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ORIGINAL ARTICLE

Pharmacokinetic interaction between tamoxifen and ondansetron in rats: non-competitive (hepatic) and competitive (intestinal) inhibition of tamoxifen metabolism by ondansetron via CYP2D subfamily and 3A1/2

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Abstract
Purpose Tamoxifen and ondansetron were commonly metabolized via rat hepatic CYP2D subfamily and 3A1/2, and ondansetron is used to treat chemotherapy-induced nausea. The purpose of this study was to report the pharmacokinetic interaction between tamoxifen and ondansetron in rats.
Methods The pharmacokinetics of tamoxifen and ondansetron were evaluated after the intravenous and oral administration of tamoxifen, ondansetron, and both drugs together to rats. The V_{max} (maximum velocity), K_m (apparent Michaelis–Menten constant), CL_{int} (intrinsic clearance), K_i (inhibition constant), and [I] (concentration of inhibitor in the liver and intestine)/K_i ratio of ondansetron were also measured.
Results The $AUC_{0-\infty}$s of tamoxifen were significantly greater after both intravenous and oral administration with ondansetron compared to those of tamoxifen alone. The significantly slower hepatic and intestinal CL_{int}s for the disappearance of tamoxifen with both drugs together were due to inhibition of metabolism of tamoxifen by ondansetron via CYP2D subfamily and 3A1/2.
Conclusions The significantly greater $AUC_{0-\infty}$ of tamoxifen after the intravenous administration of both drugs together could have possibly been attributable to a non-competitive (hepatic) inhibition of CYP2D subfamily- and 3A1/2-mediated tamoxifen metabolism by ondansetron. The significantly greater $AUC_{0-\infty}$ of tamoxifen after the oral administration of both drugs together could have been attributable to a competitive (intestinal) inhibition of CYP2D subfamily- and 3A1/2-mediated tamoxifen metabolism by ondansetron in addition to non-competitive inhibition in the liver.

Keywords Interaction between ondansetron and tamoxifen · Non-competitive and competitive inhibition · CYP2D subfamily and 3A1/2 · Rats

Abbreviations

Ae_{0-24h}	Percentage of the dose excreted in the 24-h urine
$AUC_{0-\infty}$	Total area under the plasma concentration–time curve from time zero to time infinity
C_{max}	Peak plasma concentration
CL	Time-averaged total body clearance
CL_R	Time-averaged renal clearance
CL_{NR}	Time-averaged non-renal clearance
CL_{int}	Intrinsic clearance
CYP	Cytochrome P450
F	Extent of absolute oral bioavailability
FMO	Flavin-containing monooxygenase
GI_{24h}	Percentage of the dose recovered from the gastrointestinal tract (including its contents and feces) at 24 h
HPLC	High-performance liquid chromatography
I	Inhibitor
K_i	Inhibition constant
K_m	Apparent Michaelis–Menten constant
T_{max}	Time to reach C_{max}
V_{max}	Maximum velocity
V_{ss}	Apparent volume of distribution at steady state

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Introduction

Tamoxifen, a non-steroidal selective estrogen receptor modulator, is highly efficacious in the treatment of breast cancer, particularly postmenopausal patients with tumor containing the estrogen receptor [1]. In humans, the urinary excretion, plasma protein binding, clearance, apparent volume of distribution, and half-life of tamoxifen were less than 1%, greater than 98%, 1.4 mL min^{-1} kg^{-1}, 50–60 L kg^{-1}, and 4–11 days, respectively [2]. Human liver microsome studies have shown that tamoxifen was mainly metabolized via hepatic cytochrome P450 (CYP) 3A subfamily by three oxidative pathways; *N*-desmethyltamoxifen via CYP3A subfamily, 4-hydroxytamoxifen via CYP2C9, 2D6, and 3A subfamily, and *N*-oxide via flavin-containing monooxygenase (FMO) [3, 4]. Rat liver microsome studies have shown that *N*-desmethyltamoxifen was formed via CYP3A1 and possibly via CYP1A2 and/or 3A2, 4-hydroxytamoxifen via CYP1A2 [5] and CYP2D subfamily, especially CYP2D2 [6], and *N*-oxide via FMO1 [6]. However, the hepatic and gastrointestinal first-pass effects of tamoxifen in rats in vivo did not seem to be reported.

Ondansetron, a potent and selective 5-HT_3 (5-hydroxytryptamine) receptor antagonist, is most effective in the treatment of chemotherapy-induced nausea and of nausea secondary to upper abdominal irradiation. Hepatic CYP2D subfamily and 3A1/2 (not CYP1A1/2, 2B1/2, 2C6 and 2E1) were responsible for the metabolism of ondansetron in rats [7]. After the intravenous, oral, intraportal, intragastric, and intraduodenal administration of ondansetron at a dose of 8 mg kg^{-1} to rats, its extent of absolute oral bioavailability (*F*) was 4.07%, unabsorbed fraction from the gastrointestinal tract for up to 24 h was 1.58% of the oral dose, hepatic first-pass effect after absorption into the portal vein was 64.8%, and intestinal first-pass effect was 34.2% of the oral dose [8]. The lower *F* of ondansetron in rats, 4.07% [8], than 62 ± 15% in humans [2] was mainly due to greater metabolism of ondansetron in rat liver [8].

Pharmacokinetic interactions between ondansetron and anticancer drugs have been reported. For example, ondansetron alters the systemic exposure to cyclophosphamide in breast cancer patients [9], and causes significantly smaller total area under the plasma concentration–time curve from time zero to time infinity (AUC) of high-dose cyclophosphamide and cisplatin in patients who received high-dose chemotherapy with cyclophosphamide, cisplatin, and carmustine [10]. However, no studies on pharmacokinetic interaction between tamoxifen and ondansetron have yet been reported. Thus, in the present study, pharmacokinetic interaction between tamoxifen and ondansetron was evaluated using rats as an animal model, because both the two drugs were commonly metabolized via hepatic CYP2D subfamily and 3A1/2 in rats as in humans (hepatic CYP2D6 and 3A4). The CYP isozymes responsible for the metabolism of tamoxifen and the hepatic and gastrointestinal first-pass effects of tamoxifen in rats were also reported.

Materials and methods

Chemicals

Ondansetron hydrochloride dihydrate was supplied from Dong-A Pharmaceutical Company, Ltd. (Yongin, South Korea). Tamoxifen citrate, propranolol [internal standard for the high-performance liquid chromatographic (HPLC) analysis of ondansetron], imipramine hydrochloride (internal standard for the HPLC analysis of tamoxifen), *N*,*N*-dimethylacetamide (DMA), tri(hydroxymethyl)aminomethane (Tris)-buffer, ethylenediamine tetraacetic acid (EDTA; as a disodium salt), and the reduced form of *β*-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt) were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Other chemicals were of reagent or HPLC grade.

