunction.
metabolism to 5-FU, and CYP1A and CYP3A are important for the synthesis of 5-FU from tegafur in rat liver microsomes; part of this conversion is mediated by TPase or uridine phosphorylase in the cytosol.[15] Our results show that the conversion of tegafur to 5-FU by liver microsomes was significantly reduced in HD rats, although there was no difference in the cytosolic activity. Activities of CYP1A1/2 and CYP3A1 (which correspond to the activity of 7-ethoxyresorufin O-deethylation and testosterone 6β-hydroxylation, respectively) and the immunodetectable protein of CYP3A1 in the liver of HD rats was lower than that in control rats.

**Figure 3** Pharmacokinetics after intravenous administration of S-1. Blood concentration-time curves of (a) tegafur, (b) 5-fluorouracil (5-FU), (c) 2-chloro-2,4-dihydroxypyridine (CDHP) and (d) oteracil potassium after intravenous administration of S-1 at 5 mg/kg. Points represent means ± SD (n = 4).

**Table 4** Pharmacokinetic parameters of tegafur, 5-FU, CDHP and oteracil potassium after intravenous administration of S-1 to control rats and rats with hepatic dysfunction.

<table>
<thead>
<tr>
<th></th>
<th>AUC (ng h/ml)</th>
<th>t1/2 (h)</th>
<th>Cmax (ng/ml)</th>
<th>V1 (ml/kg)</th>
<th>CL (ml/h per kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tegafur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>65274</td>
<td>3.28 ± 0.31</td>
<td>–</td>
<td>546 ± 107</td>
<td>76.6</td>
</tr>
<tr>
<td>HD</td>
<td>83030</td>
<td>4.21 ± 0.07†</td>
<td>–</td>
<td>522 ± 48</td>
<td>60.2</td>
</tr>
<tr>
<td>5-FU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>431</td>
<td>2.96 ± 0.42</td>
<td>159 ± 12</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HD</td>
<td>337</td>
<td>3.99 ± 0.75&lt;sup&gt;+&lt;/sup&gt;</td>
<td>92.5 ± 32.7&lt;?sup&gt;</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CDHP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1656</td>
<td>0.62 ± 0.02</td>
<td>–</td>
<td>297 ± 45</td>
<td>876</td>
</tr>
<tr>
<td>HD</td>
<td>1654</td>
<td>0.60 ± 0.02</td>
<td>–</td>
<td>278 ± 33</td>
<td>877</td>
</tr>
<tr>
<td>Oteracil potassium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4106</td>
<td>0.83 ± 0.12</td>
<td>–</td>
<td>325 ± 37</td>
<td>1189</td>
</tr>
<tr>
<td>HD</td>
<td>3448</td>
<td>0.64 ± 0.11</td>
<td>–</td>
<td>326 ± 39</td>
<td>1415</td>
</tr>
</tbody>
</table>

Values for half-life (t1/2), distribution volume of the central compartment (V1) and maximum plasma concentration (Cmax) are means ± SD (n = 4). Area under the plasma concentration-time curve (AUC) and clearance (CL) were calculated from mean blood concentrations. CDHP, 2-chloro-2,4-dihydroxypyridine; 5-FU, 5-fluorouracil; HD, hepatic dysfunction. *P < 0.05; †P < 0.01 vs control rats.
CYP1A1/2 and CYP3A1 in HD rats were also significantly reduced compared with control rats. The activity and amount of immunodetectable DPD protein was not altered in HD rats. These results suggest that the hepatic dysfunction induced by DMN decreased the conversion of tegafur to 5-FU because of a decrease in CYP content, but did not affect DPD activity. This alteration in HD rats led to prolongation of t1/2 and an increase in AUC of tegafur. We concluded that, in HD

**Figure 4** Pharmacokinetics after oral administration of S-1. Blood concentration–time curves of (a) tegafur, (b) 5-fluorouracil (5-FU), (c) 2-chloro-2,4-dihydroxypyridine (CDHP) and (d) oteracil potassium after oral administration of S-1 at 5 mg/kg. Points represent means ± SD (n = 4).

