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Dose-independent Pharmacokinetics of Ondansetron in Rats: Contribution of Hepatic and Intestinal First-pass Effects to Low Bioavailability

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ABSTRACT: The pharmacokinetic parameters of ondansetron were evaluated after its intravenous (at doses of 1, 4, 8 and 20 mg/kg) and oral (4, 8 and 20 mg/kg) administration to rats. The gastric, intestinal and hepatic first-pass effects of ondansetron were also evaluated after its intravenous, oral, intraportal, intragastric and intraduodenal administration at a dose of 8 mg/kg to rats. After intravenous and oral administration of ondansetron, the drug exhibits dose-independent pharmacokinetics in rats. After oral administration of ondansetron at a dose of 8 mg/kg, the unabsorbed fraction was 0.0158 of the dose, the extent of absolute oral bioavailability (F) value was 0.0407, and the hepatic and intestinal first-pass effects were 40.0% and 34.2% of the oral dose, respectively. The low F of ondansetron in rats was mainly due to considerable hepatic and intestinal first-pass effects. The lower F of ondansetron in rats (4.07%) than that in humans ($62 \pm 15\%$) was mainly due to greater hepatic metabolism of the drug in rats. Ondansetron was stable in the rat gastric juices and various buffer solutions having pHs ranging from 1 to 13. The equilibrium plasma-to-blood cells partition ratio of ondansetron was 1.74–5.31. Protein binding of ondansetron to fresh rat plasma was 53.2%. Copyright © 2008 John Wiley & Sons, Ltd.

Key words: ondansetron; dose-independent pharmacokinetics; intravenous; oral; intraportal; intragastric and intraduodenal administration; hepatic and intestinal first-pass effects; 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 [1]. In humans, the pharmacokinetic parameters of ondansetron are as follows: the time-averaged total body clearance (Cl) is 5.9 ± 2.6 ml/min/kg, the apparent volume of distribution is 1.9 ± 0.05 l/kg, the terminal half-life is 3.5 ± 1.2 h, the plasma protein binding is $73 \pm 2\%$, the urinary excretion is 5% of the dose, and the extent of absolute oral bioavailability (F) is $62 \pm 15\%$ [2]. Saynor and Dixon [3] reported that ondansetron is rapidly and extensively absorbed from the gastrointestinal tract and the F was less than 10% as a result of first-pass metabolism in rats. However, no studies on the reasons for the low F in rats have yet been reported.

The purposes of this study were to report the (1) dose-independent pharmacokinetics of ondansetron after its intravenous infusion (at doses of 1, 4, 8 and 20 mg/kg) or oral administration (at doses of 4, 8 and 20 mg/kg) to rats, (2) gastric, intestinal and hepatic first-pass effects of ondansetron after its intravenous, oral, intraportal, intragastric and intraduodenal administration at

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a dose of 8 mg/kg to rats to find the possible reasons for the low F of ondansetron, and (3) reasons why the F value in rats is smaller than that in humans.

Materials and Methods

Chemicals

Ondansetron hydrochloride dihydrate was donated from Dong-A Pharmaceutical Company, Ltd (Yongin, Republic of Korea). Propranolol [internal standard for the high-performance liquid chromatographic (HPLC) analysis of ondansetron], α_1-acid glycoprotein (AAG), the reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), tri(hydroxymethyl)aminomethane (Tris®)-buffer, uridine 5′-diphosphoglucuronic acid (UDPGA; as a trisodium salt) and dextran (mol. wt. 70000) were purchased from Sigma–Aldrich Corporation (St Louis, MO). Liver and intestinal S9 fractions of rat and human were purchased from XenoTech (Lenexa, KS). Human serum albumin (HSA; 20%) was purchased from Dong-Shin Pharmaceutical Company (Seoul, Republic of Korea). Various buffer solutions having pHs ranging from 1 to 13 were products from Shinyo Pure Chemicals (Osaka, Japan). Other chemicals were of reagent grade or HPLC grade.