Animals

The protocols for the animal studies were approved by Institute of Laboratory Animal Resources of Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats (7–11 weeks old, weighing 250–335 g) were purchased from Charles River Company Korea (Orient Bio, Seoul, South Korea). The procedures used for keeping and handling of the rats were similar to reported methods [7, 8].

In vitro studies

Measurement of V_{max}, K_m, and CL_{int} for disappearance of tamoxifen with or without ondansetron in rat hepatic and intestinal microsomes

The procedures used for preparation of hepatic [11] and intestinal [12] microsomes were similar to reported methods. Protein contents in hepatic and intestinal microsomes were measured using a reported method [13].

The V_{max} (the maximum velocity) and K_m (apparent Michaelis–Menten constant, the concentration at which the rate is one half of V_{max}) for disappearance of tamoxifen with or without ondansetron were determined as following. The following components were added to a test tube: microsomes (equivalent to 0.5 mg proteins for both hepatic and intestinal microsomes); 10 μL of methanol containing final tamoxifen concentrations of 0.2, 0.5, 1, 2, 5, and 10 μM (for both hepatic and intestinal microsomes); 10 μL of distilled water containing with or without 1 μM ondansetron

(for both hepatic and intestinal microsomes); and 50 μL of 0.1 M phosphate buffer (pH 7.4) containing 1 mM NADPH. The volume was adjusted to 0.5 mL by adding 0.1 M phosphate buffer (pH 7.4), and the components were mixed at 37°C using a thermomixer at 500 oscillations per min (opm). All the microsomal incubation conditions were in the linear range of the reaction rate. The reaction was terminated by addition of 200 μL of acetonitrile after 15-min incubation.

The kinetic constants (K_m and V_{max}) were calculated using a non-linear regression method [14]. The intrinsic clearance (CL_{int}) was calculated by dividing the V_{max} by the K_m.

Measurement of K_i of ondansetron and manner of inhibition of tamoxifen metabolism by ondansetron in rat hepatic and intestinal microsomes

The procedures used to investigate the manner of inhibition of tamoxifen metabolism by ondansetron were similar to a reported method [15]. The following components were added to a tube: microsomes (equivalent to 0.5 mg proteins for both hepatic and intestinal microsomes); 10 μL of methanol containing final tamoxifen concentrations of 0.2, 0.5, 1, and 2 μM (for hepatic microsomes) or 0.5, 1, 2, and 5 μM (for intestinal microsomes); 10 μL of distilled water containing final ondansetron (as an inhibitor for both hepatic and intestinal microsomes) concentrations of 0.05, 0.1, 0.2, 0.5, 1, and 2 μM, and 50 μL of 0.1 M phosphate buffer (pH 7.4) containing 1 mM NADPH. The volume was adjusted to 0.5 mL by adding 0.1 M phosphate buffer (pH 7.4), and the components were mixed at 37°C using a thermomixer at 500 opm. All the microsomal incubation conditions were linear. The reaction was terminated by addition of 200 μL of acetonitrile after 15-min incubation.

The kinetic constants including apparent inhibition constant (K_i) of ondansetron for disappearance of tamoxifen in hepatic and intestinal microsomes were calculated from appropriate non-linear regression models for enzyme inhibition using Graphpad Prism® (GraphPad Software, San. Diego, CA, USA).

Measurement of K_m, V_{max}, and CL_{int} for disappearance of tamoxifen with chemical inhibitors of specific CYP2D subfamily and 3A1/2 in hepatic and intestinal microsomes

Quinine hydrochloride (a main inhibitor of CYP2D subfamily in rat) and troleandomycin (a main inhibitor of CYP3A1/2 in rat) were used to determine whether CYP2D subfamily and 3A1/2 are responsible for the metabolism of tamoxifen in rat liver and intestine. The procedures used were similar to a method mentioned above to measure the K_i of ondansetron, except that 10 μL of methanol containing final tamoxifen concentrations of 0.2, 0.5, 1, 2, 5, and 10 μM were incubated with 10 μL of methanol containing 3 μM quinine hydrochloride or 10 μL of methanol containing 50 μM troleandomycin for 5 min. For the mechanism-based inhibitor (troleandomycin) and controls, the microsomes, CYP inhibitor, and NADPH were pre-incubated for 15 min, and 20 units of catalase were added to prevent auto-inactivation of CYP isozymes during the pre-incubation [16].

Measurement of rat plasma protein binding of ondansetron and tamoxifen with or without each drug

Protein binding values of ondansetron and tamoxifen with or without each other to fresh rat plasma ($n = 5$, each) were measured using equilibrium dialysis [7, 8]. Plasma (1 mL) was dialyzed against 1 mL of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran ('the buffer') in a 1-mL dialysis cell (Spectrum Medical Industries, Los Angeles, CA, USA) using a Spectra/Por 4 membrane (mol. wt. cutoff 12–14 KDa; Spectrum Medical Industries). After 4-h incubation for ondansetron and 12-h incubation for tamoxifen, two 100 μL were collected from each compartment and stored at −70°C until used for the HPLC analysis of ondansetron and tamoxifen.

In vivo studies

Intravenous and oral administration of ondansetron, tamoxifen, and both drugs together

The procedures used for pretreatment of rats including cannulation (in the early morning) of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) were similar to reported methods [7, 8, 17]. The rats were not restrained in the present study.

Ondansetron (ondansetron hydrochloride dihydrate was dissolved in distilled water: DMA = 1:1, v/v) at a dose of 8 mg kg^{-1} ($n = 6$), tamoxifen (tamoxifen citrate was also dissolved in the same vehicle) at a dose of 2 mg kg^{-1} ($n = 6$), and both drugs together ($n = 6$) were infused (total infusion volume of 2 mL kg^{-1}) for 1 min via the jugular vein. A blood sample (approximately 0.22 mL for ondansetron alone and tamoxifen alone, and 0.42 mL for both drugs) was collected via the carotid artery at 0 (control), 1 (end of the infusion), 3, 7, 15, 30, 60, 90, 120, and 150 min after the start of the intravenous infusion of ondansetron, and at 0, 1, 3, 7, 15, 30, 60, 120, 180, 240, 360, and 480 min after the start of the intravenous infusion of tamoxifen and both drugs together. 1 mL of blood collected from untreated rats was infused at 1 and 3 h, respectively (for both drugs together), to replace the blood loss due to blood

sampling. A blood sample was immediately centrifuged and a 100 μL (two 100 μL for both drugs together) of a plasma sample was collected in a 1.5-mL polyethylene tube, and was stored at −70°C (Revco ULT 1490 D-N–S; Western Mednics, Ashville, NC, USA) until used for the HPLC analysis of ondansetron and tamoxifen. The procedures used for preparation and handling of the 24-h urine sample (Ae_{0-24h}) and the gastrointestinal tract (including its contents and feces) sample at 24 h (GI_{24h}) were similar to reported methods [7, 8].

