Pharmacokinetic Drug Interactions between Ondansetron and Tamoxifen in Female Sprague-Dawley Rats with DMBA-induced Mammary Tumor

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Abstract. *Tamoxifen, which is used to treat breast cancer, and ondansetron, used for the treatment of chemotherapy-induced nausea, are commonly metabolized via cytochrome P450 (CYP) 2D subfamily and 3A1/2 in rats, as in humans. This study was conducted to investigate the pharmacokinetic interactions between ondansetron and tamoxifen after intravenous and oral administration of ondansetron (both 8 mg/kg) and/or tamoxifen (2 and 10 mg/kg for intravenous and oral administration, respectively), in rats bearing 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammarian tumors (DMBA rats), used as an animal model of human breast cancer. The total area under the plasma concentration-time curve, from time zero to infinity (AUC) of tamoxifen was significantly greater after both intravenous and oral administration with ondansetron, compared to that after administration of tamoxifen-alone. The hepatic and intestinal metabolism of tamoxifen in DMBA rats was inhibited by ondansetron. Taken together, the significant increase in tamoxifen AUC in DMBA rats after intravenous or oral administration with ondansetron may be attributed to non-competitive hepatic (intravenous) and competitive intestinal (oral) inhibition of CYP2D subfamily- and 3A1/2-mediated tamoxifen metabolism by ondansetron.*

Tamoxifen is widely used as a chemopreventive or chemotherapeutic agent in women with high risk of estrogen receptor-positive breast cancer (1). Tamoxifen undergoes extensive metabolism, mediated primarily by cytochrome *P450* (CYP) enzymes, to form many metabolites (Figure 1). Tamoxifen α-hydroxylation is catalyzed primarily by CYP3A in human hepatic microsomes (2), whereas 4-hydroxy-, *N-des*methyl- and *N*-oxide metabolites of tamoxifen are mainly formed by the action of the CYP2C9, -2D6 and -3A subfamily, the CYP3A subfamily, and flavin-containing monooxygenase (FMO), respectively (3, 4). In liver microsomes isolated from rats pre-treated with various CYP isozyme inducers and inhibitors, *N-des*methyl tamoxifen was formed *via* CYP3A1 and possibly CYP1A2 and -3A2, whereas 4-hydroxy-tamoxifen was formed *via* the CYP1A2 and -2D subfamily (5). The extent of absolute oral bioavailability (*F*) was 35.8%, the hepatic first-pass effect after absorption into the portal vein was 34.0%, and the intestinal first-pass effect was 24.2% of the orally administered dose in normal male rats (6).

Ondansetron, a potent and selective 5-hydroxytryptamine type-3 (5-HT_3) receptor antagonist, is used to treat nausea and vomiting induced by chemotherapy, radiotherapy, and surgery. Ondansetron is primarily metabolized *via* the hepatic

Abbreviations: $Ae_{0\text{-}24\ h}$: Percentage of the dose excreted in the 24-h urine; AUC: 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_{int}: intrinsic clearance; CL_{NR}: time-averaged non-renal clearance; CL_R: time-averaged renal clearance; CYP: cytochrome P450; DMA: *N,N*-dimethylacetamide; DMBA: 7,12-dimethylbenz[a]anthracene; $GI_{24\ h}$: percentage of the dose recovered from the gastrointestinal tract (including its contents and feces) at 24 h; HPLC: high-performance liquid chromatography; K_m: apparent Michaelis–Menten constant; MRT: mean residence time; NADPH: the reduced form of β-nicotinamide adenine dinucleotide phosphate; OND: ondansetron; SD: standard deviation; T_{max}: time to reach C_{max}; TMX: tamoxifen; Tris: tri(hydroxymethyl)aminomethane; V_{max}: maximum velocity; V_{ss}: apparent volume of distribution at steady state.