Rats

Protocols for the animal studies were approved by the Animal Care and Use Committee of the College of Pharmacy of Seoul National University, Seoul, Republic of Korea. Male Sprague–Dawley rats (7–9 weeks old and weighing 230–280 g) were purchased from the Samtako Bio Korea (Osan, Republic of Korea). Rats were maintained in a clean-room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of $23 \pm 2^{\circ}C$ with 12 h light (07:00–19:00) and dark (19:00–07:00) cycles and a relative humidity of $55 \pm 5\%$. Then, the rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered, pathogen-free air, with food (Sam Yang Company, Pyeongtaek, Republic of Korea) and water available *ad libitum*.

Pretreatment of rats for drug administration

Early in the morning, the jugular vein (for drug administration in the intravenous and intraportal studies) and the carotid artery (for blood sampling) of each rat were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ) while each rat was under light ether anesthesia [4]. 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). Both silastic tubes were inserted into a wire sheath to allow free movement of the rat. Then, each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, Republic of Korea) and allowed to recover from the anesthesia for 4–5 h before beginning the experiment. Rats were freely moving post-surgery. Hence, the rats were not restrained in the present study.

Intravenous study

Ondansetron hydrochloride dihydrate (dissolved in distilled water) at doses of 1 (n=8), 4 (n=10), 8 (n=10) and 20 (n=10) mg/kg as ondansetron base was infused (total infusion volume of 2 ml/kg) over 1 min via the jugular vein of each rat. A blood sample (approximately 0.22 ml) was collected via the carotid artery at 0 (control), 1 (at the end of the infusion), 3, 7, 15, 30, 45, 60, 75 (for the 1 mg/kg), 90, 120 (for the 4 mg/kg), 150, 180 (for the 8 mg/kg) and 240 (for the 20 mg/kg) min after the start of the intravenous infusion of ondansetron. A heparinized 0.9% NaCl-injectable solution (20 units/ml), 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) until used for the HPLC analysis of ondansetron [5,6]. 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 [5,6]. At the same time (24 h), as much blood as possible was collected

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via the carotid artery and each rat was killed by cervical dislocation. Then, the abdomen was opened and the entire gastrointestinal tract (including its contents and feces) of each rat was removed, transferred into a beaker containing 50 ml of methanol (to facilitate the extraction of ondansetron), and cut into small pieces using scissors. After stirring with a glass rod for 1 min, two 100 μl aliquots of the supernatant were collected from each beaker and stored at −70°C until used for the HPLC analysis of ondansetron [5,6].

After cannulation of the jugular vein, the abdomen was opened and the bile duct was cannulated with polyethylene tube while each rat was under light ether anesthesia [4]. The abdomen was sutured. Other procedures were similar to those for the 'pretreatment of rats for drug administration'. Ondansetron hydrochloride dihydrate at a dose of 8 mg/kg as ondansetron base was infused via the jugular vein of rats (n=5), and the 0–24 h bile sample was collected for the measurement of biliary excretion of ondansetron.

Oral study

Ondansetron hydrochloride dihydrate (the same solution used in the intravenous study) at doses of 4 (n=7), 8 (n=8) and 20 (n=8) mg/kg as ondansetron base was administered orally (total oral volume of 5 ml/kg) using a feeding tube to rats. Blood sampling time schedules were 0, 3, 5, 10, 20, 30, 40, 50, 60 (for the 4 mg/kg), 90 (for the 8 mg/kg) and 120 (for the 20 mg/kg) min after oral administration of ondansetron. Other procedures were similar to those for the intravenous study.

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

After cannulation of the carotid artery and the jugular vein, the abdomen was opened, the vein from the cecum was cannulated, and the cannula was pushed forward about 4.0 cm toward the liver through the portal vein to minimize impaired blood-flowing into the portal vein [7]. The abdomen was sutured. Other procedures were similar to those for the 'pretreatment of rats for drug administration'. Ondansetron hydrochloride dihydrate (the same solution used in the intravenous study) at a dose of 8 mg/kg as ondansetron base was infused over 30 min via the jugular vein and the portal vein for the intravenous (n=5) and intraportal (n=6) administration, respectively, with the assistance of an infusion pump (Model 2400-006; Harvard Instrument, Southnatick, MA). At the same time, an equal volume (2 ml/kg) of distilled water was also infused over 30 min via the portal vein for the intravenous study and via the jugular vein for the intraportal study. A blood sample (approximately 0.22 ml) was collected via the carotid artery at 0, 15, 30 (at the end of the infusion), 31, 33, 37, 45, 60, 75, 90, 120, 150 and 180 min after the start of the infusion of ondansetron. After centrifugation, a 100 μl aliquot of plasma sample was kept at −70°C until used for the HPLC analysis of ondansetron [5,6].