Ondansetron (the same solution used in the intravenous study) at a dose of 8 mg kg^{-1} ($n = 6$), tamoxifen (the same solution used in the intravenous study) at a dose of 10 mg kg^{-1} ($n = 7$), and both drugs together ($n = 6$) were orally administered (total oral volume of 5 mL kg^{-1}) using a gastric gavage tube. A blood sample (approximately 0.22 mL for ondansetron alone and tamoxifen alone, and 0.42 mL for both drugs) was collected via the carotid artery at 0, 3, 5, 10, 20, 30, 40, 50, 60, 75, and 90 min after the oral administration of ondansetron, and at 0, 10, 30, 60, 90, 120, 180, 240, 480, and 720 min after the oral administration of tamoxifen and both drugs. Other procedures were similar to those for the intravenous study.

Measurement of the hepatic first-pass effect of tamoxifen in rats

The procedures used for the cannulation of the carotid artery, the jugular vein, and the vein from the caecum were similar to a reported method [18]. Tamoxifen (2 mg kg^{-1}) was infused (2 mL kg^{-1}) for 30 min into the jugular and the portal veins for the intravenous ($n = 4$) and intraportal ($n = 5$) administration, respectively, with the assistance of an infusion pump (Model 2400-006; Harvard Instrument, Southnatick, MA, USA). At the same time, an equal volume of the vehicle [distilled water:DMA (1:1, v/v)] was also infused for 30 min into the portal and the jugular veins for the intravenous and intraportal administration, respectively.

Measurement of the gastrointestinal first-pass effect of tamoxifen in rats

Rats were fasted overnight with free access to water. The surgical procedures and other procedures were similar to those for above mentioned hepatic first-pass effects of tamoxifen. For the intraportal infusion ($n = 5$), tamoxifen (10 mg kg^{-1}) was infused (2 mL kg^{-1}) via the portal vein for 30 min, and 2 mL kg^{-1} of the vehicle was instilled into the stomach using a 23-gauge needle. For the intragastric instillation ($n = 4$), 2 mL kg^{-1} of the vehicle was infused via the portal vein for 30 min, and the same dose of tamoxifen was instilled into the stomach.

Measurement of hepatic and intestinal concentrations of ondansetron after the intravenous and oral administration of ondansetron and tamoxifen

The same doses of ondansetron and tamoxifen were administered intravenously and orally. At 1, 5, 15, 30, and 60 min for the intravenous administration and 5, 15, 30, 60, and 120 min for the oral administration, approximately 1 g of liver (for the intravenous and oral administration) and small intestine (for the oral administration) was excised and blotted on tissue paper ($n = 3$ at each time point for each route of administration). A blood sample was also collected. The tissue samples were homogenized with four volumes of 0.9% NaCl injectable solution and centrifuged at 9,000×g for 10 min. Two 100 μL of the supernatant and a plasma sample were collected and stored at −70°C until used for the HPLC analysis of ondansetron.

HPLC analysis of ondansetron and tamoxifen

Concentrations of ondansetron in the samples were determined by a slight modification of reported HPLC methods [19, 20]; 50 μL of pH 9 buffer solution and 20 μL of distilled water containing 50 μg mL^{-1} of propranolol (internal standard) were added to 100 μL of sample. And then, the mixture was extracted with 0.5 mL of dichloromethane. After vortex-mixing for 30 s and centrifugation at 15,000×g for 10 min, the organic layer was transferred to a new tube and evaporated under a gentle stream of nitrogen gas at room temperature. The residue was reconstituted in 100 μL of the mobile phase and 75 μL was injected directly onto the reversed-phase (C_{18}; Symmetry; 100 mm l × 4.6 mm. i.d.; particle size, 3.5 μm; Waters, Milford, MA, USA) HPLC column. The mobile phase, 0.02 M sodium phosphate monobasic solution:acetonitrile (70:30, v/v; adjusted pH to 4.0 with 85% phosphoric acid), was run at a flow rate of 1.0 mL min^{-1}, and the column eluent was monitored using an ultraviolet detector at 305 nm at room temperature. The retention times of ondansetron and propranolol (internal standard) were approximately 2.2 and 3.6 min, respectively. The detection limits of ondansetron in rat plasma and urine samples were all 0.02 μg mL^{-1}. The coefficients of variation (intra- and inter-day) were below 5.39%.

Concentrations of tamoxifen in the samples were determined by a slight modification of reported HPLC methods [21, 22]. Briefly, 100 μL of a biological sample was deproteinized with 200 μL of acetonitrile containing 2 μg mL^{-1} of imipramine (internal standard). After vortex-mixing and centrifugation (15,000×g for 5 min), the upper organic layer was transferred to a new tube and evaporated under a gentle stream of nitrogen gas at room temperature. The residue was reconstituted in 100 μL of the mobile phase and

75 μL was injected directly onto a reversed-phase (C_{18}; Symmetry; 250 mm l × 4.6 mm. i.d.; particle size, 5 μm; SynChrom, Inc., Lafayette, IN, USA) HPLC column. The mobile phase, acetonitrile:0.05 M ammonium acetate (75:25, v/v; adjusted pH to 6.4 with acetic acid) was run at a flow rate of 1.0 mL min^{-1}, and the column eluent was monitored using an ultraviolet detector at 280 nm at room temperature. The retention times of imipramine (internal standard) and tamoxifen were approximately 7 and 9 min, respectively. The detection limits of tamoxifen in the rat plasma and urine samples were all 0.05 μg mL^{-1}. The coefficients of variation (intra- and inter-day) were below 9.28%.

Pharmacokinetic analysis

Standard methods [23] were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis (WinNonlin®; Pharsight Corporation, Mountain View, CA, USA); the $AUC_{0-\infty}$ [24], the time-averaged total body, renal, and non-renal clearances (CL, CL_R, CL_{NR}, respectively), the terminal half-life ($t_{1/2}$), the first moment of AUC (AUMC), the mean residence time (MRT), the apparent volume of distribution at a steady state (V_{ss}), and the F [17]. 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 deemed to be significantly using a Social Package of Statistical Sciences (SPSS) posteriori analysis of variance (ANOVA) among the three means for the unpaired data and then individual differences among groups were determined using Duncan's multiple range test, or an unpaired t test between the two means for the unpaired data. All data are expressed as mean ± SD except median (ranges) for T_{max}.

