JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 853–861
© 2008 The Authors
Received October 29, 2007
Accepted March 7, 2008
DOI 10.1211/jpp.60.7.0006
ISSN 0022-3573

Effects of cytochrome P450 (CYP) inducers and inhibitors on ondansetron pharmacokinetics in rats: involvement of hepatic CYP2D subfamily and 3A1/2 in ondansetron metabolism

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Abstract

The types of hepatic microsomal cytochrome P450 (CYP) isozymes responsible for the in-vivo metabolism of ondansetron in rats have not been reported. In this study, ondansetron at a dose of 8 mg kg^{-1} was administered intravenously to rats pretreated with various inducers of CYP isozymes, such as 3-methylcholanthrene, orphenadrine citrate, isoniazid and dexamethasone phosphate (the main inducers of CYP1A1/2, 2B1/2, 2E1 and 3A1/2 in rats, respectively), and inhibitors, such as SKF-525A (a non-specific inhibitor of CYP isozymes), sulfaphenazole, quinine hydrochloride and troleandomycin (the main inhibitors of CYP2C6, 2D subfamily and 3A1/2 in rats, respectively). In rats pretreated with quinine hydrochloride and troleandomycin, the time-averaged non-renal clearance of ondansetron was significantly slower (48.9 and 13.2% decrease, respectively) than that in control rats. In rats pretreated with dexamethasone phosphate, the time-averaged non-renal clearance was significantly faster (18.2% increase) than that in control rats. The results suggest that ondansetron is primarily metabolized via the CYP2D subfamily and 3A1/2 in rats.

Introduction

Ondansetron, a potent and selective 5-HT_3 (5-hydroxytryptamine) receptor antagonist, has been used for the treatment of chemotherapy- and radiotherapy-induced nausea and emesis (Oxford et al 1992). Therapeutic failure sometimes occurs due to metabolism of ondansetron via hepatic microsomal cytochrome P450 (CYP) isozymes (Janicki 2005). Ondansetron is coadministered with other drugs in most clinical situations. Gilbert et al (1998) reported that ondansetron alters the systemic exposure to cyclophosphamide in breast cancer patients, and Cagnoni et al (1999) reported that ondansetron causes a significantly smaller total area under the plasma concentration–time curve from time zero to infinity (AUC) of high-dose cyclophosphamide and cisplatin in patients. Thus, identifying the types of CYP isozymes responsible for the metabolism of ondansetron is important in order to determine possible therapeutic failure and drug interactions.

The following results on ondansetron have been reported in humans (Laethem & Serabjit-Singh 2000). Ondansetron is metabolized to 8-hydroxyondansetron (40% of the given dose), 7-hydroxyondansetron (20% of the dose), 6-hydroxyondansetron (less than 5% of the dose), and *N*-desmethylondansetron (very minor amount) (Pritchard 1992). CYP2D6 (Fisher et al 1994), the 3A subfamily (Fisher et al 1994) and 1A1/2 (Dixon et al 1995) are responsible for the metabolism of ondansetron. CYP1A1/2 plays the most important role, CYP2D6 plays a relatively minor role, and the involvement of the CYP3A subfamily seems important only at relatively high concentrations of ondansetron (Dixon et al 1995). In rats, ondansetron is extensively and rapidly metabolized to two primary products, 7-hydroxyondansetron and desmethylondansetron, based on rat liver slices and isolated hepatocytes (Worboys et al 1996). 8-Hydroxyondansetron, the major product in humans, is also formed in rat microsomes in addition to the above two metabolites (Worboys et al 1996). To our knowledge, no studies on the types of CYP isozymes responsible for the metabolism of ondansetron in rats in-vivo have been reported. This study was performed to determine the CYP isozymes responsible for the metabolism of ondansetron in rats in-vivo using various

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Funding: This study was supported in part by a grant from the Seoul City Collaborative Project among the Industry, Academy, and Research Institute, Korea.

chemical inhibitors and inducers of CYP isozymes, and using selected chemical inhibitors of CYP isozymes in rat hepatic microsomes.

Materials and Methods

Chemicals

Ondansetron hydrochloride dihydrate was supplied from Dong-A Pharmaceutical Company (Yongin, South Korea). Propranolol, the internal standard for the high-performance liquid chromatographic (HPLC) analysis of ondansetron, 3-methylcholanthrene, a main inducer of CYP1A1/2 in rats (Williams et al 1979; Choi et al 1991), orphenadrine citrate, a main inducer of CYP2B1/2 in rats (Murray et al 2003), isoniazid, a main inducer of CYP2E1 in rats (Ryan et al 1985), dexamethasone phosphate, a main inducer of CYP3A1/2 in rats (Arlotto et al 1987; Ross et al 1993; Correia 1995), SKF-525A, a non-specific inhibitor of CYP isozymes in rats (Conney 1971), sulfaphenazole, a main inhibitor of CYP2C6 in rats (Kobayashi et al 2003), quinine hydrochloride, an inhibitor of the CYP2D subfamily in rats (Steiner et al 1988; Kobayashi et al 1989), troleandomycin, a main inhibitor of CYP3A1/2 in rats (Sinclair et al 2000), 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 grade or HPLC grade.