Measurement of the gastric and intestinal first-pass effects of ondansetron in rats

Rats were fasted overnight with free access to water. The surgical procedures and other procedures were similar to those for the hepatic first-pass effects of ondansetron. For the intraportal infusion (n=4), ondansetron hydrochloride dihydrate (the same solution used in the intravenous study) at a dose of 8 mg/kg as ondansetron base was infused via the portal vein for 30 min, and 2 ml/kg of distilled water was instilled into the stomach and duodenum, respectively, using a 23 gauge needle. For the intraduodenal instillation (n=5), 2 ml/kg of distilled water was instilled into the stomach and infused via the portal vein for 30 min, respectively, and the same dose of ondansetron base was instilled into the duodenum. For the intragastric instillation (n=3), 2 ml/kg of distilled water was instilled into the duodenum and infused via the portal vein for 30 min, respectively, and the same dose of ondansetron base was instilled into the stomach.

Disappearance of ondansetron in rat tissue homogenates

The procedures used were similar [8] to a reported method [9]. Approximately 1 g of each rat liver, kidney, heart, stomach, lung, small intestine, large intestine, muscle and brain was excised (n=4) after cervical dislocation. Each

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tissue sample was rinsed with cold 0.9% NaCl-injectable solution, blotted dry with tissue paper and weighed. Then, each tissue was homogenized (Ultra-Turrax T25; Janke & Kunkel, IKA-Labortechnik, Staufeni, Germany) with 4 volumes of 0.25 M sucrose. Metabolic activity was initiated by adding a 550 µl aliquot of Tris®-buffer (pH 7.4), a 50 µl aliquot of distilled water containing 0.5 µg/ml of ondansetron base (the same solution used in the intravenous study), a 100 µl aliquot of Tris®-buffer (pH 7.4) containing 1 mM NADPH and 3.3 mM UDPGA to an Eppendorf tube containing a 300 µl aliquot of the 9000 × *g* supernatant fraction (S9 fraction) of each tissue homogenate in a thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany) kept at 37°C and 500 oscillations/min (opm). To terminate the enzyme activity, a 0.5 ml of dichloromethane was added after 30 min incubation. Then, a 50 µl aliquot of buffer solution (pH 9) and a 20 µl aliquot of distilled water containing 50 µg/ml of propranolol (internal standard) were added. The amount of ondansetron remaining in each tissue was determined by reported HPLC methods [5,6].

Disappearance of ondansetron in liver and intestinal S9 fraction of rat and human

The procedures used were similar [8] to a reported method [9]. Metabolic activity was initiated by adding a 50 µl aliquot of distilled water containing 1 µM of ondansetron base to an Eppendorf tube containing a 550 µl aliquot of Tris®-buffer (pH 7.4), a 100 µl aliquot of Tris®-buffer (pH 7.4) containing 1 mM NADPH and 3.3 mM UDPGA, a 300 µl aliquot of the liver (20 mg/ml) or intestinal (4 mg/ml) S9 fraction of rat or human in a thermomixer kept at 37°C and 500 oscillations/min (opm). At 0, 5 and 30 min for the intestinal S9 fraction and 0, 5 and 15 min for the liver S9 fraction, a 100 µl aliquot was transferred to an Eppendorf tube containing a 0.5 ml of dichloromethane, a 50 µl aliquot of buffer solution (pH 9) and a 20 µl aliquot of distilled water containing 50 µg/ml of propranolol (internal standard). The amount of ondansetron remaining was determined by reported HPLC methods [5,6].