Results

In vitro studies

V_{max}, K_m, and CL_{int} for disappearance of tamoxifen with and without ondansetron in rat hepatic and intestinal microsomes

The V_{max}, K_m, and CL_{int} for disappearance of tamoxifen with and without ondansetron in hepatic microsomes are listed in Table 1a. Compared to without ondansetron, the V_{max} and CL_{int} were significantly slower (by 40.2 and 34.9%, respectively) with ondansetron. However, the K_ms were comparable between with and without ondansetron. This suggests that the maximum velocity for disappearance of tamoxifen (primarily metabolism) was significantly slower, affinity of the enzyme(s) for tamoxifen was not altered, and formation of metabolite(s) of tamoxifen significantly decreased with ondansetron. The above data suggest that the inhibition of hepatic metabolism of tamoxifen by ondansetron was a non-competitive manner.

The V_{max}, K_m, and CL_{int} for disappearance of tamoxifen with and without ondansetron in intestinal microsomes are also listed in Table 1a. Compared to without ondansetron, the K_m and CL_{int} were significantly higher (by 197%) and significantly slower (by 41.4%), respectively, with ondansetron. However, the V_{max}s were comparable between with and without ondansetron. The above data suggest that the inhibition of intestinal metabolism of tamoxifen by ondansetron was a competitive manner.

V_{max}, K_m, and CL_{int} for disappearance of tamoxifen with and without quinine and troleandomycin in hepatic and intestinal microsomes

The V_{max}, K_m, and CL_{int} for disappearance of tamoxifen with and without quinine and troleandomycin in hepatic and intestinal microsomes also listed in Table 1b. Compared to without quinine, the CL_{int}s for disappearance of tamoxifen were significantly slower for both hepatic and intestinal microsomes (by 21.0 and 36.3%, respectively), with quinine. Compared to without troleandomycin, the CL_{int}s for disappearance of tamoxifen were also significantly slower for both hepatic and intestinal microsomes (by 24.2 and 26.9%, respectively), with troleandomycin. Compared to without quinine and troleandomycin, the hepatic CL_{int}s for disappearance of ondansetron were significantly slower with quinine and troleandomycin (by 71.8 and 24.2%, respectively) [7]. Compared to without quinine and troleandomycin, the intestinal CL_{int}s for disappearance of ondansetron were significantly slower (by 29.8 and 35.9%, respectively), with quinine and troleandomycin (data not shown). The above data indicate that both tamoxifen and ondansetron were commonly metabolized via CYP2D subfamily and 3A1/2 in rats.

Non-competitive and competitive inhibition of tamoxifen metabolism by ondansetron in rat hepatic and intestinal microsomes, respectively

To investigate the kinetics of the inhibitory effects of ondansetron on tamoxifen metabolism, tamoxifen disappearance rate in rat hepatic microsomes was examined in various concentrations of ondansetron. The Lineweaver–Burk [25] and Dixon [26] plots for disappearance of tamoxifen in hepatic microsomes are shown in Fig. 1. The Lineweaver–Burk

Table 1 Mean (± standard deviation) V_{max}, K_m, and CL_{int} for disappearance of tamoxifen (a) with and without ondansetron and (b) with and without chemical inhibitors of CYP2D subfamily (quinine) and 3A1/2 (troleandomycin) in hepatic and intestinal microsomes

Parameter	Without ondansetron	With ondansetron (1 μM)
Hepatic	$n = 4$	$n = 4$
V_{max} (nmol min^{-1} mg^{-1} protein)	1.23 ± 0.200	0.735 ± 0.111**
K_m (μM)	46.5 ± 7.31	42.0 ± 2.28
CL_{int} (μL min^{-1} mg^{-1} protein)	0.0269 ± 0.00468	0.0175 ± 0.00289*
Intestinal	$n = 3$	$n = 3$
V_{max} (nmol min^{-1} mg^{-1} protein)	0.162 ± 0.0254	0.286 ± 0.0738
K_m (μM)	17.0 ± 2.87	50.5 ± 5.57***
CL_{int} (μL min^{-1} mg^{-1} protein)	0.00958 ± 0.000907	0.00561 ± 0.000821**

Parameter	Control	With quinine (3 μM)	With troleandomycin (50 μM)
Hepatic	$n = 3$	$n = 3$	$n = 3$
V_{max} (nmol min^{-1} mg^{-1} protein)	1.24 ± 0.142	0.877 ± 0.0373[a]	0.840 ± 0.0685[a]
K_m (μM)	44.1 ± 5.51	39.8 ± 4.80	39.5 ± 4.28
CL_{int} (μL min^{-1} mg^{-1} protein)	0.0281 ± 0.00208	0.0222 ± 0.00196[a]	0.0213 ± 0.00863[a]
Intestinal	$n = 3$	$n = 3$	$n = 3$
V_{max} (nmol min^{-1} mg^{-1} protein)	0.155 ± 0.0261	0.227 ± 0.0616	0.164 ± 0.0304
K_m (μM)	16.3 ± 3.49	37.1 ± 8.87[b]	23.9 ± 5.11
CL_{int} (μL min^{-1} mg^{-1} protein)	0.00959 ± 0.000916	0.00611 ± 0.000317[a]	0.00701 ± 0.00125[a]

* $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared to without ondansetron

[a] Significantly different ($P < 0.05$) from control

[b] Significantly different ($P < 0.05$) from other groups

plots (Fig. 1a) exhibited a series of lines converging on the *x*-axis [the inverse of the substrate (tamoxifen) concentration], also suggesting a non-competitive inhibition of tamoxifen metabolism by ondansetron in the liver. The Dixon plots (Fig. 1c) for the various substrate concentrations are linear and converge on the *x*-axis, which further indicates a non-competitive inhibition of tamoxifen metabolism by ondansetron in the liver. From these data, the apparent K_i of ondansetron in the liver was 2.23 (1.42–3.05) μM.

The Lineweaver–Burk and Dixon plots for disappearance of tamoxifen in intestinal microsomes are also shown in Fig. 1. The Lineweaver–Burk plots (Fig. 1b) exhibited a series of lines crossing the *y*-axis (the inverse of tamoxifen disappearance) at the same point, also suggesting a competitive inhibition of tamoxifen metabolism by ondansetron in the intestine. The Dixon plots (Fig. 1d) for the various substrate concentrations are linear and converge above the *x*-axis, which further suggests a competitive inhibition of tamoxifen metabolism by ondansetron in the intestine. From these data, the apparent K_i of ondansetron in the intestine was 2.10 (1.45–2.75) μM.

