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Short Communication

Effects of tesmilifene, a substrate of CYP3A and an inhibitor of P-glycoprotein, on the pharmacokinetics of intravenous and oral docetaxel in rats

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Abstract

Objectives It has been reported that docetaxel is a P-glycoprotein substrate and is metabolized via the cytochrome P450 (CYP) 3A subfamily in rats. Tesmilifene is a substrate of the CYP3A subfamily and is an inhibitor of P-glycoprotein. Thus, the effects of various doses of tesmilifene on the pharmacokinetics of intravenous and orally administered docetaxel have been investigated in rats.

Methods Docetaxel (20 mg/kg as base) was administered intravenously and orally without and with tesmilifene (5, 10, and 20 mg/kg) in rats.

Key findings After intravenous administration of docetaxel with tesmilifene, the values of nonrenal clearance (CL_{NR}) and area under the plasma concentration–time (AUC) for docetaxel were comparable with those without tesmilifene. Tesmilifene did not increase the values of AUC or of absolute oral bioavailability (F) for docetaxel after oral administration of docetaxel with tesmilifene.

Conclusions The inhibition for the metabolism of docetaxel via hepatic and intestinal CYP3A subfamily, and inhibition of P-glycoprotein-mediated efflux of docetaxel in the intestine by tesmilifene were almost negligible. The extremely low value of F for docetaxel was due to the incomplete absorption from the gastrointestinal tract and considerable first-pass metabolism of docetaxel in rats.

Keywords docetaxel; P-glycoprotein; pharmacokinetics; rats; tesmilifene

Introduction

There has been interest for the development of oral dosage forms of cytotoxic drugs, from the aspect of patient convenience and for pharmacoeconomics.[1,2] However, the low extent of absolute oral bioavailability (F) of taxanes has limited the development for oral dosage forms due to the high affinity of drugs for the multidrug efflux pump, P-glycoprotein (P-gp), in the mucosa of the gastrointestinal tract. This has resulted in limitation of the absorption of the orally administered taxanes because of the direct efflux of taxanes into the gut lumen.[3,4] Oral administration with ciclosporin, a P-gp inhibitor, resulted in prolonged exposure of taxanes.[5]

Docetaxel (Taxotere), a member of the taxane class, has antitumour activity; the disruption of the equilibrium within the microtubule system by docetaxel ultimately leads to cell death.[6,7] Among mice, rats, dogs, and cancer patients, docetaxel is exclusively eliminated by hepatic metabolism and biliary excretion as a parent drug and its hydroxylated metabolites.[8,9] The intestinal first-pass effects of docetaxel via cytochrome P450 (CYP) 3A subfamily could be the main reason for the low value of F in humans and mice, in addition to the high affinity of the drug for P-gp as a substrate.[4,10,11]

Tesmilifene, *N*,*N*-diethyl-2-[4-(phenylmethyl)phenyl] ethanamine, is a novel antihistaminic and chemopotentiating agent; the survival benefit for patients co-administrated tesmilifene and doxorubicin is greater than that with single administration of doxorubicin.[12,13] Tesmilifene is reported as a substrate of CYP1A1, 2D6 and 3A4, and an inhibitor of P-gp for the efflux of paclitaxel and doxorubicin.[11,12–14] Also, the higher concentration of tesmilifene

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shows the inhibition effect of P-gp *in vitro*, overcoming drug resistance to paclitaxel.[15] However, the in-vivo interaction between docetaxel and tesmilifene seems not to have been reported. Thus, this study has investigated the effects of tesmilifene on the pharmacokinetics of intravenous and oral docetaxel in rats.

Materials and Methods

Chemicals

Docetaxel trihydrate and tesmilifene were supplied from Central Research Institute, Shin Poong Pharmaceutical Company, Ltd (Ansan, South Korea). Butyl 4-hydroxybenzoate (internal standard for the high-performance liquid chromatographic (HPLC) analysis of docetaxel) and paclitaxel (internal standard for the liquid chromatography for the tandom mass spectrometry (LC-MS/MS) of docetaxel) were purchased from Sigma–Aldrich Corporation (St Louis, MO, USA). *N*,*N*-Dimethylacetamide (DMA) was a product from Burdick & Jackson (Muskegon, MI, USA). Other chemicals were of reagent or HPLC grade.