Tissue distribution of ondansetron after intravenous infusion

Ondansetron hydrochloride dihydrate (the same solution used in the intravenous study) at a dose of 8 mg/kg as ondansetron base was infused over 1 min via the jugular vein of rats. Approximately 1 g each of rat liver, kidney, heart, stomach, lung, small intestine, large intestine, muscle and brain was quickly excised after 5 and 60 min, respectively (n=5 at each time), after exsanguination. Each tissue sample was rinsed with cold 0.9% NaCl-injectable solution, blotted dry with tissue paper, and weighed. Then, each tissue was homogenized with 4 volumes of 0.9% NaCl-injectable solution in a tissue homogenizer and centrifuged. Two 100 µl aliquots of plasma or supernatant of each tissue homogenates were stored at −70°C until used for the HPLC analysis of ondansetron [5,6].

Stability of ondansetron

The procedures used were similar to a reported method [8]. Ondansetron hydrochloride dihydrate stock solution in distilled water was spiked into each test tube containing rat plasma, urine, or five gastric juices, and various buffer solutions having pHs ranging from 1 to 13 to produce an ondansetron base concentration of 5 µg/ml. Each sample was incubated in a water-bath shaker [37 ± 2 C, 50 oscillations/min (opm)] for 24 h, except for 4 h for the rat gastric juices.

Factors affecting protein binding of ondansetron to 4% HSA using equilibrium dialysis

Various factors affecting the protein binding of ondansetron to 4% HSA were evaluated (n=3, each) using equilibrium dialysis [10]. One ml of 4% HSA (20% HSA was diluted in isotonic Sørensen phosphate buffer of pH 7.4) was dialysed against 1 ml of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (v/v) dextran ('the buffer') to minimize volume shift [11] in a 1 ml dialysis cell (Fisher Scientific, Fair Lawn, NJ) using a Spectra/Por 4 membrane (mol. wt cutoff of 12–14 KDa; Spectrum Medical Industries Inc., Los Angeles, CA). Similar experiments were also performed with fresh rat (n=4) and human (n=3) plasma.

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In vitro distribution kinetics of ondansetron between plasma and blood cells of rat blood

The procedures used were similar to a reported method [12]. Heparinized blood (1 ml; freshly withdrawn via the carotid artery from seven unanesthetized rats and blood pooled together) was pipetted into each glass test tube (22 tubes for each concentration). The initial ondansetron base concentrations in rat blood were 0.5, 1 and 5 μg/ml, respectively. After 30 s gentle mixing manually, each sample was incubated in a water-bath shaker (37 ± 2°C, 50 opm). At 0, 1, 3, 5, 7, 10, 15, 30, 60, 90 and 120 min, the blood sample was centrifuged and the plasma sample was collected. Whole blood concentrations of ondansetron were also measured at each time by adding 2 volumes of distilled water to facilitate the hemolysis and to increase the reproducibility of an HPLC assay [12] of ondansetron.

HPLC analysis of ondansetron

Concentrations of ondansetron in the samples were determined by a slight modification of reported HPLC methods [5,6]. In brief, a 50 μl aliquot of buffer solution (pH 9) and a 20 μl aliquot of distilled water containing 50 μg/ml 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 (15000 × *g*, 10 min), the upper aqueous layer was discarded. 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 injected directly onto a reversed-phase (C_{18}; Symmetry®; 100 mm. ℓ. × 4.6 mm. i.d.; particle size, 3.5 μm; Waters, Milford, MA) 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, 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 limit of ondansetron in plasma and urine samples were all 0.02 μg/ml. The coefficients of variation (intra- and inter-day) were below 5.39%.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (*AUC*) was calculated using the trapezoidal rule–extrapolation method [13]. 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 [14] were used to calculate the following pharmacokinetic parameters using a non-compartment analysis (WinNonlin®; professional edition version 2.1; Pharsight, Mountain View, CA); 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*), the apparent volume of distribution at steady states (V_{ss}), and the *F*. The *F* value was calculated based on the *AUC* after intravenous administration of ondansetron at a dose of 8 mg/kg, since the *AUC* values were dose-proportional at four intravenous doses studied (Table 1). The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly read from the experimental data. The mean 'true' fraction of the oral dose of ondansetron unabsorbed (F_{unabs}) from the gastrointestinal tract was estimated using a reported equation [15].

$$GI_{oral,\,24\,h} = F_{unabs} + (F \times GI_{intravenous,\,24\,h}) \qquad (1)$$

in which $GI_{24\,h}$ is the total amount of ondansetron recovered from the gastrointestinal tract (including its contents and feces) at 24 h.