Rat plasma protein binding of ondansetron and tamoxifen with and without each other to fresh rat plasma

Protein binding (bound fraction) values of ondansetron with and without tamoxifen, and of tamoxifen with and without ondansetron to fresh rats' plasma were 71.2 ± 4.09, 72.9 ± 6.17, 98.7 ± 0.481, and 98.9 ± 0.234%, respectively; the values of both drugs were comparable between with and without each other.

In vivo studies

Pharmacokinetics of ondansetron after the intravenous administration of ondansetron with and without simultaneous intravenous administration of tamoxifen

The mean arterial plasma concentration–time profiles of ondansetron after its intravenous administration with and without tamoxifen are shown in Fig. 2a. The relevant pharmacokinetic parameters are listed in Table 2. The pharmacokinetic parameters of ondansetron listed in Table 2 were comparable (not significantly different) between with and without tamoxifen.

Pharmacokinetics of tamoxifen after the intravenous administration of tamoxifen with and without simultaneous intravenous administration of ondansetron

The mean arterial plasma concentration–time profiles of tamoxifen after its intravenous administration with and without ondansetron are shown in Fig. 2c. The relevant pharmacokinetic parameters are also listed in Table 2.

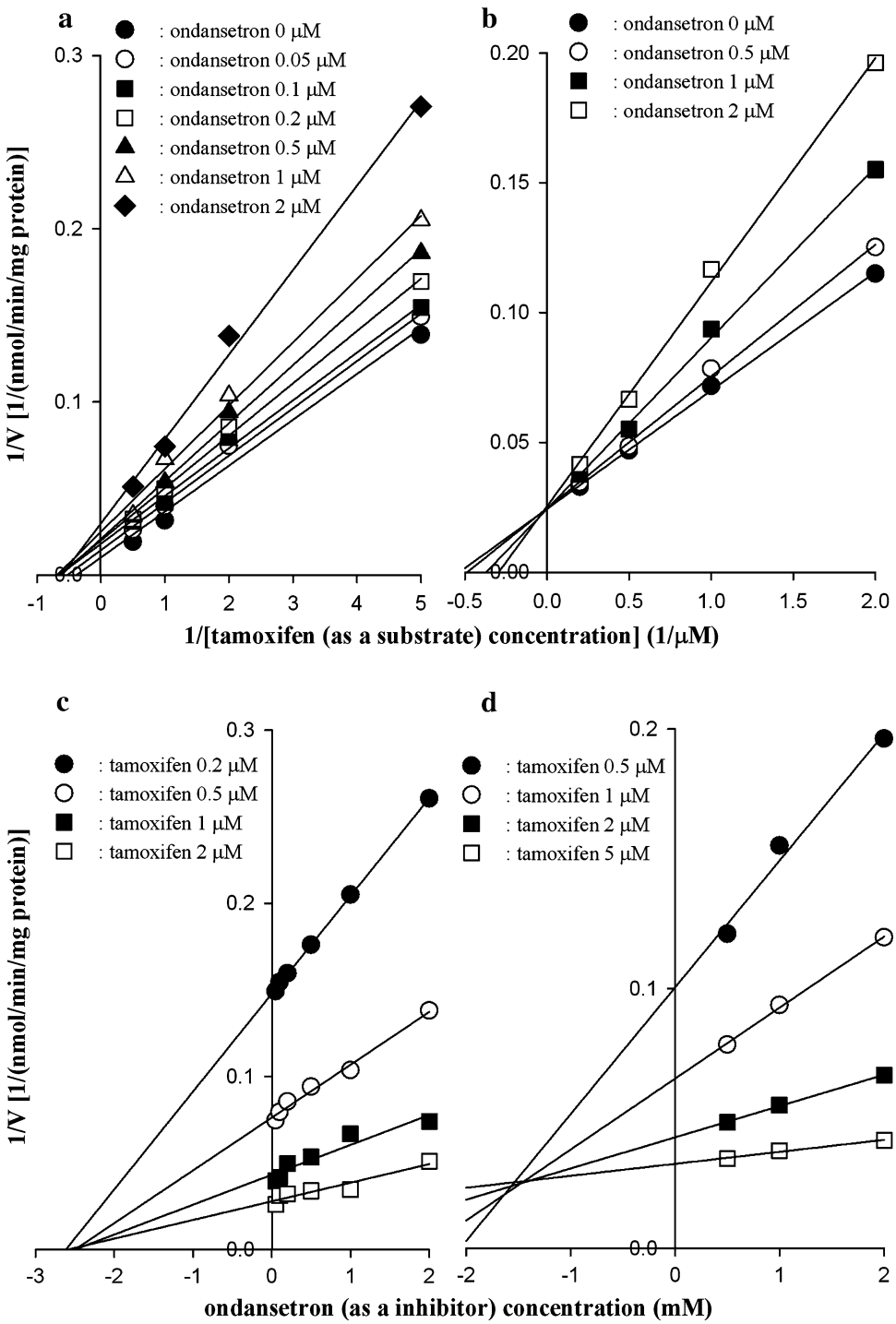

Fig. 1 Lineweaver–Burk (**a**, **b**) and Dixon (**c**, **d**) plots showing inhibition of disappearance of tamoxifen by ondansetron in rat hepatic (**a**, **c**) and intestinal (**b**, **d**) microsomes. The 'v' represents the velocity for disappearance of tamoxifen

Compared to without ondansetron, changes in the pharmacokinetic parameters of tamoxifen with ondansetron are as follows: the $AUC_{0-\infty}$ was significantly greater (by 22.9%) and CL was significantly slower (by 20.1%).

After the intravenous administration of tamoxifen at a dose of 2 mg kg^{-1} to 5 rats with bile duct cannulation, the 24-h biliary excretion of tamoxifen was almost negligible; the mean value was 0.0756 ± 0.0174% of the dose.