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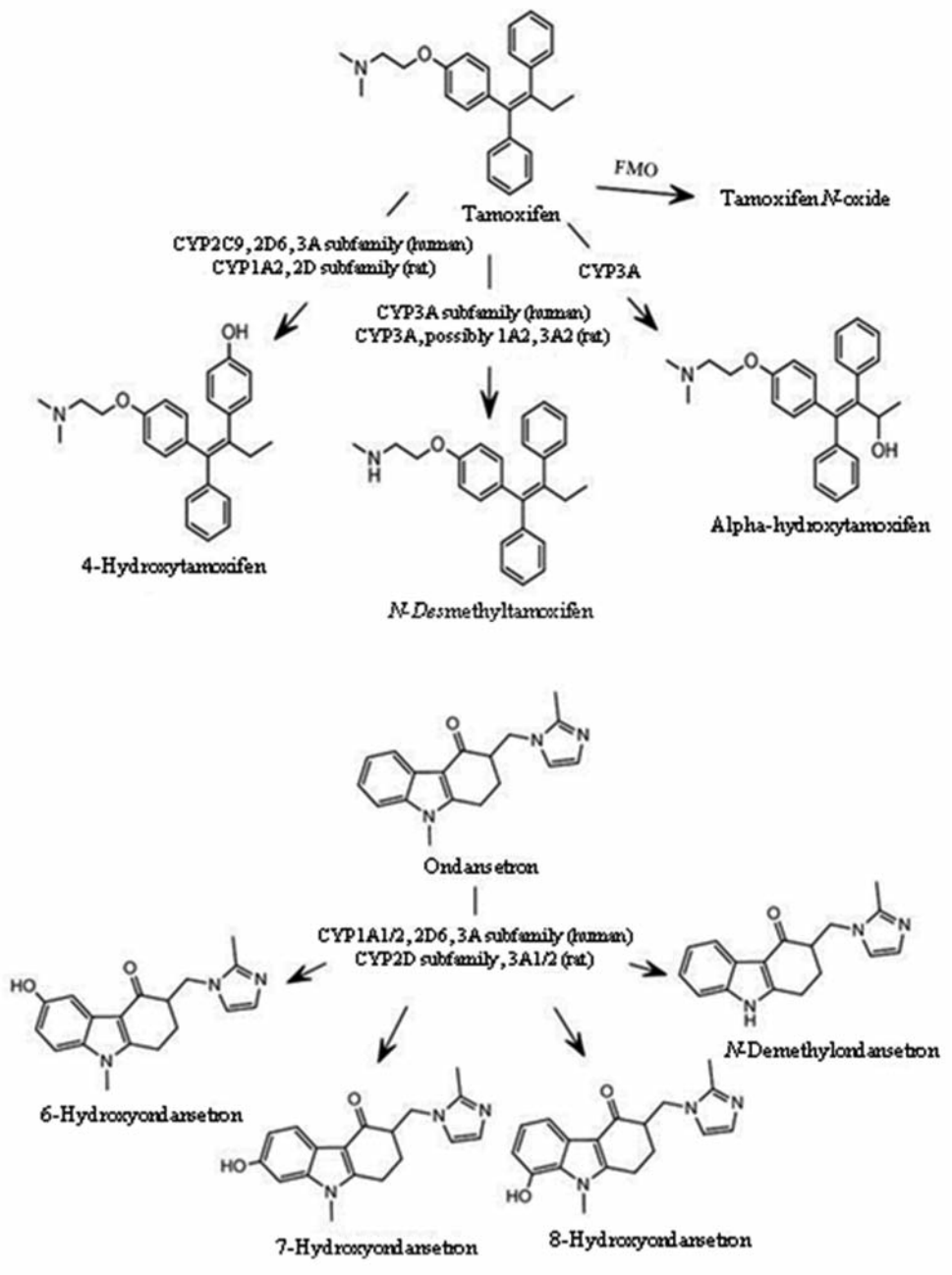

Figure 1. *Metabolic pathways of tamoxifen and ondansetron.*

CYP2D6, -3A subfamily, and -1A1/2 in humans (7, 8). In rats, ondansetron is metabolized by the CYP2D subfamily and -3A1/2 (9) (Figure 1). The *F* value was reported to be 4.07%; the hepatic first-pass effect after absorption into the portal vein was 64.8%, the intestinal first-pass effect was 34.2% of the oral dose, and the unabsorbed fraction from the gastrointestinal tract was maintained at 1.58% for up to 24 h in rats (10).

Ondansetron is commonly co-administered with other drugs, including most anticancer agents currently in clinical use. Pharmacokinetic interaction between ondansetron and anticancer drugs has been reported. For example, in patients with breast cancer who received high-dose chemotherapy with cyclophosphamide, cisplatin and carmustine (11), the total area under the plasma concentration-time curve from time zero to infinity (AUC) of high-dose cyclophosphamide and cisplatin

was significantly reduced by ondansetron. Concomitantly administered ondansetron also altered the systemic exposure to cyclophosphamide in patients with breast cancer (12). An *in vitro* study using human hepatic microsomes has shown that the formation of tamoxifen metabolites *via* CYP2D6 and -3A4 was significantly inhibited by black cohosh (13).

Although different CYP isozymes are responsible for the metabolism of ondansetron in rats and humans (14, 15), metabolism *via* the CYP2D and -3A subfamilies is common in both species. Notably, the degree of homology between rat and human CYP2D and -3A subfamily proteins is very high (16). Ondansetron and tamoxifen are both metabolized by the CYP2D subfamily and -3A1/2 in rats. Thus, a significant increase in the AUC of tamoxifen following intravenous or oral administration of both drugs together was expected as a consequence of inhibition of hepatic and/or intestinal CYP2D subfamily and -3A1/2 by ondansetron (6).