Statistical analysis

A value of $p < 0.05$ was deemed to be statistically significant using a Duncan's multiple range test of Social Package of Statistical Sciences (SPSS) *posteriori* analysis of variance (ANOVA) among the three or four means for the unpaired data. All data are expressed as mean ± SD, except median (ranges) for T_{max}.

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Table 1. Mean (± SD) pharmacokinetic parameters of ondansetron after its intravenous administration at various doses to rats

Parameter	1 mg/kg (n=8)	4 mg/kg (n=10)	8 g/kg (n=10)	20 mg/kg (n=10)
Body weight (g)	263 ± 2.89	266 ± 9.62	265 ± 7.07	267 ± 10.8
AUC[a] (μg min/ml)	28.6 ± 12.7	86.1 ± 23.5	214 ± 57.7	507 ± 44.3
Terminal half-life (min)	(24.4 ± 4.91)[b]	28.0 ± 3.73	31.0 ± 2.88	41.0 ± 6.47
MRT (min)	(11.7 ± 1.45)[b]	16.4 ± 2.82	15.5 ± 4.06	22.5 ± 4.26
Cl (ml/min/kg)	40.9 ± 15.7	49.6 ± 13.4	39.8 ± 10.4	39.7 ± 3.55
Cl_r (ml/min/kg)	1.09 ± 0.688[c]	0.930 ± 0.308	0.924 ± 0.379	0.612 ± 0.261
Cl_{nr} (ml/min/kg)	39.8 ± 15.7	48.8 ± 13.5	38.9 ± 10.3	39.1 ± 3.58
V_{ss} (ml/kg)	(496 ± 206)[b]	832 ± 300	636 ± 279	900 ± 212
$Ae_{0–24\,h}$ (% of i.v. dose)	2.97 ± 1.93[c]	2.10 ± 0.979	2.38 ± 0.933	1.56 ± 0.746
$GI_{24\,h}$ (% of i.v. dose)	0.422 ± 0.342	0.170 ± 0.0840	0.121 ± 0.0437	0.150 ± 0.0929[d]

[a]Dose-normalized (based on the 1 mg/kg) values were compared when statistical analysis was performed.
[b]The numbers in parentheses at an ondansetron dose of 1 mg/kg were not included in statistics, since the detection of ondansetron in plasma was shorter than those at 4, 8 and 20 mg/kg.
[c]1 mg/kg was significantly different ($p<0.05$) from 20 mg/kg.
[d]20 mg/kg was significantly different ($p<0.05$) from 1, 4 and 8 mg/kg.

Results

Pharmacokinetics of ondansetron after intravenous administration to rats

For the intravenous administration of ondansetron at doses of 1, 4, 8 and 20 mg/kg to rats, the mean arterial plasma concentration–time profiles of the drug are shown in Figure 1(a), and the relevant pharmacokinetic parameters are listed in Table 1. Note that the dose-normalized (based on the 1 mg/kg) AUC values of ondansetron were comparable (not significantly different) among the four intravenous doses studied; the values were 28.6 ± 12.7, 21.5 ± 5.89, 26.8 ± 7.22 and 25.3 ± 2.21 μg min/ml for 1, 4, 8 and 20 mg/kg, respectively. The slope between log AUC values and log doses of ondansetron was close to 1.0 (the value was 0.991). Moreover, other pharmacokinetic parameters of ondansetron listed in Table 1 were also not significantly different among the four intravenous doses studied except the Cl_r and the percentage of the intravenous dose of ondansetron excreted in the 24 h urine as unchanged drug ($Ae_{0–24\,h}$) and $GI_{24\,h}$. Since the Cl_r, the $Ae_{0–24\,h}$, and the $GI_{24\,h}$ values were very small, the contribution of these values to other pharmacokinetics of ondansetron would be minor. The above data indicate that ondansetron exhibits dose-independent pharmacokinetics after four intravenous doses studied in rats. Worboys *et al*. [16] reported a similar terminal half-life (48.0 ± 38.6 min) and Cl (35.0 ± 8.1 ml/min) of ondansetron after its intravenous administration at a dose of 10 mg/kg to five rats.