Pharmacokinetics of ondansetron after the oral administration of ondansetron with and without simultaneous oral administration of tamoxifen

The mean arterial plasma concentration–time profiles of ondansetron after its oral administration with and without tamoxifen are shown in Fig. 2b. The relevant pharmacokinetic parameters are also listed in Table 2. Absorption of

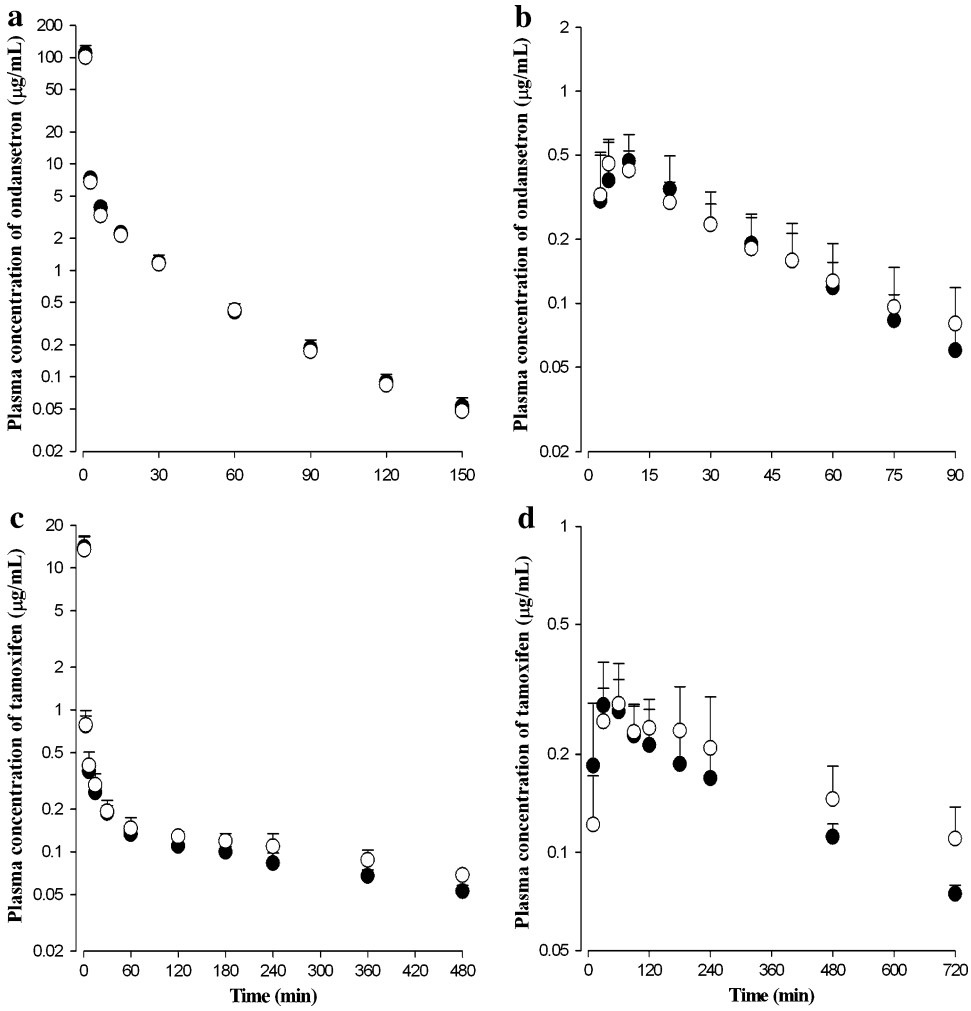

Fig. 2 Mean arterial plasma concentration–time profiles of ondansetron without (*filled circle* $n = 6$ for both routes of administration) and with (*open circle* $n = 6$ for both routes of administration) tamoxifen (**a** and **b** for the intravenous and oral administration, respectively) and tamoxifen without (*filled circle* $n = 6$ and 7 for the intravenous and oral administration, respectively) and with (*open circle* $n = 6$ for both routes of administration) ondansetron (**c** and **d** for the intravenous and oral administration, respectively) after the intravenous infusion of ondansetron at a dose of 8 mg kg^{-1}, tamoxifen at a dose of 2 mg kg^{-1}, or both drugs together and after the oral administration of ondansetron at a dose of 8 mg kg^{-1}, tamoxifen at a dose of 10 mg kg^{-1}, or both drugs together to rats. *Bars* represent standard deviation

ondansetron was rapid; ondansetron was detected in plasma at the first blood sampling time point (3 min) with rapid T_{max} (5–10 min) for both with and without tamoxifen. The pharmacokinetic parameters of ondansetron listed in Table 2 were also comparable (not significantly different) between with and without tamoxifen.

Pharmacokinetics of tamoxifen after the oral administration of tamoxifen with and without simultaneous oral administration of ondansetron

The mean arterial plasma concentration–time profiles of tamoxifen after its oral administration with and without ondansetron are shown in Fig. 2d. The relevant pharmacokinetic parameters are also listed in Table 2. Absorption of tamoxifen was also rapid; tamoxifen was detected in plasma at the first blood sampling time point (15 min) with rapid T_{max} (30–60 min) for both with and without ondansetron. Compared to without ondansetron, the $AUC_{0-\infty}$ of tamoxifen was also significantly greater (by 32.7%) with ondansetron.

Hepatic first-pass effect of tamoxifen

The $AUC_{0-\infty}$s of tamoxifen (Fig. 3a) after its intravenous and intraportal administration at a dose of 2 mg kg^{-1} to rats were 73.9 ± 14.4 and 48.8 ± 10.3 μg min mL^{-1}, respectively. The $AUC_{0-\infty}$ of tamoxifen after its intraportal administration was significantly smaller (by 34.0%) than that after its intravenous administration, suggesting that the hepatic first-pass effect of tamoxifen after absorption into the portal vein was approximately 34.0% in rats.

Gastrointestinal first-pass effect of tamoxifen in rats

The $AUC_{0-\infty}$s of tamoxifen (Fig. 3b) after its intraportal and intragastric administration at a dose of 10 mg kg^{-1} to rats were 265 ± 10.9 and 201 ± 10.6 μg min mL^{-1}, respectively. The $AUC_{0-\infty}$ of tamoxifen after its intragastric administration was significantly smaller (by 24.2%) than that after its intraportal administration, suggesting that the gastrointestinal first-pass effect of tamoxifen was 24.2% of the oral dose in rats.