Animals

The protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of the Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats (7–11 weeks old, 230–350 g) were purchased from the Samtako Bio Korea (Osan, South Korea). Rats were maintained in a clean-room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea) at a temperature of 23 ± 2°C, with a 12-h light–dark cycle (light on 0700–1900 hours) and a relative humidity of 55 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered, pathogen-free air, with food (Agribrands Purina Korea, Pyeongtaek, South Korea) and water freely available.

Pretreatment of rats with CYP inducers and inhibitors

Rats received a single intravenous injection of 80 $mg kg^{-1}$ (2 mL) of sulfaphenazole dissolved in distilled water with a minimum amount of NaOH to give a pH of approximately 8.0 (Ogiso et al 1999) (the SPT group), a single intraperitoneal injection of 50 $mg kg^{-1}$ (3.3 mL) of SKF-525A dissolved in 0.9% NaCl-injectable solution (Conney 1971) (the SKT group), 500 $mg kg^{-1}$ (5 mL) of troleandomycin dissolved in 0.9% NaCl-injectable solution acidified to pH 4.0 with HCl (Sinclair et al 2000) (the TMT group), or 20 $mg kg^{-1}$ (5 mL) of quinine hydrochloride dissolved in 0.9% NaCl-injectable solution (Kobayashi et al 1989) (the QNT group), three daily intraperitoneal injections of 50 $mg kg^{-1}$ (5 mL) of dexamethasone phosphate dissolved in 0.9% NaCl-injectable solution (Arlotto et al 1987; Ross et al 1993; Correia 1995) (the DXT group), 150 $mg kg^{-1}$ (3 mL) of isoniazid dissolved in 0.9% NaCl-injectable solution (Ryan et al 1985) (the INT group), or 60 $mg kg^{-1}$ (5 mL) of orphenadrine citrate dissolved in 0.9% NaCl-injectable solution (Murray et al 2003) (the OPT group), or four daily intraperitoneal injections of 20 $mg kg^{-1}$ (3.3 mL) of 3-methylcholanthrene dissolved in corn oil (Williams et al 1979; Choi et al 1991) (the MCT group). Control groups received an intraperitoneal (or intravenous) injection of 5 $mL kg^{-1}$ of 0.9% NaCl-injectable solution for the DXC (INC, OPC), SKC (QNC, TMC) and SPC groups, or 3.3 $mL kg^{-1}$ of corn oil for the MCC group. In each group 'T' and 'C' refer to pretreatment and control, respectively. During the pretreatment, the rats had free access to food and water.

Pretreatment of rats for the intravenous study

Early in the morning, the jugular vein (for drug administration) and the carotid artery (for blood sampling) of each rat were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ, USA), while each rat was under light ether anaesthesia (Kim et al 1993). Both cannulae were exteriorized to the dorsal side of the neck, where each cannula was terminated with a long silastic tube (Dow Corning, Midland, MI, USA). Both silastic tubes were inserted into a wire sheath to allow free movement of the rat. Rats were then housed individually in rat metabolic cages (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from the anaesthesia for 4–5 h before beginning the experiment. The rats were not restrained during the study.

Intravenous study

An experiment was performed just after the injection for the SPC and SPT groups (Ogiso et al 1999; Bae et al 2005), after 1 h for the SKT (Conney 1971) and QNT (Tomkins et al 1997) groups, after 2 h for the SKC (QNC, TMC) and TMT groups (Wrighton et al 1985; Arlotto et al 1987; Sinclair et al 2000), on Day 4 for the DXC (INC, OPC), DXT, INT and OPT groups (Ryan et al 1985; Arlotto et al 1987; Ross et al 1993; Sinclair et al 2000; Murray et al 2003), and on Day 5 for the MCT and MCC groups (Williams et al 1979; Choi et al 1991).

Ondansetron hydrochloride dihydrate (dissolved in distilled water) at a dose of 8 $mg kg^{-1}$ as free ondansetron was administered intravenously over 1 min via the jugular vein of the control groups (n = 5 for the DXC (INC, OPC) group; n = 6 for the MCC and SPC groups; n = 8 for the SKC (QNC, TMC) group) and the pretreatment groups (n = 5 for the MCT and SPT groups; n = 6 for the INT and OPT groups; n = 7 for the SKT, DXT and QNT groups; n = 8 for the TMT group). A blood sample (~0.22 mL) was collected via the carotid artery at time 0 (control) and at 1 (at the end of the infusion), 3, 7, 15, 30, 45, 60, 75, 90, 120, 150 and 180 min after the start of the intravenous infusion of ondansetron. A heparinized 0.9%

NaCl-injectable solution (20 units mL^{-1}; 0.3 mL) was used to flush the cannula immediately after each blood sampling to prevent blood clotting. Blood samples were immediately centrifuged and a 100-μL aliquot of each plasma sample was stored at −70°C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC, USA) until used for the HPLC analysis of ondansetron. At the end of 24 h, each metabolic cage was rinsed with 5 mL of distilled water and the rinsings were combined with the 24-h urine sample. After measuring the exact volume of the combined urine sample, two 100-μL aliquots of the combined urine sample were stored at −70°C until used for the HPLC analysis of ondansetron. At the same time (24 h), each rat was exsanguinated and killed by cervical dislocation.