Animals

The protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of Seoul National University, Seoul, South Korea. Male Sprague-Dawley rats (6–9-weeks old; 240–280 g) were purchased from Charles River Company Korea (Orient, Seoul, South Korea). All rats were maintained under the same conditions as reported by Choi *et al*.[16]

Intravenous and oral administration of docetaxel with or without tesmilifene

The procedures used for pretreatment of rats including the cannulation (early in the morning) of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) were similar to a reported method.[16]

Docetaxel (docetaxel trihydrate was dissolved in DMA : distilled water = 1 : 1, v / v) at a dose of 20 mg/kg as base without (control; $n = 9$) and with 5 ($n = 10$), 10 ($n = 9$), or 20 ($n = 6$) mg/kg tesmilifene was simultaneously administered intravenously for 1 min in rats. The total injection volume was 2 ml/kg. Tesmilifene (dissolved in ethanol) was diluted in distilled water. Blood samples (each approximately 220 μl) were collected via the carotid artery at 0 (control), 1 (end of the infusion), 5, 15, 30, 60, 90, 120, 180, 240, 300 and 360 min after the start of intravenous infusion of docetaxel. Heparinized 0.9% NaCl-injectable solution (20 U/ml; 0.3 ml) was used to flush each cannula immediately after each blood sampling to prevent blood clotting. After centrifugation of a blood sample, 100 μl plasma was stored at –70°C until used for the analysis of docetaxel. The procedures used for the preparation and handling of the 24-h urine sample ($Ae_{0\text{–}24\,h}$) and the gastrointestinal tract (including its contents and faeces) sample at 24 h ($GI_{24\,h}$) were similar to a reported method.[16]

Docetaxel (the same solution used in the intravenous study) at a dose of 20 mg/kg as base without ($n = 6$) and with 5 ($n = 7$), 10 ($n = 7$) or 20 ($n = 8$) mg/kg tesmilifene was simultaneously administered orally in rats using a gastric gavage tube after overnight fasting with free access to water. The total oral volume was 5 ml/kg. Blood samples were collected via the carotid artery at 0, 3, 5, 15, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min after oral administration of docetaxel. Other procedures were similar to those in the intravenous study.

HPLC and LC-MS/MS analyses of docetaxel

Concentrations of docetaxel were determined using LC-MS/MS (plasma samples in oral study) or HPLC (other samples) method. For LC-MS/MS analysis, 150 μl acetonitrile containing 25 ng/ml paclitaxel (internal standard) and 100 μl 0.1% acetic acid were added to 100 μl of a biological sample. After vortex-mixing and centrifugation, 10 μl supernatant was directly injected onto a C_{18} column (Symmetry BEH phenyl; 100 × 2.1 mm i.d.; particle size, 1.7 μm; Waters, Milford, MA, USA). The mobile phase, 0.1% acetic acid : acetonitrile (50 : 50, v / v), was run at a flow rate of 0.3 ml/min. An ABI/MDS Sciex model API 4000 triple quadrupole mass spectrometer was used. The source temperature was set at 250°C, ion spray voltage was 5500 V, gases were set at 50 for the nebulizin and curtain gas, and at 20 and 50 for the auxiliary and CAD gases, respectively. The MS/MS transition of docetaxel measured was 808.5 → 527.1 and that of paclitaxel was 854.3 → 286.2. The detection limit of docetaxel in rat plasma was 0.1 ng/ml, based on a signal-to-noise ratio of 3. The linearity was investigated in the ranges of 0.1–500 ng/ml and the linear regression equation for docetaxel in rat plasma was $y = 0.0512\,x + 0.0154$ ($R^2 = 1$; $n = 3$), where y is the peak area ratio of analyte to internal standard, and x is the plasma concentration of docetaxel.