Table 2 Mean ± SD pharmacokinetic parameters of ondansetron and tamoxifen after the intravenous and oral administration of ondansetron (at a dose of 8 mg kg^{-1} for both routes of administration), tamoxifen (at a dose of 2 and 10 mg kg^{-1} for the intravenous and oral administration, respectively), and both drugs together to rats

Parameter	Ondansetron		Tamoxifen	
	Without tamoxifen	With tamoxifen	Without ondansetron	With ondansetron
Intravenous	$n = 6$	$n = 6$	$n = 6$	$n = 6$
$AUC_{0-\infty}$ (μg min mL^{-1})	239 ± 28.7	222 ± 27.4	89.5 ± 5.00	110 ± 7.64**
Terminal half-life (min)	30.2 ± 1.02	28.5 ± 2.07	339 ± 93.6	401 ± 57.7
MRT (min)	15.2 ± 1.39	15.6 ± 1.70	385 ± 125	474 ± 66.5
CL (mL min^{-1} kg^{-1})	33.9 ± 3.98	36.5 ± 4.62	22.4 ± 1.33	17.9 ± 1.06**
CL_R (mL min^{-1} kg^{-1})	0.523 ± 0.326	0.293 ± 0.116	NC	NC
CL_{NR} (mL min^{-1} kg^{-1})	33.4 ± 3.95	36.2 ± 4.62	NC	NC
V_{ss} (mL kg^{-1})	516 ± 80.3	574 ± 121	8,570 ± 2,565	8,509 ± 1412
$Ae_{0-24\ h}$ (% of dose)	1.55 ± 1.00	0.814 ± 0.329	BD	BD
$GI_{24\ h}$ (% of dose)	NM	NM	BD	BD
Oral	$n = 6$	$n = 6$	$n = 7$	$n = 6$
$AUC_{0-\infty}$ (μg min mL^{-1})	20.5 ± 5.24	22.2 ± 4.79	159 ± 26.5	211 ± 50.5*
Terminal half-life (min)	29.9 ± 1.55	38.9 ± 10.8	421 ± 97.6	474 ± 97.4
CL_R (mL min^{-1} kg^{-1})	1.48 ± 0.895	1.22 ± 0.249	NC	NC
C_{max} (μg mL^{-1})	0.505 ± 0.154	0.496 ± 0.117	0.298 ± 0.114	0.286 ± 0.0549
T_{max} (min)	10.0 (5.00–10.0)	7.50 (5.00–10.0)	30.0 (30.0–60.0)	60.0 (30.0–60.0)
$Ae_{0-24\ h}$ (% of dose)	0.379 ± 0.245	0.345 ± 0.122	BD	BD
$GI_{24\ h}$ (% of dose)	NM	NM	10.4 ± 3.30	9.11 ± 5.70

BD below the detection limit, *NC* not calculable, *NM* not measured

* $P < 0.05$, ** $P < 0.01$ compared to without ondansetron

Hepatic and intestinal concentrations of ondansetron after the intravenous and oral administration of ondansetron and tamoxifen

Concentrations of ondansetron as an inhibitor ([I]) in the liver and intestine after the intravenous and oral administration of ondansetron and tamoxifen to rats are listed in Table 3. After the intravenous administration of both drugs together, the [I] of ondansetron in the liver for up to 5 min was higher than the hepatic K_i of ondansetron (2.23 μM). The [I]/K_i ratios in the liver were 2.59, 1.68, 0.578, 0.364, and 0.157 for 1, 5, 15, 30, and 60 min, respectively. After the oral administration of both drugs, the [I] of ondansetron in the liver and intestine for up to 5 and 60 min were higher than or close to the hepatic and intestinal K_i of ondansetron, respectively (2.23 and 2.10 μM, respectively). The [I]/K_i ratios in the liver were 1.60, 0.807, 0.409, and 0.130 for 5, 15, 30, and 60 min, respectively, and the ratios in the intestine were 6.86, 2.47, 3.56, 0.629, and 0.435 for 5, 15, 30, 60, and 120 min, respectively.

Discussion

The $AUC_{0-\infty}$s of ondansetron were dose-proportional after its intravenous (at doses of 1, 4, 8, and 20 mg kg^{-1}) and oral (at doses of 4, 8, and 20 mg kg^{-1}) administration to rats [8]. Thus, a dose of 8 mg kg^{-1} of ondansetron was chosen in the present study. Tamoxifen was administered intravenously at a dose of 2 mg kg^{-1} and orally at a dose of 10 mg kg^{-1} to rats [27]. Thus, the doses of 2 and 10 mg kg^{-1} for the intravenous and oral administration of tamoxifen, respectively, were chosen in the present study.

After the intravenous administration of tamoxifen at dose of 2 mg kg^{-1} to rats, the Ae_{0-24h} was below the detection limit (Table 2), suggesting that intravenous tamoxifen is almost completely eliminated via a non-renal (CL_{NR}) route. The contribution of the gastrointestinal (including the biliary) excretion of tamoxifen to its CL_{NR} did not seem to be considerable; the GI_{24h} of tamoxifen was also below the detection limit (Table 2). Moreover, the 24-h biliary excretion of tamoxifen was less than 0.0756% of the intravenous dose at 2 mg kg^{-1} as mentioned earlier. Thus, the changes in the CL of tamoxifen listed in Table 2 could represent the changes in its metabolic clearance. It has been reported that changes in the CL_{NR} of ondansetron could also represent the changes in its metabolism in rats [8].

After the intravenous administration of both drugs together, the $AUC_{0-\infty}$ of tamoxifen was significantly greater than that of without ondansetron, possibly due to significantly slower CL of tamoxifen with ondansetron