However, no studies have examined possible interactions between tamoxifen and ondansetron in breast cancer or in an animal model for human breast cancer, such as breast tumor-bearing rats. Therefore, the present study aimed to evaluate the pharmacokinetic interaction between tamoxifen and ondansetron by using rats with 7,12-dimethylbenz[a] anthracene (DMBA)-induced mammary tumor (DMBA rats). In this specific animal model, rats develop tumors that closely resemble those found in human breast cancer (17).

Materials and Methods

Chemicals. Ondansetron hydrochloride dihydrate was obtained from Dong-A Pharmaceutical Company, Ltd. (Yongin, Korea). Propranolol [internal standard for high-performance liquid chromatographic (HPLC) analysis of ondansetron], tamoxifen citrate, imipramine hydrochloride (internal standard for the HPLC analysis of tamoxifen), tri(hydroxymethyl)aminomethane (Tris)-buffer, ammonium acetate, sodium phosphate monobasic, dichloromethane, DMBA, olive oil, *N,N*-dimethylacetamide (DMA), 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 protocol for this animal study was approved by the Institute of Laboratory Animal Resources of Seoul National University (Seoul, Korea). Female Sprague-Dawley rats (6-7 weeks old, weighing 160-180 g) were purchased from Charles River Company Korea (Orient Bio Inc., Seoul, Korea). They were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of 20 to 23˚C with 12-h light (07:00-19:00) and 12-h dark (19:00-07:00) cycles and relative humidity of 50±5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy), under filtered pathogen-free air and with food (Agribrands Purina Korea, Pyeongtaek, Korea) and water available *ad libitum*.

Induction of mammarian tumors in rats. Mammarian tumors were induced in rats by using DMBA (dissolved in olive oil) at a dose of 5 mg (in 1 ml) per rat by using a slightly modified version of a previously reported method (18, 19). DMBA was administered to rats that were approximately 6 to 7 weeks old because of age-dependent susceptibility of the mammarian gland to chemically-induced carcinogenesis (20).

Measurement of V_{max}, K_m and CL_{int} for disappearance of tamoxifen, alone and in combination with ondansetron. Hepatic and intestinal microsomes were prepared as described previously (21). Microsomal proteins isolated from the liver and intestine were quantitated using the Bradford protein assay (22).

The maximum velocity (V_{max}) and apparent Michaelis–Menten constant (K_m), the concentration at which the rate is one-half of V_{max}, for the disappearance of tamoxifen administered-alone and in combination with ondansetron were determined using the following *in vitro* assay. The following components were added to a test tube: microsomes (equivalent to 0.5 mg of hepatic or intestinal microsomal protein); 10 μl of methanol containing tamoxifen at final concentrations of 0.2, 0.5, 1, 2, 5 and 10 μM (for both hepatic and intestinal microsomes); 10 μl of distilled water 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 reaction was terminated after 15-min incubation by transferring 100 μl of the reaction mixture to a tube containing 200 μl of acetonitrile.

All microsomal incubation conditions were within the linear range of the reaction rate. The kinetic constants (K_m and V_{max}) for tamoxifen disappearance were determined using non-linear regression (23). The intrinsic clearance (CL_{int}) was calculated by dividing the V_{max} by the K_m.

Intravenous and oral administration of ondansetron and tamoxifen, alone and in combination to DMBA rats. The procedures used for the pre-treatment of rats, including cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the rat intravenous study), were previously described (6, 18, 24). The rats were not restrained in the present study. In the previous study, the intravenous doses of ondansetron and tamoxifen were 8 and 2 mg/kg, respectively, while orally administered doses of ondansetron and tamoxifen to rats were 8 and 10 mg/kg, respectively (6).

Ondansetron (ondansetron hydrochloride dihydrate, dissolved in distilled water:DMA 1:1, v/v) at a dose of 8 mg/kg (n=7), tamoxifen (tamoxifen citrate, dissolved in the same vehicle) at a dose of 2 mg/kg (n=8), or both drugs together (n=6 or 8), were infused for 1 min *via* the jugular vein (the total infusion volume was 2 ml/kg). A blood sample (approximately 0.22 ml) was collected *via* the carotid artery at 0 (control), 1 (end of the infusion), 3, 7, 15, 30, 45, 60, 90, 120, 150 and 180 min after the start of intravenous infusion of ondansetron. Samples (0.22 ml) were also collected at 0 (control), 1 (end of the infusion), 3, 7, 15, 30, 60, 120, 180, 240, 360 and 480 min after the start of the intravenous infusion of tamoxifen-alone and after both drugs together. Blood was centrifuged immediately and 100 μl of plasma were removed and transferred to a 1.5-ml polyethylene tube and stored at −70˚C (Revco ULT 1490 D-N-S; Western Medics, Ashville, NC, USA) until used for HPLC analysis. The procedures used for the preparation and handling of the 24-h urine samples ($Ae_{0\text{-}24\ h}$) and the gastrointestinal tract (including its contents and feces) samples at 24 h (GI24 h) were similar to a reported method (18, 25).