For HPLC analysis, 1 ml ethylacetate and 100 μl methanol containing 5 μg/ml butyl 4-hydroxybenzoate (internal standard) were added to 100 μl of a biological sample. After vortex-mixing and centrifugation, the organic layer was collected and dried (Dry Thermobath; Eyela, Tokyo, Japan) under a gentle stream of nitrogen gas at 40°C. Then, a 100 μl mobile phase was added to reconstitute the residue, and 50 μl supernatant was directly injected onto a C_{18} column (Symmetry; 300 × 4.6 mm i.d.; particle size, 5 μm; Waters μ bondapak; Waters, Milford, MA, USA). The mobile phase, distilled water : acetonitrile (60 : 40, v / v), was run at a flow rate of 1.0 ml/min with an ultraviolet detector at 227 nm, at room temperature. The detection limits of docetaxel in rat plasma, urine, and gastrointestinal tract samples were 0.1, 0.2 and 0.5 μg/ml, respectively; the linearities were investigated in the ranges of 0.1–500, 0.2–50, and 0.2–50 μg/ml, respectively. The linear regression equations for docetaxel were $y = 0.169\,x + 0.109$ ($R^2 = 0.995$; $n = 3$), $y = 0.139\,x - 0.0429$ ($R^2 = 0.999$; $n = 3$) and $y = 0.109\,x + 0.0507$ ($R^2 = 0.999$; $n = 3$), respectively.

Pharmacokinetic analysis

Standard methods were used to calculate the following pharmacokinetic parameters using a noncompartmental analysis (WinNonlin; professional edition version 2.1; Pharsight, Mountain View, CA, USA); the total area under the plasma concentration–time curve from time zero to infinity (AUC; for intravenous study) or up to the last measured time, 6 h, in plasma ($AUC_{0\text{–}6\,h}$; for oral study), terminal half-life ($t^l/_2$), time-averaged total body, renal and nonrenal clearances (CL, CL_R,

and CL_{NR}, respectively), mean residence time (*MRT*) and apparent volume of distribution at a steady state (Vd_{ss}).[17,18] The *F* value was calculated by dividing the $AUC_{0\text{–}6\,h}$ after oral administration by the $AUC_{0\text{–}6\,h}$ after intravenous administration. The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the experimental data.

Statistical analysis

A P value < 0.05 was considered to be statistically significant using Tukey's multiple range test of Social Package of Statistical Sciences (SPSS; version 13.0) posteriori analysis of variance among the four means for the unpaired data. All data are expressed as mean ± SD except median (ranges) for T_{max}.

Results

For the simultaneous intravenous and oral administration of docetaxel 20 mg/kg as base without or with various doses of tesmilifene (5, 10 or 20 mg/kg) in rats, the mean arterial plasma concentration–time curves of docetaxel are shown in Figure 1. The relevant pharmacokinetic parameters are listed in Table 1. After simultaneous intravenous administration of docetaxel with various doses of tesmilifene, no significant changes in the pharmacokinetic parameters of docetaxel were observed, except for the significantly longer (by 39.3%) terminal half-life with 20 mg/kg tesmilifene compared with 10 mg/kg.

After simultaneous oral administration of docetaxel with various doses of tesmilifene, absorption of docetaxel from the rat gastrointestinal tract was rapid; docetaxel was detected in plasma at the early sampling time point (3 or 5 min) and rapidly reached T_{max} (5 min). Changes in the pharmacokinetic parameters of docetaxel with tesmilifene were as follows: the $Ae_{0\text{–}24\,h}$ with 10 mg/kg tesmilifene and the $GI_{24\,h}$ with 20 mg/kg tesmilifene were significantly smaller (by 90.5 and 47.9%, respectively), compared with the values for those without tesmilifene.