Measurement of kinetic parameters for the disappearance of ondansetron in the presence of chemical inhibitors of specific CYP450 isozymes (quinine and troleandomycin) in rat hepatic microsomes

To identify the hepatic CYP isozymes responsible for the metabolism of ondansetron in rats, the specific chemical inhibitors of CYP isozymes were used. The procedures used were similar to reported methods (Shim et al 2004; Oh et al 2007). The livers (~8 g) of control rats (n = 6) were homogenized (Ultra-Turrax T25; Janke & Kunkel, IKA-Labortechnik, Staufeni, Germany) in approximately 20 mL of ice-cold buffer of 0.154 M KCl/50 mM Tris-HCl in 1 mM EDTA (pH 7.4) at 4°C in an ice-bath. The homogenates were centrifuged (10 000 g, 30 min) and the supernatant fraction was further centrifuged (100 000 g, 90 min). The microsomal protein content was measured using a reported method (Bradford 1976).

The maximum velocity (V_{max}) and the apparent Michaelis–Menten constant (K_m; the concentration at which the rate is one half of the V_{max}) for the disappearance of ondansetron were determined after incubating the above microsomal fraction (equivalent to 0.5 mg protein), a 50-μL aliquot of distilled water containing the final ondansetron concentrations of 0.05, 0.1, 0.2, 0.5, 1, 2, and 5 μM as free ondansetron, a 5-μL aliquot of methanol containing 3 μM quinine or 50 μM troleandomycin (Monostory et al 2005), and a 50-μL aliquot of 0.1 M phosphate buffer (pH 7.4) containing 1 mM NADPH in a final volume of 0.5 mL by adding 0.1 M phosphate buffer (pH 7.4) in a water-bath shaker (37°C, 500 oscillations min^{-1}). For the studies on the mechanism-based inhibitor (troleandomycin) and controls, the hepatic microsomes and NADPH were preincubated for 15 min, and 20 units of catalase were added to prevent auto-inactivation of CYP isozymes during preincubation of microsomes with NADPH (Huskey et al 1995). All of the above microsomal incubation conditions were linear. The reaction was terminated by addition of 0.5 mL of dichloromethane after 5 min incubation. The kinetic constants (K_m and V_{max}) for the disappearance of ondansetron were calculated using a non-linear regression method (Duggleby 1995). The intrinsic clearances (CL_{int}) for the disappearance of ondansetron were calculated by dividing the V_{max} by the K_m.

Measurement of rat plasma protein binding of ondansetron using equilibrium dialysis

Protein binding of ondansetron to fresh plasma from the INC (OPC), INT, OPT, MCC, MCT, SKC (QNC, TMC), SKT, QNT and TMT rats was determined using equilibrium dialysis (Shim et al 2000) at an ondansetron concentration of 0.5 μg mL^{-1}. Plasma (1 mL) was dialysed 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 (molecular weight cut-off 12–14 kDa; Spectrum Medical Industries). After 4 h incubation, two 50-μL aliquots were removed from the buffer and plasma compartments, respectively, and stored at −70°C until used for the HPLC analysis of ondansetron.

HPLC analysis of ondansetron

Concentrations of ondansetron in the samples were determined by a slight modification of reported HPLC methods (Depot et al 1997; Bauer et al 2002). In brief, a 50-μL aliquot of pH 9 buffer solution and a 20-μL aliquot of distilled water containing 50 μg mL^{-1} of propranolol (internal standard) were added to a 100-μL aliquot of sample. Then, the mixture was extracted with 0.5 mL of dichloromethane. After vortex-mixing for 30 s and centrifugation (15 000 g, 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 a 100-μL aliquot of the mobile phase and a 75-μL aliquot was directly injected onto a reversed-phase (C_{18} Symmetry; 100 mm length × 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 UV 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 limit of ondansetron in the rat plasma and urine samples was 0.02 μg mL^{-1}. The coefficients of variation (intra- and inter-day) were below 5.39%.

Pharmacokinetic analysis

The AUC was calculated using the trapezoidal rule–extrapolation method (Chiou 1978). The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods (Gibaldi & Perrier 1982) were used to calculate the following pharmacokinetic parameters using a non-compartment analysis (WinNonlin professional edition version 2.1; Pharsight, Mountain View, CA, USA): the time-averaged total body, renal and non-renal clearances (CL, CL_R and CL_{NR}, respectively), the terminal half-life, the first moment of AUC (AUMC), the mean residence time

(MRT), and the apparent volume of distribution at steady state (V_{ss}).

Statistical analysis

A value of $P < 0.05$ was deemed to be significant using SPSS (Chicago, IL, USA) posteriori analysis of variance among the four 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 ± s.d.

Results

Pharmacokinetics of ondansetron after its intravenous administration to rats pretreated with various CYP inducers

For the intravenous administration of ondansetron at a dose of 8 mg kg^{-1} to DXT, INT, OPT, MCT and control (DXC (INC, OPC) and MCC) rats, the mean arterial plasma concentration–time profiles of ondansetron are shown in Figure 1, and the relevant pharmacokinetic parameters are listed in Table 1.