Ondansetron (prepared in the same manner as in the intravenous study) at a dose of 8 mg/kg (n=5), tamoxifen (same preparation as in the intravenous study) at a dose of 10 mg/kg (n=6), or both drugs together (n=6 or 7) were orally administered using a feeding tube (total administered volume, 5 ml/kg). Blood samples (approximately 0.22 ml) were collected *via* the carotid artery at 0, 3, 5, 10, 20, 30, 40, 50, 60, 75 and 90 min after oral administration of ondansetron and 0, 10, 30, 60, 90, 120, 180, 240, 480, 720 and 1080 min after oral administration of tamoxifen and after both drugs in combination. Procedures for handling samples were similar to those used for the intravenous study.

HPLC analysis of ondansetron and tamoxifen. Concentrations of ondansetron in all samples were determined using a previously reported HPLC method (10). Fifty microliters of a buffered solution (pH 9) and 20 μl of distilled water containing 50 μg/ml of propranolol (internal standard) were added to 100 μl of the sample. The mixture was extracted with 0.5 ml of dichloromethane by vortexing for 30 s, then centrifuging at 15,000 ×g for 10 min. The organic layer was transferred to a clean 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 were injected directly onto a reversed-phase HPLC column (C_{18}; Symmetry®; 100 mm *l*. × 4.6 mm i.d.; particle size, 3.5 μm; Waters, Milford, MA, USA). The mobile phase consisted of a solution of 0.02 M sodium phosphate monobasic:acetonitrile (70:30), with 85% phosphoric acid used to adjust the pH to 4.0. The flow rate was 1.0 ml/min and the column eluent was monitored using an ultraviolet detector at 305 nm at room temperature. The retention times for ondansetron and propranolol were approximately 2.2 and 3.6 min, respectively. The quantification limit of ondansetron in rat plasma and urine samples was 0.02 μg/ml.

Concentration of tamoxifen in all samples was determined using a previously reported HPLC method (6). One hundred microliters of biological sample was deproteinized with 200 μl of acetonitrile containing 2 μg/ml of imipramine (internal standard). After vortexing and then centrifuging at 15,000 ×g for 5 min, the upper organic layer was transferred to a clean 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 were injected directly onto a reversed-phase HPLC column (C18; Symmetry®; 250 mm l×4.6 mm i.d.; particle size, 5 μm; SynChrom Inc., Lafayette, IN, USA). The mobile phase consisted of acetonitrile:0.05 M ammonium acetate buffer (75:25), with the pH adjusted to 6.4 using acetic acid. The flow-rate was 1.0 ml/min, and the column eluent was monitored using an ultraviolet detector at 280 nm at room temperature. The retention times for imipramine and tamoxifen were approximately 7 and 9 min, respectively. The quantification limit of tamoxifen in the rat plasma and urine samples was 0.05 μg/ml.

Pharmacokinetic analysis. The AUC was calculated using the trapezoidal rule-extrapolation method (26). The area from the last datum point to infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods (27) were used to calculate the following pharmacokinetic parameters by using non-compartmental analysis (WinNonlin®; Pharsight Corporation, Mountain View, CA, USA). These parameters included the time-averaged total body, renal, and non-renal clearances (CL, CL_R and CL_{NR}, respectively); the terminal half-life ($t_{1/2}$); the mean residence time (MRT); and the apparent volume of distribution at steady state (V_{ss}). The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were derived directly from the experimental data.

Statistical analysis. A *p*-value of <0.05 was deemed to be statistically significant by using an unpaired *t*-test. All data were expressed as the mean±standard deviation (SD) except median (ranges) for T_{max}.

Table I. *In vitro maximum velocity (V_{max}), apparent Michaelis–Menten constant (K_m) and intrinsic clearance (CL_{int}) for the disappearance of tamoxifen-alone and in combination with ondansetron (OND) in 7,12-dimethylbenz[a]anthracene (DMBA)-treated rats.*

Parameter	Without OND	With OND
Hepatic	(n=3)	(n=3)
V_{max} (nmol/min/mg protein)	0.525±0.0643	0.383±0.0671
K_m (μM)	21.3±3.64	22.9±9.56
CL_{int} (μl/min/mg protein)	24.9±2.95	17.9±4.47
Intestinal	(n=3)	(n=4)
V_{max} (nmol/min/mg protein)	0.129±0.0117	0.151±0.0262
K_m (μM)	8.98±1.73	17.0±2.08**
CL_{int} (μl/min/mg protein)	14.6±1.53	8.86±1.21**

Data are the mean±standard deviation (SD). **p<0.01.