Discussion

Docetaxel 5–20 mg/kg and tesmilifene 1–20 mg/kg have been used previously in pharmacokinetic studies in rats. Thus, 20 mg/kg docetaxel and 5, 10, and 20 mg/kg tesmilifene were chosen for this study.[11,19,20]

The $Ae_{0\text{–}24\,h}$ values of intravenous docetaxel were less than 0.955% of the dose (Table 1), indicating that intravenous docetaxel was almost completely metabolized in rats. The contribution of the gastrointestinal excretion of docetaxel to the CL_{NR} of the drug was almost negligible; the values for $GI_{24\,h}$ were less than 3.03% of the intravenous dose (Table 1). Thus, the values of CL_{NR} for docetaxel listed in Table 1 could have represented its metabolic clearance in rats. Additionally, the changes in the CL_{NR} for docetaxel could have represented the changes in its metabolism.

After simultaneous intravenous administration of docetaxel with various doses of tesmilifene the *AUC*, *CL* and CL_{NR} values for docetaxel were comparable with those without tesmilifene (Table 1). Although tesmilifene has been reported to be a substrate of the CYP3A subfamily, comparable CL_{NR} values of docetaxel (Table 1) suggested that the effect (inhibition) of various does of tesmilifene (5–20 mg/kg) for the metabolism of docetaxel via hepatic CYP3A subfamily was almost negligible in rats.[8]

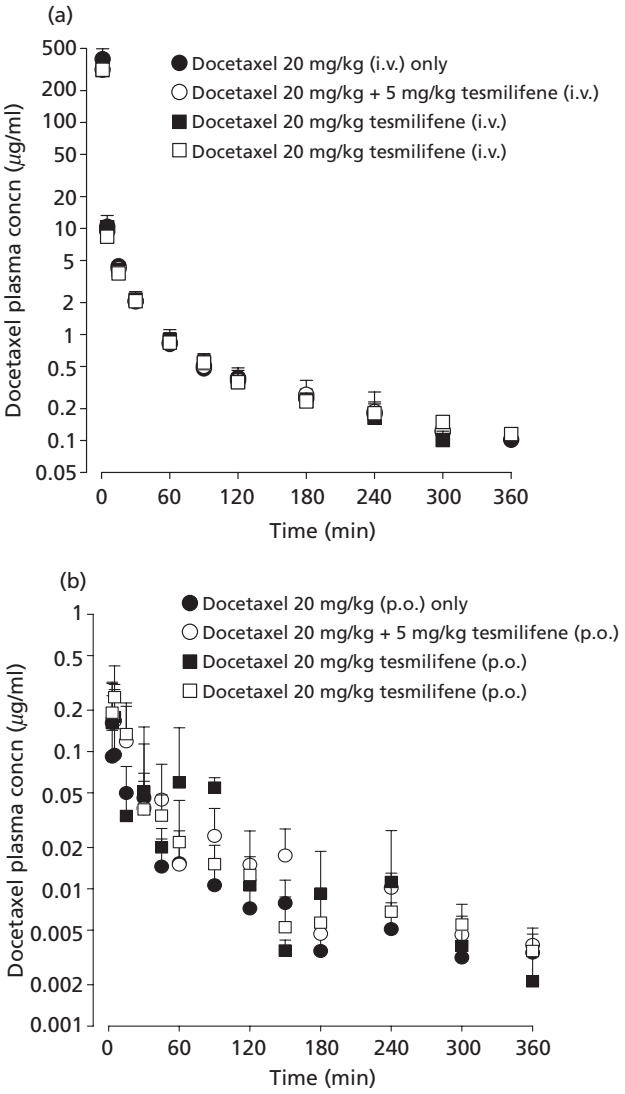

Figure 1 Mean arterial plasma concentration–time profiles of docetaxel. (a) Intravenous or (b) oral administration of docetaxel 20 mg/kg without (n = 9 and 6 for intravenous and oral administration, respectively) or with 5 (n = 10 and 7 for intravenous and oral administration, respectively), 10 (n = 9 and 7 for intravenous and oral administration, respectively), or 20 mg/kg tesmilifene (n = 6 and 8 for intravenous and oral administration, respectively) in rats. Vertical bars represent SD.