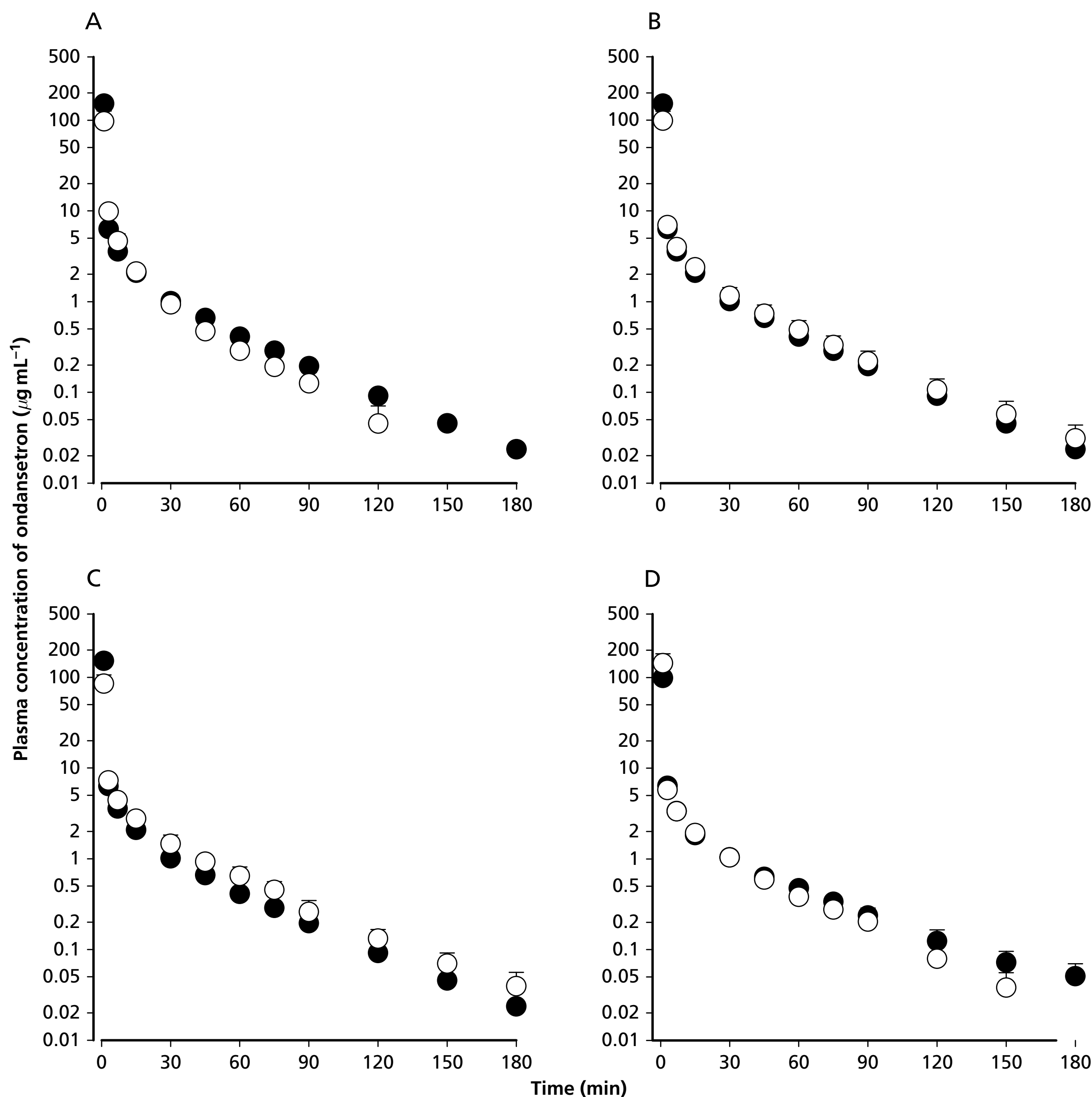

Figure 1 Mean arterial plasma concentration–time profiles of ondansetron after 1-min intravenous infusion at a dose of 8 mg kg^{-1} to rats pretreated with enzyme inducers (open symbols) such as dexamethasone (A), isoniazid (B), orphenadrine (C) and 3-methylcholanthrene (D), and their respective control rats (closed symbols). Vertical bars represent the standard deviation (some vertical bars are within the symbols).

Table 1 Pharmacokinetic parameters of ondansetron after intravenous administration at a dose of 8 $mg\,kg^{-1}$ to rats pretreated with dexamethasone (DXT), isoniazid (INT), orphenadrine (OPT) and 3-methylcholanthrene (MCT), and control rats (DXC (INC, OPC) and MCC)