**Table 5** Pharmacokinetic parameters of tegafur, 5-FU, CDHP, and oteracil potassium after oral administration of S-1 to control rats and rats with hepatic dysfunction

<table>
<thead>
<tr>
<th></th>
<th>AUC (ng h/ml)</th>
<th>t1/2 (h)</th>
<th>Cmax (ng/ml)</th>
<th>tmax (h)</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tegafur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>58556</td>
<td>3.10 ± 0.36</td>
<td>7204 ± 586</td>
<td>1.0 ± 0.0</td>
<td>0.90</td>
</tr>
<tr>
<td>HD</td>
<td>80219</td>
<td>6.37 ± 2.57</td>
<td>4960 ± 914†</td>
<td>2.8 ± 1.3*</td>
<td>0.97</td>
</tr>
<tr>
<td>5-FU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>684</td>
<td>1.92 ± 0.23</td>
<td>178 ± 46</td>
<td>1.3 ± 0.5</td>
<td>–</td>
</tr>
<tr>
<td>HD</td>
<td>591</td>
<td>4.17 ± 1.24</td>
<td>74.5 ± 46.7*</td>
<td>2.0 ± 0.5</td>
<td>–</td>
</tr>
<tr>
<td>CDHP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>529</td>
<td>2.45 ± 0.52</td>
<td>259 ± 30</td>
<td>0.5 ± 0.0</td>
<td>0.32</td>
</tr>
<tr>
<td>HD</td>
<td>483</td>
<td>7.96 ± 4.36</td>
<td>149 ± 42†</td>
<td>0.8 ± 0.3</td>
<td>0.29</td>
</tr>
<tr>
<td>Oteracil potassium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>180</td>
<td>3.60 ± 3.04</td>
<td>64.1 ± 16.1</td>
<td>0.6 ± 0.3</td>
<td>0.04</td>
</tr>
<tr>
<td>HD</td>
<td>265</td>
<td>4.41 ± 2.41</td>
<td>58.3 ± 26.7</td>
<td>2.3 ± 0.5*</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Values for maximum plasma concentration (Cmax) and time to Cmax (tmax) are means ± SD (n = 4). Area under the plasma-concentration–time curve (AUC) was calculated from mean blood concentration. The bioavailability (F) was calculated by dividing the AUC after oral administration by the AUC after intravenous administration (AUCoral/AUCiv). CDHP, 2-chloro-2,4-dihydroxypyridine; 5-FU, 5-fluorouracil; HD, hepatic dysfunction. *P < 0.05; †P < 0.01 vs control rats.
rats, the alteration of tegafur and 5-FU is attributed to decreased conversion of tegafur to 5-FU, which induces the decrease in $C_{\text{max}}$ of 5-FU and prolongation of the $t_{1/2}$. After oral administration, significant decreases in $C_{\text{max}}$ of tegafur, 5-FU and CDHP, and prolongation of $t_{\text{max}}$ of tegafur, 5-FU and oteracil potassium were observed. Prolongation of the $t_{1/2}$ of tegafur, 5-FU and CDHP was also observed after oral administration. A previous report suggested that DMN treatment induced a decrease in the rate of hepatic blood flow.\textsuperscript{[23]} We suggest that the decrease in the absorption rate treatment induced a decrease in the rate of hepatic blood flow.\textsuperscript{[23]} We suggest that the decrease in the absorption rate of each compound is due to the decrease in the hepatic blood flow rate induced by DMN treatment, which led to the decrease in $C_{\text{max}}$ and prolongation of $t_{\text{max}}$. Furthermore, the blood concentration–time profile of CDHP showed very rapid elimination after intravenous administration; we propose that the flip-flop phenomenon (elimination rate is much larger than absorption rate) occurred in both groups after oral administration. Because of this flip-flop phenomenon, the blood concentration–time profile of CDHP from 6 h mainly reflects the absorption phase of the agent and showed the different $t_{1/2}$ values between oral and intravenous administration. Reduction in the absorption rate in HD rats and the flip-flop phenomenon of CDHP are likely to explain the prolongation of $t_{1/2}$ of CDHP observed in HD rats compared with control rats. We also speculate that the alteration in the blood concentration–time profile of 5-FU in HD rats after oral administration of S-1 can be attributed to the decreased absorption rate of tegafur and CDHP, and to the decrease in the elimination rate of 5-FU. Because of these characteristic profiles of S-1 in HD rats, although $C_{\text{max}}$ of 5-FU significantly decreased, the $t_{1/2}$ of 5-FU was prolonged and the AUC of 5-FU was less affected by hepatic dysfunction.

Conclusions

The blood concentration–time profiles of tegafur, 5-FU and CDHP were altered after the oral administration of S-1 due to changes in the elimination rate of tegafur induced by a decrease in the conversion of tegafur to 5-FU, as well as the absorption rate of tegafur, CDHP and oteracil potassium. Hepatic dysfunction had a lesser effect on the AUC of 5-FU after the oral administration of S-1 in rats because of the characteristic profiles of tegafur and CDHP in HD rats. Because previous reports\textsuperscript{[24,25]} suggest that the anti-tumour activity of 5-FU depends on exposure to the agent, we believe that patients with hepatic dysfunction can expect a similar response to S-1 as normal patients without requiring dose modification.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

20. Yoshisue K et al. Effects of 5-fluorouracil on the drug-metabolizing enzymes of the small intestine and the consequent...