Results

Determination of V_{max}, K_m and CL_{int} for disappearance of tamoxifen-alone and in combination with ondansetron. The V_{max}, K_m and CL_{int} for disappearance of tamoxifen-alone and in combination with ondansetron in the hepatic microsomes are listed in Table I. The CL_{int} and V_{max} of tamoxifen with ondansetron were considerably slower (by 28.1%, p=0.086 and 27.0%, p=0.058, respectively) than the corresponding values for tamoxifen-alone. However, the K_m for tamoxifen-alone was comparable to that of tamoxifen with ondansetron. This suggests that the V_{max} for the disappearance of tamoxifen and formation of one or more tamoxifen metabolites (primarily metabolism) was considerably reduced, but the affinity of metabolizing enzymes for tamoxifen was not altered. These data indicate that in DMBA rats, ondansetron inhibited the hepatic metabolism of tamoxifen in a non-competitive manner.

The V_{max}, K_m and CL_{int} for the disappearance of tamoxifen-alone and in combination with ondansetron in the intestinal microsomes are also listed in Table I. The K_m and CL_{int} of tamoxifen with ondansetron were significantly higher (by 89.3%) and significantly slower (by 39.3%), respectively, than those of tamoxifen-alone. However, the V_{max} for tamoxifen-alone was comparable to that for tamoxifen with ondansetron. This suggests that the affinity of metabolizing enzymes for tamoxifen when administered with ondansetron, significantly decreased and the formation

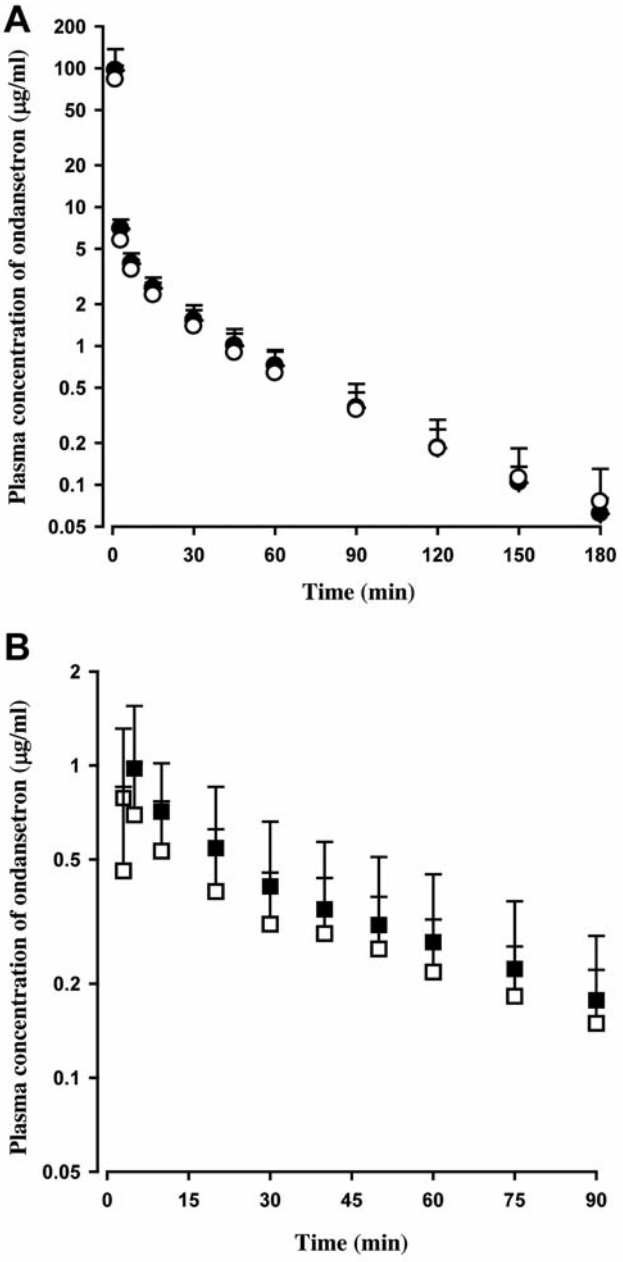

Figure 2. *Mean arterial plasma concentration–time profiles for rats treated with 7,12-dimethylbenz[a]anthracene (DMBA) after intravenous administration of ondansetron at 8 mg/kg with (○; n=8) and without (●; n=7) tamoxifen at 2 mg/kg (A) and after same-dose given orally with (□; n=6) and without (■; n=5) tamoxifen at 10 mg/kg (B). Bars represent the standard deviations (SD).*

of one or more tamoxifen metabolites was significantly slower, but the V_{max} for disappearance of tamoxifen (primarily metabolism) was not altered. These data indicate that in DMBA rats, ondansetron inhibited the intestinal metabolism of tamoxifen in a competitive manner.