Parameter	DXC, INC, OPC (n = 5)	DXT (n = 7)	INT (n = 5)	OPT (n = 6)	MCC (n = 6)	MCT (n = 5)
Initial bodyweight (g)	253 ± 16.0	269 ± 6.90	257 ± 14.4	233 ± 18.9	283 ± 45.9	292 ± 38.5
Final bodyweight (g)[a]	275 ± 16.2	219 ± 12.4	258 ± 7.58	232 ± 14.7	297 ± 51.1	301 ± 43.6
AUC ($\mu g\ min\,mL^{-1}$)[b]	267 ± 34.0	228 ± 28.0	232 ± 24.0	241 ± 23.7	219 ± 17.7	251 ± 43.5
Terminal half-life (min)[c]	30.1 ± 2.87	22.4 ± 3.43	31.6 ± 3.22	32.7 ± 6.89	33.6 ± 4.87	25.5 ± 5.27
MRT (min)[d]	13.1 ± 2.22	11.3 ± 2.40	17.3 ± 4.01	20.7 ± 2.35	18.6 ± 3.03	12.8 ± 2.48
CL ($mL\,min^{-1}\ kg^{-1}$)[b]	30.3 ± 3.99	35.5 ± 4.25	34.7 ± 3.65	33.4 ± 3.17	36.8 ± 3.15	32.7 ± 5.62
CL_R ($mL\,min^{-1}\ kg^{-1}$)[e]	0.635 ± 0.193	0.373 ± 0.103	0.736 ± 0.287	0.511 ± 0.188	0.674 ± 0.300	0.815 ± 0.386
CL_{NR} ($mL\,min^{-1}\ kg^{-1}$)[b]	29.7 ± 4.02	35.1 ± 4.17	34.0 ± 3.78	32.9 ± 5.35	36.1 ± 3.24	31.9 ± 5.25
V_{ss} ($mL\,kg^{-1}$)[d]	404 ± 115	406 ± 111	599 ± 146	685 ± 71.9	682 ± 96.6	423 ± 123
$A_{e0-24\,h}$ (% of i.v. dose)[e]	2.13 ± 0.678	1.03 ± 0.236	2.17 ± 0.982	1.56 ± 0.604	1.85 ± 0.889	2.36 ± 0.845

Values are mean ± s.d. AUC, total area under the plasma concentration–time curve from time zero to infinity; MRT, mean residence time; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged non-renal clearance; V_{ss}, apparent volume of distribution at steady state; $A_{e0-24\,h}$, percentage of the dose excreted in the 24-h urine. [a]Control (DXC, INC, OPC) and INT groups were significantly different ($P < 0.05$) compared with DXT and OPT groups. [b]Control (DXC, INC, OPC) groups were significantly different ($P < 0.05$) compared with the DXT group. [c]Control (DXC, INC, OPC), INT and OPT groups were significantly different ($P < 0.05$) compared with the DXT group. The MCT group was significantly different ($P < 0.05$) compared with the MCC group. [d]Control (DXC, INC, OPC) and DXT groups were significantly different ($P < 0.05$) compared with INT and OPT groups. The MCT group was significantly different ($P < 0.01$) compared with the MCC group. [e]Control (DXC, INC, OPC) and INT groups were significantly different ($P < 0.05$) compared with the DXT group.

The mean plasma concentrations of ondansetron declined in a polyexponential fashion for all groups of rats.

Compared with dexamethasone control rats (DXC rats), the AUC was significantly smaller (14.6% decrease), the terminal half-life was significantly shorter (25.6% decrease), the CL and the CL_{NR} were significantly faster (17.2 and 18.2% increase, respectively), the CL_R was significantly slower (41.3% decrease), and the percentage of the intravenous dose of ondansetron excreted in the 24-h urine as unchanged drug ($A_{e0-24\,h}$) was significantly smaller (51.6% decrease) in DXT rats.

Compared with isoniazid control rats (INC rats), the MRT was significantly longer (32.1% increase) and the V_{ss} was significantly larger (48.3% increase) in INT rats.

Compared with orphenadrine control rats (OPC rats), the MRT was significantly longer (58.0% increase) and the V_{ss} was significantly larger (69.6% increase) in OPT rats.

Compared with 3-methylcholanthrene control rats (MCC rats), the terminal half-life and the MRT were significantly shorter (24.1 and 31.2% decrease) and the V_{ss} was significantly smaller (38.0% decrease) in MCT rats.

The bodyweight gain decreased in DXT rats compared with that in control rats (DXC rats), as reported in other studies (Bae et al 2005; Choi & Lee 2006; Lee et al 2006).

Pharmacokinetics of ondansetron after intravenous administration to rats pretreated with various CYP inhibitors

For the intravenous administration of ondansetron at a dose of 8 $mg\,kg^{-1}$ to SKT, QNT, TMT, SPT and control (SKC (QNC, TMC) and SPC) rats, the mean arterial plasma concentration–time profiles of ondansetron are shown in Figure 2, and the relevant pharmacokinetic parameters are listed in Table 2. The mean plasma concentrations of ondansetron also declined in a polyexponential fashion for all groups of rats.

Compared with SKF-525A control rats (SKC rats), the AUC was significantly greater (204% increase), the terminal half-life and the MRT were significantly longer (231 and 685% increase, respectively), the CL and the CL_{NR} were significantly slower (66.4 and 69.1% decrease, respectively), the V_{ss} was significantly larger (145% increase), and the $A_{e0-24\,h}$ was significantly greater (306% increase) in SKT rats.