Considering the plasma protein binding value of docetaxel in rats (80%), the CL_R values of docetaxel (Table 1) were estimated from the free (unbound to plasma proteins) fraction of docetaxel in plasma ($CL_{R,fu}$).[21] The $CL_{R,fu}$ values thus estimated were 0.373, 0.945, 1.35 and 1.31 ml/min/kg for without and with 5, 10 and 20 mg/kg tesmilifene, respectively. These values were considerably slower than the reported glomerular filtration rate (creatinine clearance) in rats (5.24 ml/min/kg).[22] The above data indicated that docetaxel was mainly reabsorbed in rat renal tubules and reabsorption of docetaxel decreased (inhibited) by tesmilifene. The CL_R, $Ae_{0\text{–}24\,h}$, and $GI_{24\,h}$ values for docetaxel were very small (Table 1).

Table 1 Pharmacokinetic parameters of docetaxel after its simultaneous intravenous or oral administration without and with tesmilifene in rats

Parameter	Docetaxel without tesmilifene	Docetaxel with tesmilifene		
	Control	5 mg/kg	10 mg/kg	20 mg/kg
Intravenous	($n = 9$)	($n = 10$)	($n = 9$)	($n = 6$)
AUC (μg min/ml)	868 ± 126	755 ± 122	746 ± 187	742 ± 74.2
$t^l/_2$ (min)	129 ± 32.0	132 ± 20.0	112 ± 15.8	156 ± 15.8[a]
MRT (min)	32.7 ± 12.8	39.1 ± 13.9	30.9 ± 6.54	43.5 ± 3.02
CL (ml/min/kg)	23.6 ± 4.55	27.2 ± 4.68	28.2 ± 6.47	27.2 ± 3.21
CL_R (ml/min/kg)	0.0746 ± 0.0478	0.189 ± 0.122	0.270 ± 0.206	0.262 ± 0.108
CL_{NR} (ml/min/kg)	24.2 ± 5.58	27.9 ± 2.34	29.2 ± 6.59	27.0 ± 3.16
Vd_{ss} (ml/kg)	804 ± 443	1070 ± 403	900 ± 382	1190 ± 212
$Ae_{0\text{–}24\,h}$ (% of dose)	0.311 ± 0.210	0.647 ± 0.395	0.879 ± 0.587	0.955 ± 0.372
$GI_{24\,h}$ (% of dose)	2.54 ± 0.959	3.03 ± 1.38	2.89 ± 0.528	2.05 ± 1.05
Oral	($n = 6$)	($n = 7$)	($n = 7$)	($n = 8$)
$AUC_{0\text{–}6\,h}$ (μg min/ml)	3.96 ± 1.32	6.91 ± 2.70	6.38 ± 1.01	6.70 ± 2.09
C_{max} (μg/ml)	0.123 ± 0.0474	0.204 ± 0.151	0.244 ± 0.103	0.270 ± 0.159
T_{max} (min)	5 (3–30)	5 (3–45)	5 (3–60)	5 (3–15)
$Ae_{0\text{–}24\,h}$ (% of dose)	1.29 ± 0.937	0.459 ± 0.175	0.123 ± 0.0933[b]	0.490 ± 0.339
$GI_{24\,h}$ (% of dose)	40.5 ± 10.4	29.5 ± 14.5	23.1 ± 12.2	21.1 ± 9.21[c]
F (%)	0.466	0.941	0.869	0.936