Table II. *Pharmacokinetic parameters of ondansetron at a dose of 8 mg/kg for both intravenous and oral administration alone and in combination with tamoxifen (TMX) to 7,12-dimethylbenz[a]anthracene (DMBA)-treated rats.*

Parameter	Without TMX	With TMX
Intravenous	(n=7)	(n=8)
AUC (μg×min/ml)	257±50.8	225±48.7
Terminal half-life (min)	35.6±2.23	39.1±5.56
MRT (min)	23.9±5.11	24.7±8.41
CL (ml/min/kg)	32.4±7.47	36.9±6.83
CL_R (ml/min/kg)	0.777±0.290	1.28±0.469
CL_{NR} (ml/min/kg)	31.6±7.34	35.6±6.65
V_{ss} (ml/kg)	786±307	872±199
$Ae_{0\text{-}24}$ h (% of dose)	2.44±0.916	3.49±1.07
Oral	(n=5)	(n=6)
AUC (μg×min/ml)	44.5±17.7	43.7±22.2
Terminal half-life (min)	48.9±9.31	49.1±6.24
C_{max} (μg/ml)	1.01±0.531	0.837±0.342
T_{max} (min)[a]	5.00 (5.00-10.0)	5.00 (5.00-10.0)
CL_R (ml/min/kg)	1.47±0.473	1.24±0.590
$Ae_{0\text{-}24\ h}$ (% of dose)	0.735±0.444	0.502±0.471

$Ae_{0\text{-}24\ h}$: Percentage of the dose excreted in the 24-h urine; AUC: total area under the plasma concentration–time curve from time zero to time infinity; CL: time-averaged total body clearance; C_{max}: peak plasma concentration; CL_{NR}: time-averaged non-renal clearance; CL_R: time-averaged renal clearance; $GI_{24\ h}$: percentage of the dose recovered from the gastrointestinal tract (including its contents and feces) at 24 h; MRT: mean residence time; T_{max}: time to reach C_{max}; V_{ss}: apparent volume of distribution at steady state. Data are the mean±standard deviation (SD). [a]Median (range).

Pharmacokinetics of ondansetron following intravenous or oral administration-alone and in combination with intravenous or oral administration of tamoxifen to DMBA rats. The mean arterial plasma concentration-time profiles of intravenously administered ondansetron with and without co-administration of tamoxifen are shown in Figure 2A, and the relevant pharmacokinetic parameters are listed in Table II. Following intravenous co-administration of tamoxifen and ondansetron to DMBA rats, the pharmacokinetic parameters of ondansetron were comparable (not significantly different) to those in rats administered ondansetron-alone.

Following oral administration of ondansetron with and without tamoxifen, the mean arterial plasma concentration–time profiles of ondansetron are shown in Figure 2B and the relevant pharmacokinetic parameters are also listed in Table II. Absorption of ondansetron was very rapid; ondansetron was detected in plasma at the first blood sampling time point (3 min), with a rapid T_{max} (5-10 min), in both groups of rats. The pharmacokinetic parameters of ondansetron listed in Table II were also comparable between the two groups.

Pharmacokinetics of tamoxifen following intravenous or oral administration alone, and in combination with intravenous or oral administration of ondansetron to DMBA rats. The mean