Compared with quinine control rats (QNC rats), the AUC was significantly greater (90.8% increase), the terminal half-life and the MRT were significantly longer (46.3 and 182% increase, respectively), the CL and the CL_{NR} were significantly slower (47.4 and 48.9% decrease, respectively), the V_{ss} was significantly larger (46.8% increase), and the $A_{e0-24\,h}$ was significantly greater (108% increase) in QNT rats.

Compared with troleandomycin control rats (TMC rats), the terminal half-life and the MRT were significantly longer (40.0 and 76.7% increase, respectively), the CL, the CL_R, and the CL_{NR} were significantly slower (14.0, 41.2 and 13.2% decrease, respectively), and the V_{ss} was significantly larger (48.9% increase) in TMT rats.

The pharmacokinetic parameters of ondansetron were not significantly different between the SPC and SPT rats.

K_m, V_{max} and CL_{int} for the disappearance of ondansetron in the presence of chemical inhibitors of specific CYP450 isozymes in rat hepatic microsomes

The V_{max}, K_m and CL_{int} for the disappearance of ondansetron in the microsomal fractions of control rats with and without

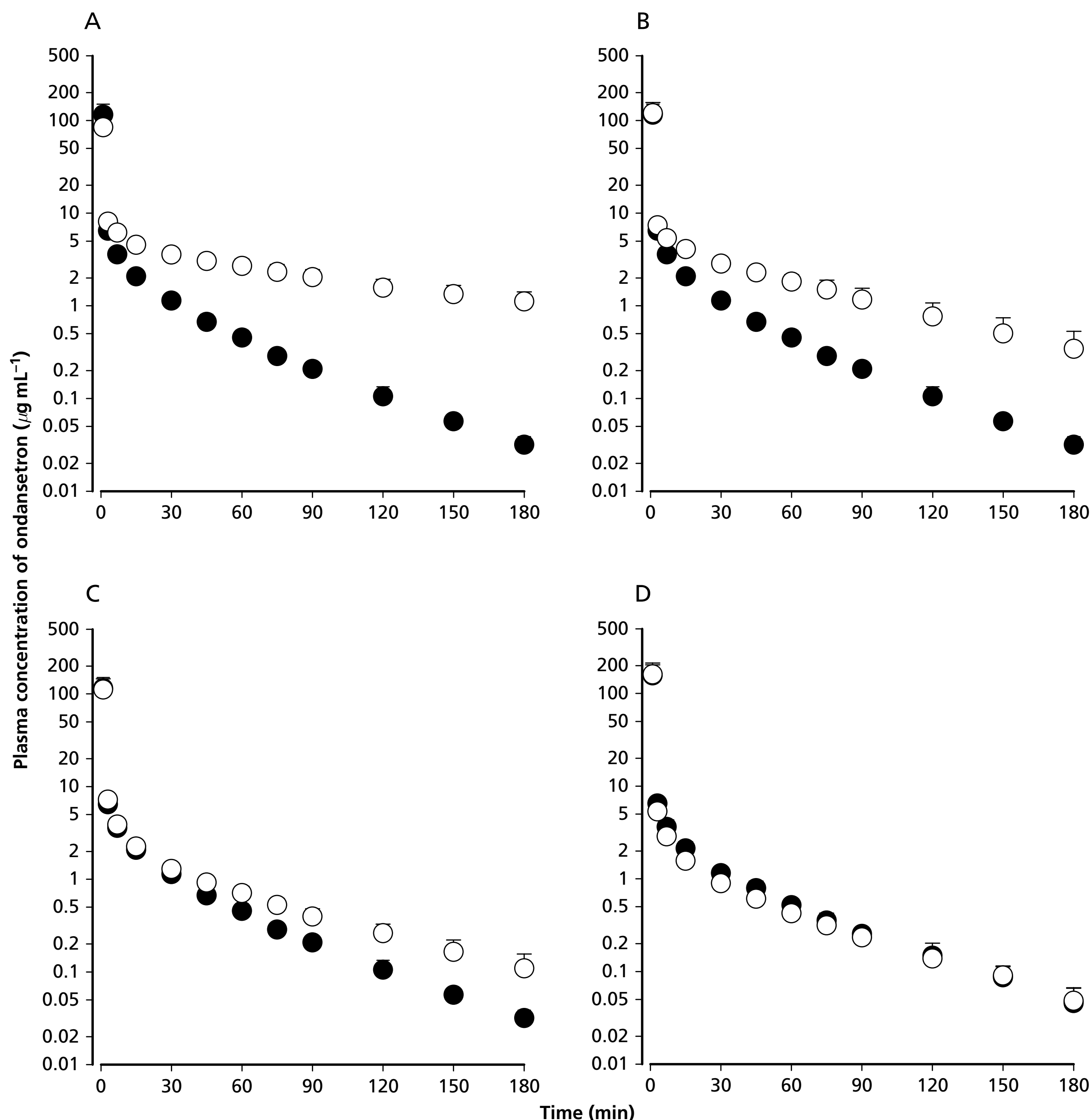

Figure 2 Mean arterial plasma concentration–time profiles of ondansetron after 1-min intravenous infusion at a dose of 8 mg kg^{-1} to rats pretreated with enzyme inhibitors (open symbols), such as SKF 525-A (A), quinine (B), troleandomycin (C) and sulfaphenazole (D), and their respective control rats (closed symbols). Vertical bars represent standard deviation (some vertical bars are within the symbols).

quinine or troleandomycin are listed in Table 3. The V_{max} in the presence of quinine or troleandomycin was significantly slower (78.4 or 95.4% decrease, respectively) than that in control rats. The K_m in the presence of quinine or troleandomycin was lower (24.7% decrease for the quinine, $P = 0.262$ or 56.8% decrease for the troleandomycin, $P < 0.05$) than that in control rats. The CL_{int} in the presence of quinine or troleandomycin was significantly slower (71.8 or 88.0% decrease, respectively) than that in control rats.