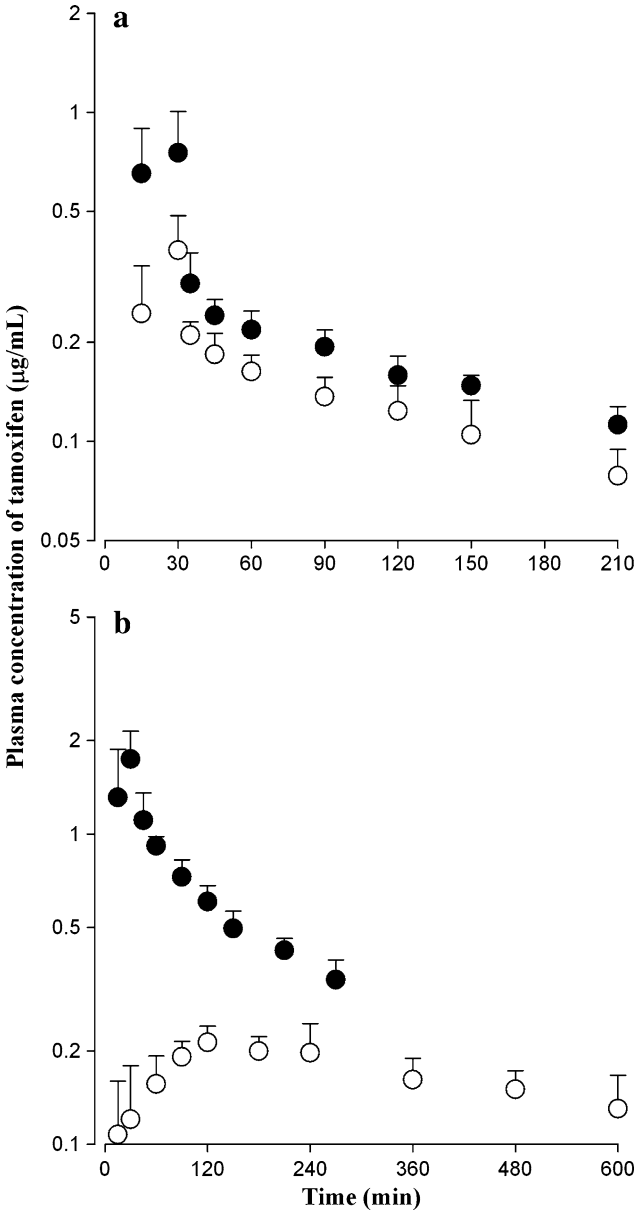

Fig. 3 Mean arterial plasma concentration–time profiles of tamoxifen **a** after its intravenous (*filled circle* $n = 4$) and intraportal (*open circle* $n = 5$) administration at a dose of 2 mg kg^{-1} and **b** after its intraportal (*filled circle* $n = 5$) and intragastric (*open circle* $n = 4$) administration at a dose of 10 mg kg^{-1} to rats. *Bars* represent standard deviation

(Table 2). The slower CL of tamoxifen could have been at least partly supported by the significantly slower in vitro hepatic CL_{int} for disappearance of tamoxifen by ondansetron (Table 1a) because, tamoxifen is a drug with very close to an intermediate hepatic extraction ratio (hepatic first-pass effect of 34.0%). The free fractions of tamoxifen in plasma were comparable between with and without ondansetron and effects of ondansetron on the hepatic blood flow rate did not seem to be reported in rats. The slower hepatic CL_{int} for disappearance of tamoxifen (greater $AUC_{0-\infty}$ of tamoxifen) by ondansetron (Table 1) could be supported by the following. After the intravenous administration of both drugs, the $[I]/K_i$ ratio of ondansetron in the liver was greater than 2.0 only at 1 min and the ratios were greater than 0.01 for up to the last measured time point, 60 min (Table 3). Bachmann and Lewis [28] suggest that if, for a putative inhibitory drug–drug interaction, the $[I]/K_i$ ratio is >2.0, the drug–drug interaction is due to CYP inhibition, but the ratio is <0.01, the mechanism is not attributable to CYP inhibition. Thus, the above data suggest that the slower CL (greater $AUC_{0-\infty}$) of tamoxifen with both drugs together could have been attributable to inhibition of hepatic metabolism of tamoxifen by ondansetron via CYP2D subfamily and 3A1/2.

After the oral administration of both drugs together, the $AUC_{0-\infty}$ of tamoxifen with ondansetron was also significantly greater than that of without ondansetron (Table 3). This could possibly have been supported by the significantly slower both in vitro hepatic and intestinal CL_{int}s for disappearance of tamoxifen by ondansetron (Table 1). The above data suggest that intestinal metabolism of tamoxifen could also be inhibited by ondansetron via CYP2D subfamily and 3A1/2. After the oral administration of both drugs, the $[I]/K_i$ ratios in the liver were greater than 0.01 for up to the last measured time point, 60 min, and the ratios in intestine were greater than 2.0 for up to 30 min, and greater than 0.01 for up to the last measured time point, 120 min (Table 3). The above data suggest that the contribution of inhibition of metabolism of tamoxifen by ondansetron in the intestine to the greater oral $AUC_{0-\infty}$ of tamoxifen was greater than that of hepatic inhibition. This could explain the greater oral $AUC_{0-\infty}$ of tamoxifen (32.7% increase) than 22.9% increase after the intravenous $AUC_{0-\infty}$ of tamoxifen (Table 2).

The CYP3A subfamily expressed in rat intestine is different from that in rat liver [29]. Although CYP2D subfamily is little expressed in both rat liver and intestine, CYP2D subfamily expressed in rat intestine is unlikely to play a significant role for metabolism of drugs compared to that expressed in rat liver [29]. Thus, those factors could contribute to a different manner of inhibition of tamoxifen metabolism by ondansetron between liver (non-competitive inhibition) and intestine (competitive inhibition) in rats.

Although the CYP isozymes responsible for the metabolism of ondansetron are somewhat different between rats (CYP2D subfamily and 3A1/2) and humans (CYP2D6, 3A subfamily, and 1A1/2) [30, 31], the CYP2D and 3A subfamilies are common. The protein homologies of human and rat CYP2D and 3A subfamilies have been reported that these were very close [32].

In summary, after the intravenous administration of tamoxifen and ondansetron, the $AUC_{0-\infty}$ of tamoxifen was significantly greater than that of without ondansetron.

Table 3 Mean ± SD concentrations (μM) of ondansetron in the liver and intestine after the intravenous and oral administration of ondansetron and tamoxifen to rats

Time (min)	Intravenous Liver	Time (min)	Oral	
			Liver	Intestine
1	5.78 ± 1.73 (0.815 ± 0.193)	5	3.56 ± 1.11 (6.00 ± 1.07)	14.4 ± 9.73 (24.6 ± 14.5)
5	3.75 ± 0.116 (1.24 ± 0.0243)	15	1.80 ± 1.50 (3.11 ± 0.523)	5.19 ± 2.44 (10.6 ± 2.35)
15	1.29 ± 0.336 (0.904 ± 0.240)	30	0.912 ± 0.876 (5.49 ± 1.90)	7.47 ± 2.90 (57.2 ± 14.1)
30	0.812 ± 0.197 (1.04 ± 0.203)	60	0.289 ± 0.195 (6.28 ± 6.83)	1.32 ± 1.98 (16.7 ± 23.0)
60	0.349 ± 0.077 (1.14 ± 0.0579)	120		0.965 ± 0.468 (19.5 ± 11.9)

The values in parentheses represent the tissue-to-plasma (T/P) ratios. $n = 3$ at each time for each route of administration

This could have possibly been due to a non-competitive inhibition of CYP2D subfamily- and 3A1/2-mediated hepatic tamoxifen metabolism by ondansetron. After the oral administration of tamoxifen and ondansetron, the $AUC_{0-\infty}$ of tamoxifen was also significantly greater than that of without ondansetron. This could have mainly been due to a competitive inhibition of CYP2D subfamily- and 3A1/2-mediated intestinal tamoxifen metabolism by ondansetron in addition to non-competitive inhibition of hepatic metabolism of tamoxifen by ondansetron. The present rat data should be extrapolated carefully in humans.

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