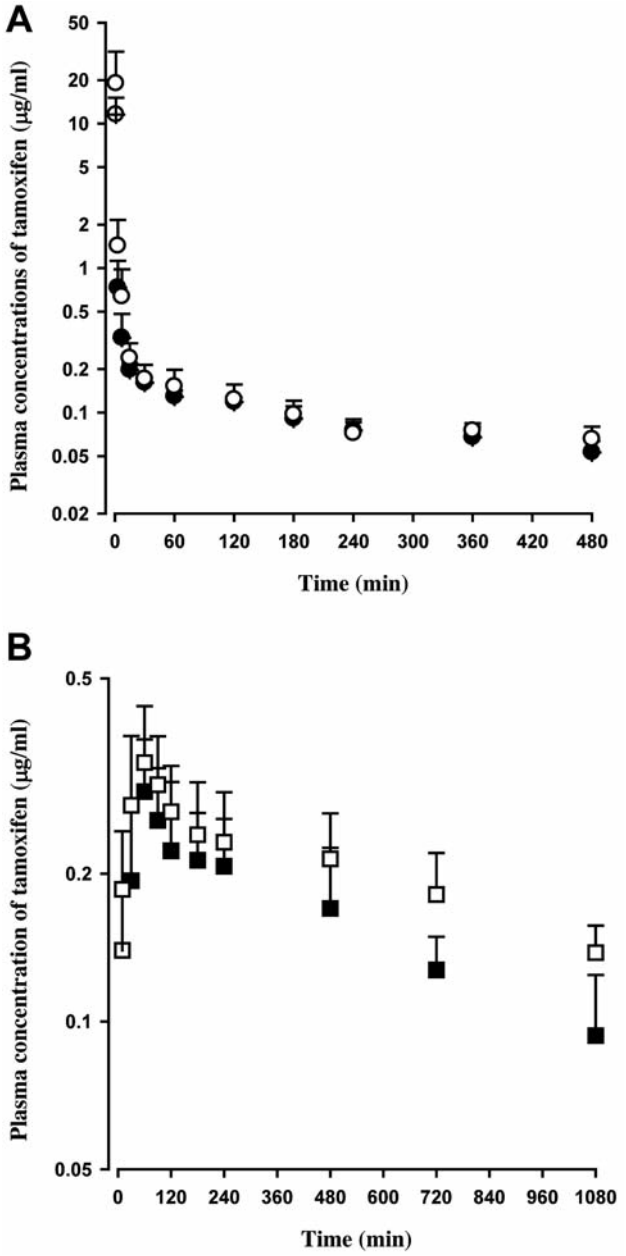

Figure 3. *Mean arterial plasma concentration–time profiles for rats treated with 7,12-dimethylbenz[a]anthracene (DMBA) after intravenous administration of tamoxifen at 2 mg/kg with (○; n=6) and without (●; n=5) ondansetron at 8 mg/kg (A) and after oral administration of tamoxifen at 10 mg/kg with (□; n=6) and without (■; n=7) same-dose of ondansetron (B). Bars represent standard deviations (SD).*

arterial plasma concentration-time profiles of tamoxifen after intravenous administration with and without ondansetron are shown in Figure 3A and the relevant pharmacokinetic parameters are listed in Table III. Compared to tamoxifen-alone, tamoxifen co-administered with ondansetron had significantly greater AUC (by 43.0%) and significantly slower CL (by 29.0%).

Table III. *Pharmacokinetic parameters of tamoxifen at doses of 2 and 10 mg/kg for intravenous and oral administration, respectively, alone and in combination with ondansetron (OND) to 7,12-dimethylbenz[a] anthracene (DMBA)-treated rats.*

Parameter	Without OND	With OND
Intravenous	(n=5)	(n=6)
AUC (μg min/ml)	98.6±16.3	141±26.7*
Terminal half-life (min)	525±143	706±159
MRT (min)	612±187	773±191
CL (ml/min/kg)	20.7±3.54	14.7±3.37*
V_{ss} (ml/kg)	12,300±2,140	11,700±5,360
Oral	(n=6)	(n=7)
AUC (μg min/ml)	204±54.8	340±68.5**
Terminal half-life (min)	525±101	674±122
C_{max} (μg/ml)	0.294±0.0816	0.371±0.0884
T_{max} (min)[a]	60.0 (60.0)	60.0 (30.0-60.0)
$GI_{24\ h}$ (% of dose)	14.7±4.96	10.4±2.77

AUC: Total area under the plasma concentration–time curve from time zero to time infinity; CL: time-averaged total body clearance; Cmax: peak plasma concentration; $GI_{24\ h}$: percentage of the dose recovered from the gastrointestinal tract (including its contents and feces) at 24 h; MRT: mean residence time; T_{max}: time to reach C_{max}; V_{ss}: apparent volume of distribution at steady state. Data are the mean±standard deviation (SD). *p<0.05 and **p<0.01. [a]Median (range).

The mean arterial plasma concentration–time profiles of tamoxifen following oral administration-alone and together with ondansetron are shown in Figure 3B. The relevant pharmacokinetic parameters are also listed in Table III. Absorption of tamoxifen was rapid; tamoxifen was detected in plasma at the first blood sampling time point (10 min), with a rapid T_{max} (30-60 min) in both groups of rats. The AUC of tamoxifen with ondansetron was significantly greater (by 66%) than that of tamoxifen-alone in DMBA rats.

Discussion

Changes in the CL of tamoxifen could possibly reflect changes in its metabolic clearance in rats, because intravenous tamoxifen is almost completely eliminated *via* a non-renal route, and the $GI_{24\ h}$ and 24-h biliary excretion of tamoxifen were also almost negligible (6). Similarly, changes in the CL_{NR} of ondansetron, as shown in Table II, could also represent changes in metabolism of the drug in rats (10).