Rat plasma protein binding of ondansetron using equilibrium dialysis

Protein binding (bound fraction) values of ondansetron to fresh plasma from INC (OPC), INT, OPT, MCC, MCT, SKC (QNC, TMC), SKT, QNT and TMT rats were 78.0 ± 1.90, 75.2 ± 3.88, 77.9 ± 2.54, 76.5 ± 5.72, 77.2 ± 6.43, 74.0 ± 7.51, 71.4 ± 1.53, 67.6 ± 6.78 and 72.7 ± 4.00%, respectively. The values were not significantly different between pretreatment and control rats.

Table 2 Pharmacokinetic parameters of ondansetron after intravenous administration at a dose of $8\,mg\,kg^{-1}$ to rats pretreated with SKF 525-A (SKT), quinine (QNT), troleandomycin (TMT), and sulfaphenazole (SPT), and control rats (SKC (QNC, TMC) and SPC)

Parameter	SKC, QNC, TMC (n = 8)	SKT (n = 7)	QNT (n = 7)	TMT (n = 8)	SPC (n = 6)	SPT (n = 5)
Bodyweight (g)	278 ± 19.3	286 ± 16.7	283 ± 30.4	271 ± 18.1	292 ± 43.6	296 ± 36.0
AUC ($\mu g\,min\,mL^{-1}$)[a]	238 ± 32.0	723 ± 168	454 ± 73.8	275 ± 30.3	287 ± 62.3	264 ± 37.6
Terminal half-life (min)[b]	33.5 ± 3.36	111 ± 41.2	49.0 ± 9.76	46.9 ± 9.66	35.9 ± 6.31	40.4 ± 7.70
MRT (min)[c]	16.3 ± 3.17	128 ± 49.8	46.0 ± 13.7	28.8 ± 7.28	16.7 ± 2.77	16.5 ± 3.41
CL ($mL\,min^{-1}\,kg^{-1}$)[d]	34.2 ± 4.80	11.5 ± 2.40	18.0 ± 2.77	29.4 ± 3.23	29.1 ± 6.94	30.9 ± 4.49
CL_R ($mL\,min^{-1}\,kg^{-1}$)[e]	0.927 ± 0.221	1.28 ± 0.541	1.03 ± 0.291	0.545 ± 0.318	0.872 ± 0.152	1.15 ± 0.536
CL_{NR} ($mL\,min^{-1}\,kg^{-1}$)[d]	33.3 ± 4.85	10.3 ± 2.36	17.0 ± 2.68	28.9 ± 2.96	28.3 ± 6.92	29.7 ± 3.98
V_{ss} ($mL\,kg^{-1}$)[f]	568 ± 167	1390 ± 248	834 ± 171	846 ± 242	489 ± 151	516 ± 144
$A_{e0-24\,h}$ (% of iv dose)[g]	2.76 ± 0.758	11.2 ± 4.34	5.74 ± 1.44	1.78 ± 0.886	3.12 ± 0.830	3.62 ± 1.41

Values are mean ± s.d. AUC, total area under the plasma concentration–time curve from time zero to time infinity; MRT, mean residence time; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged non-renal clearance; V_{ss}, apparent volume of distribution at steady state; $A_{e0-24\,h}$, percentage of the dose excreted in the 24-h urine. [a]Control (SKC, QNC, TMC) and TMT groups were significantly different ($P < 0.05$) compared with SKT and QNT groups. [b]Control (SKC, QNC, TMC) groups were significantly different ($P < 0.05$) compared with SKT, QNT, and TMT groups. [c]Control (SKC, QNC, TMC) groups were significantly different ($P < 0.05$) compared with SKT, QNT, and TMT groups. Control (SKC, QNC, TMC) groups were significantly different ($P < 0.05$) compared with the QNT group. [d]Each group was significantly different ($P < 0.05$), except the SPC and SPT groups. [e]Control (SKC, QNC, TMC), SKT, and QNT groups were significantly different ($P < 0.05$) compared with the TMT group. [f]Control (SKC, QNC, TMC), SKT, TMT and QNT groups were significantly different ($P < 0.05$). [g]Control (SKC, QNC, TMC) and TMT groups were significantly different ($P < 0.05$) compared with SKT and QNT groups.