Biliary excretion of ondansetron after intravenous administration to rats

After intravenous administration of ondansetron at a dose of 8 mg/kg to five rats with bile duct cannulation, the 24 h biliary excretion of ondansetron was almost negligible; the mean value was 0.125 ± 0.0627% of the dose.

Pharmacokinetics of ondansetron after oral administration to rats

For the oral administration of ondansetron at doses of 4, 8 and 20 mg/kg to rats, the mean arterial plasma concentration–time profiles are shown in Figure 1(b), and the relevant pharmacokinetic parameters are listed in Table 2. After oral administration of ondansetron, the absorption of the drug from the rat gastrointestinal tract was rapid; ondansetron was detected in plasma from the first blood sampling time (3 min) and rapidly reached T_{max} (5–10 min) for all the three oral doses studied. Saynor and Dixon [3] also reported that ondansetron is rapidly absorbed from rat gastrointestinal tract. Note that the dose-normalized (based on the 4 mg/kg) AUC values of ondansetron were also comparable (not significantly different) among the three doses studied; the values were 5.20 ± 1.84, 4.36 ± 1.01

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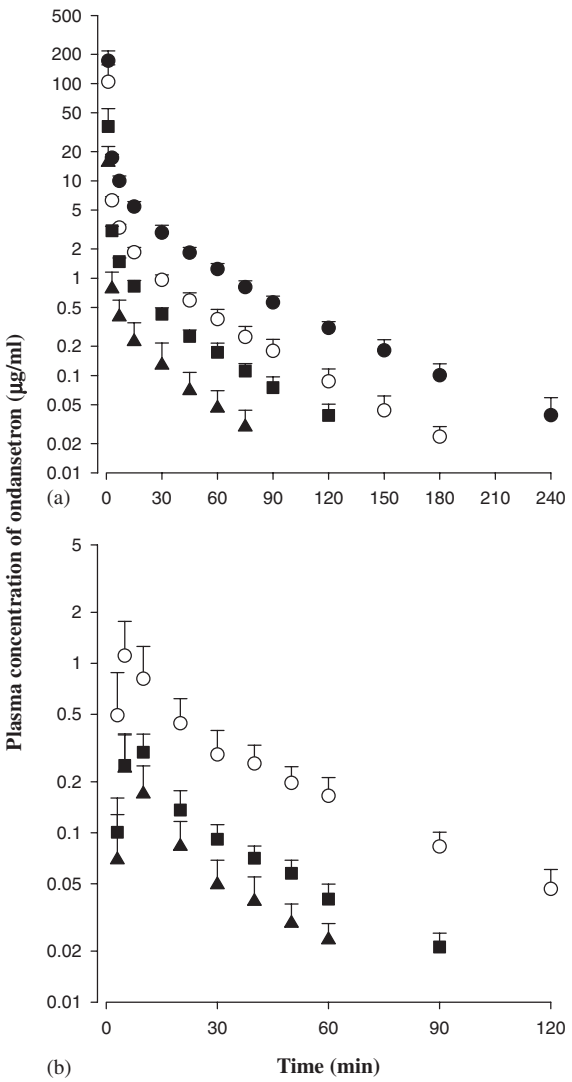