Docetaxel was administered at a dose of 20 mg/kg (control). Values are mean ± SD. *AUC*, total area under the plasma concentration–time curve from time zero to infinity; $t^l/_2$, terminal half-life; *MRT*, mean residence time; *CL*, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged nonrenal clearance; Vd_{ss}, apparent volume of distribution at steady state; $Ae_{0\text{–}24\,h}$, percentage of the dose excreted in the urine up to 24 h; $GI_{24\,h}$, percentage of the dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h; $AUC_{0\text{–}6\,h}$, total area under the plasma concentration–time curve from time zero to 6 h; C_{max}, maximum plasma concentration; T_{max}, time to reach C_{max}; *F*, extent of absolute oral bioavailability. [a]Tesmilifene (20 mg/kg) group was significantly different ($P < 0.05$) from tesmilifene (10 mg/kg) group (*P* value for the overall treatment effect (analysis of variance) was 0.007). [b]Tesmilifene (10 mg/kg) group was significantly different ($P < 0.05$) from without tesmilifene group (*P* value for the overall treatment effect (analysis of variance) was 0.018). [c]Tesmilifene (20 mg/kg) group was significantly different ($P < 0.05$) from without tesmilifene group (*P* value for the overall treatment effect (analysis of variance) was 0.035).

After simultaneous oral administration of docetaxel with tesmilifene, the *AUC* values for docetaxel were comparable with that for without tesmilifene (Table 1). This indicated that the inhibitory effects of tesmilifene on the P-gp-mediated efflux of docetaxel in the intestine and intestinal CYP3A subfamily were almost negligible.[23]

The extremely low values of *F* (less than 1%) for docetaxel (Table 1) could have been due, at least partly, to the incomplete absorption of docetaxel from the gastrointestinal tract in rats. For comparison, the mean 'true' fractions unabsorbed (F_{unabs}) after oral administration of docetaxel in rats without and with tesmilifene were estimated based on the following reported equations:[24]

$$0.405 = F_{unabs} + (0.00466 \times 0.0254) \quad \text{without tesmilifene} \tag{1}$$

$$0.295 = F_{unabs} + (0.00941 \times 0.0303) \quad 5\ \text{mg/kg tesmilifene} \tag{2}$$

$$0.231 = F_{unabs} + (0.00869 \times 0.0289) \quad 10\ \text{mg/kg tesmilifene} \tag{3}$$

$$0.211 = F_{unabs} + (0.00936 \times 0.0205) \quad 20\ \text{mg/kg tesmilifene} \tag{4}$$

in which 0.405 (0.295, 0.231 and 0.211, respectively), 0.0254 (0.0303, 0.0289 and 0.0205, respectively) and 0.00466 (0.00941, 0.00869 and 0.00936, respectively) are the $GI_{24\,h}$ values after oral and intravenous administration and *F*, respectively, for without tesmilifene (or with 5, 10 or 20 mg/kg tesmilifene, respectively). The F_{unabs} values thus estimated were 40.5, 29.5, 23.1 and 21.1% for without and with 5, 10 and 20 mg/kg tesmilifene, respectively. Thus, the percentages of the docetaxel oral doses absorbed up to 24 h were 59.5, 70.5, 76.9 and 78.9% of the oral dose, respectively. The values for *F* were extremely low, even considering the unabsorbed fractions of docetaxel up to 24 h. This suggested considerable first-pass (gastric and/or intestinal) metabolism of docetaxel in rats as reported in humans.[3,4,10] It has been reported that first-pass metabolism of docetaxel by the gut wall is likely to be the major cause of its low *F*.[10]

Conclusions

The doses of tesmilifene used in this study did not considerably inhibit the metabolism of docetaxel via the hepatic CYP3A subfamily, P-gp mediated efflux of docetaxel in the intestine, or the metabolism of docetaxel via the intestinal CYP3A subfamily in rats. The extremely low values of *F* for docetaxel could have been due to the incomplete absorption and considerable first-pass effect of docetaxel in rats. Due to the extremely low values of *F* for docetaxel with various doses of tesmilifene, the development of an oral dosage form of docetaxel with tesmilifene does not seem to be appropriate,

unless the efficacy of oral docetaxel with tesmilifene is considerably greater than 'without tesmilifene'.

Declarations

Conflict of interest

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

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