Table 3 V_{max}, K_m, and CL_{int} for the disappearance of ondansetron with or without specific chemical inhibitors of CYP isozymes

Parameter	Without quinine (n = 3)	With quinine (n = 3)	Without troleandomycin (n = 3)	With troleandomycin (n = 4)
V_{max} ($nmol\,min^{-1}$ (mg protein)$^{-1}$)	2.25 ± 0.313	0.485 ± 0.212*	2.41 ± 0.671	0.111 ± 0.0648*
K_m (μM)	17.4 ± 3.64	13.1 ± 6.18	18.7 ± 4.11	7.51 ± 5.50*
CL_{int} ($\mu L\,min^{-1}$ (mg protein)$^{-1}$)	0.133 ± 0.0261	0.0375 ± 0.00181*	0.128 ± 0.0125	0.0159 ± 0.00291*

Values are mean ± s.d. V_{max}, maximum velocity; K_m, apparent Michaelis–Menten constant; CL_{int}, intrinsic clearance. *Significantly different ($P < 0.05$) compared with respective controls.

Discussion

Following the intravenous administration of ondansetron at doses of 1–20 $mg\,kg^{-1}$ to male Sprague–Dawley rats, the AUC was dose-proportional (unpublished data). An ondansetron dose of $8\,mg\,kg^{-1}$ was arbitrarily chosen in the present study.

After intravenous administration of ondansetron, the contribution of the gastrointestinal (including the biliary) excretion of unchanged drug to the CL_{NR} of ondansetron was almost negligible; the percentages of the intravenous doses of ondansetron at 1–20 $mg\,kg^{-1}$ recovered from the gastrointestinal tract (including its contents and faeces) at 24 h were almost negligible, less than 0.422% of the dose, and the 24-h biliary excretion of ondansetron was less than 0.231% of the intravenous dose at $8\,mg\,kg^{-1}$ with bile duct cannulation (unpublished data). Thus, the CL_{NR} of ondansetron listed in Tables 1 and 2 could represent the metabolic clearance of the drug. Additionally, changes in the CL_{NR} of ondansetron could represent changes in the metabolism of the drug in rats.

To determine whether hepatic CYP isozymes are involved in the metabolism of ondansetron in rats, SKF-525A (a non-specific inhibitor of hepatic CYP isozymes in rats) was administered to rats. In SKT rats, the CL_{NR} of ondansetron was significantly slower than that in control rats (Table 2), indicating that ondansetron is metabolized via hepatic CYP isozymes in rats. Thus, various inducers or inhibitors of hepatic CYP isozymes were administered to rats to determine what types of hepatic CYP isozymes are involved in the metabolism of ondansetron in rats. In DXT (a main inducer of CYP3A1/2 in rats) rats, the CL_{NR} of ondansetron was significantly faster than that in control rats (Table 1). In contrast, in QNT and TMT (main inhibitors of the CYP2D subfamily and 3A1/2 in rats, respectively) rats, the CL_{NR} of ondansetron was significantly slower than that in control rats (Table 2). The above data suggest that the CYP2D subfamily and 3A1/2 could contribute to the metabolism of ondansetron in rats. The results in Table 3 suggest that, in the presence of quinine or troleandomycin, the maximum velocity for the disappearance (primarily metabolism) of ondansetron was significantly slower, the affinity of the enzyme(s) for the ondansetron increased, and the metabolism of ondansetron was significantly slower compared with control rats. The above results also support the suggestion that ondansetron is metabolized via the hepatic CYP2D subfamily and 3A1/2 in rats.

After intravenous administration to INT, OPT, SKT, QNT and TMT rats, the V_{ss} of ondansetron became significantly larger compared with that in control rats (Tables 1 and 2). However, the V_{ss} of ondansetron in MCT rats was significantly smaller than that in control rats (Table 1). The above results could not be mainly due to an alteration in the free (unbound to plasma protein) fraction of ondansetron in plasma from INT, OPT, MCT, SKT, QNT and TMT rats. As mentioned earlier, the plasma protein binding values were comparable between pretreatment and control rats. Although the exact reason is not clear, the changes in V_{ss} of ondansetron in the INT, OPT, MCT, SKT, QNT and TMT rats could have been due to changes in the affinity of rat tissues for ondansetron. This could be due to changes in P-glycoprotein (P-gp) in the brain, liver, intestine and kidney, since it has been reported that ondansetron is a substrate for the P-gp in rats (Scott et al 2006). No studies on the changes in P-gp in rats by isoniazid, orphenadrine, SKF-525A and troleandomycin have been reported. However, quinine slightly increased multidrug resistance 1 gene expression (producing high levels of P-gp) (Belhoussine et al 1997).

The various inducers and inhibitors of CYP isozymes used in this study could have effects on various isozymes (Correia 1995), but only the CYP isozyme that significantly changed was mentioned in this study. Thus, the present results are confined to the main CYP isozymes changed by the various inducers and inhibitors of CYP isozymes.

Although the CYP isozymes responsible for the metabolism of ondansetron in rats, the CYP2D subfamily and 3A1/2, are somewhat different to those in humans, CYP2D6, 3A subfamily, and 1A1/2 (Fischer et al 1994; Dixon et al 1995), the CYP2D and 3A subfamilies are common. The protein homology of CYP2D and 3A subfamilies between humans and rats has been reported (Lewis 1996). Thus, the results on possible drug interactions of ondansetron in rats could be helpful if extrapolated to humans.

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