After both intravenous and oral administration of ondansetron-alone and in combination with tamoxifen to DMBA rats, the pharmacokinetic parameters of ondansetron were comparable between the two groups of rats. These data suggest that the pharmacokinetic profile of ondansetron is not affected by tamoxifen in DMBA rats (Table II). No effect of tamoxifen on the pharmacokinetics of ondansetron has also been reported in healthy normal male rats (6).

After intravenous administration of tamoxifen-alone and in combination with ondansetron to DMBA rats, the CL of tamoxifen with ondansetron was significantly slower (by 29.0%) compared to tamoxifen-alone. Thus, the AUC of tamoxifen was significantly greater (by 43.0%) when co-administered with ondansetron (Table III), presumably due to a reduction in hepatic CL_{int} (by 28.1%) of tamoxifen by ondansetron, as observed *in vitro* (Table I). In rats, tamoxifen has an intermediate hepatic extraction ratio (34.0%) (6); thus, its hepatic clearance depends both on its intrinsic hepatic clearance/free (unbound to plasma proteins) fraction in plasma and the hepatic blood flow rate (28). Although we did not quantify plasma protein binding of tamoxifen in DMBA rats, there have not been any reports of altered plasma protein binding in breast cancer patients or in DMBA-treated rats. In normal male rats, the effect of ondansetron on the plasma protein binding of tamoxifen was negligible (6). While the hepatic blood flow rate in DMBA rats might have increased (29), its contribution to tamoxifen metabolism did not seem significant. Because V_{max} for disappearance of tamoxifen with ondansetron was considerably slower than that of tamoxifen-alone (p=0.058), the significant reduction in CL (and significant increase in AUC) of tamoxifen with ondansetron could be attributed to a non-competitive inhibition of the CYP2D subfamily- and 3A1/2-mediated hepatic tamoxifen metabolism by ondansetron. Non-competitive inhibition of hepatic tamoxifen metabolism mediated by the CYP2D subfamily and 3A1/2 has been also reported in healthy normal male rats (6). When ondansetron was co-administered with tamoxifen, the extent to which the AUC of tamoxifen increased was greater in DMBA-treated rats (43.0%) than in normal male rats (22.9%) (6). This could be explained, in part, by gender differences in the metabolism of ondansetron, indicating that longer exposure to ondansetron may have occurred in female rats compared to male rats (30).

After oral administration of both drugs together to DMBA rats, the AUC of tamoxifen with ondansetron was significantly greater (by 66.7%) compared to that of tamoxifen-alone (Table III). This could have been caused by significantly slower intestinal (39.3%) and hepatic (28.1%) CL_{int} for the disappearance of tamoxifen by ondansetron, as was shown by *in vitro* results (Table I). Therefore, the significant increase in AUC of tamoxifen when orally administered with ondansetron could be attributed to competitive inhibition of the CYP2D subfamily- and 3A1/2-mediated intestinal tamoxifen metabolism by ondansetron, resulting from a significantly higher K_m for tamoxifen in addition to non-competitive inhibition of hepatic metabolism of tamoxifen by ondansetron in DMBA rats. Competitive inhibition of the CYP2D subfamily- and 3A1/2-mediated intestinal tamoxifen metabolism has been reported in healthy normal male rats (6). Similarly, the amplitude of the increase in AUC of tamoxifen when administered with ondansetron in DMBA rats (66.7%) was also greater than that in healthy male rats (32.7%) (6). This most likely occurred as a consequence of gender differences in ondansetron metabolism (30) and/or significant decreases in intestinal *CYP3A* mRNA expression and protein levels in DMBA rats compared to healthy normal rats (31).

The contrasting mechanisms for inhibition of tamoxifen metabolism by ondansetron in the liver (non-competitive inhibition) *versus* the intestine (competitive inhibition) of rats may be caused by the unique expression of the CYP3A subfamily in the intestine and by the contribution of the CYP2D subfamily to tamoxifen metabolism in the liver. This assumption is based on the observations that CYP2D subfamily expression is very low in both the rat liver and intestine, and because the CYP2D subfamily in the rat intestine does not seem to play as significant a role in tamoxifen metabolism as it does in the rat liver (32).

In summary, the significantly greater AUC of tamoxifen in DMBA rats after its intravenous administration with ondansetron may be attributed to the non-competitive inhibition of the hepatic CYP2D subfamily- and 3A1/2-mediated tamoxifen metabolism by ondansetron. In contrast, the significantly greater AUC of tamoxifen in DMBA rats after its oral administration with ondansetron could be attributed to the competitive inhibition of the intestinal CYP2D subfamily- and 3A1/2-mediated tamoxifen metabolism by ondansetron, in addition to non-competitive hepatic inhibition. These findings suggest that potential pharmacokinetic drug interactions may occur in patients during clinical practice and these interactions should be critically considered first, in order to avoid or minimize the risk of drug interactions.

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