Figure 1. Mean arterial plasma concentration–time profiles of ondansetron after its intravenous infusion at doses of 1 (▲; n=8), 4 (■; n=10), 8 (; n=10) and 20 (●; n=10) mg/kg (a) and oral administration at doses of 4 (▲; n=7), 8 (■; n=8) and 20 (○; n=8) mg/kg (b) to rats. Bars represent standard deviation

and 6.39 ± 3.03 μg min/ml for 4, 8 and 20 mg/kg, respectively. The slope between log *AUC* values and log doses of ondansetron was close to 1.0 (the value was 0.961). Hence, the *F* values were also comparable; the values were 0.0604, 0.0407 and 0.0631 for the oral doses of 4, 8 and 20 mg/kg, respectively. Moreover, the other pharmacokinetic parameters of ondansetron listed in Table 2 were also not significantly different among the three oral doses studied, except the T_{max} and the C_{max}. The above data also indicate that ondansetron exhibits dose-independent pharmacokinetics after the three oral doses studied in rats. Saynor and Dixon [3] also reported a similar *F* value of ondansetron, <10%, after its oral administration at a dose of 1 mg/kg to four rats.

Hepatic first-pass effect of ondansetron in rats

For the intravenous and intraportal administration of ondansetron at a dose of 8 mg/kg to rats, the mean arterial plasma concentration–time profiles of the drug are shown in Figure 2(a). The *AUC* values of ondansetron following its intravenous and intraportal administration were 218 ± 64.8 and 68.8 ± 10.7 μg min/ml, respectively. The *AUC* of ondansetron following the intraportal administration was significantly smaller (68.4% decrease) than that following the intravenous administration, suggesting that the hepatic extraction ratio of ondansetron after absorption into the portal vein was approximately 0.684 in rats.

Gastric and intestinal first-pass effects of ondansetron in rats

For the intraportal, intragastric and intraduodenal administration of ondansetron at a dose of 8 mg/kg to rats, the mean arterial plasma concentration–time profiles of the drug are shown in Figure 2(b). The *AUC* values of ondansetron following intraportal, intragastric and intraduodenal administration were 74.3 ± 20.3, 53.4 ± 13.3 and 48.9 ± 15.5 μg min/ml, respectively. The *AUC* values were comparable between the intragastric and intraduodenal administration, suggesting that the gastric first-pass effect of ondansetron was almost negligible, if any, in rats. However, the *AUC* value following the intraduodenal administration was significantly smaller (34.2% decrease) than that following the intraportal administration, suggesting that the intestinal extraction ratio of ondansetron is 0.342 in rats.

Disappearance of ondansetron in rat tissue homogenate

The percentages of spiked amount of ondansetron remaining in each tissue homoge-

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Table 2. Mean (± SD) pharmacokinetic parameters of ondansetron after its oral administration at various doses to rats

Parameter	4 mg/kg (n=7)	8 mg/kg (n=8)	20 mg/kg (n=8)
Body weight (g)	235 ± 5.00	244 ± 18.0	239 ± 5.48
AUC[a] (μg min/ml)	5.20 ± 1.84	8.71 ± 2.02	32.0 ± 15.1
Terminal half-life (min)	26.5 ± 12.9	28.8 ± 5.82	35.8 ± 8.26
C_{max}[a] (μg/ml)	0.240 ± 0.138	0.311 ± 0.104	1.13 ± 0.588[b]
T_{max}[c] (min)	5.00 (5.00)	7.50 (5.00–10.00)[d]	5.00 (5.00)
Cl_r (ml/min/kg)	1.79 ± 1.46	1.88 ± 0.889	1.45 ± 0.745
$Ae_{0-24\,h}$ (% of oral dose)	0.206 ± 0.141	0.186 ± 0.0692	0.236 ± 0.150
$GI_{24\ h}$ (% of oral dose)	0.389 ± 0.790	1.58 ± 2.01	0.290 ± 0.376
F	0.0604	0.0407	0.0631

[a]Dose-normalized (based on the 4 mg/kg) values were compared when statistical analysis was performed.
[b]20 mg/kg was significantly different ($p<0.05$) from 4 and 8 mg/kg.
[c]Median (ranges).
[d]8 mg/kg was significantly different ($p<0.05$) from 4 and 20 mg/kg.

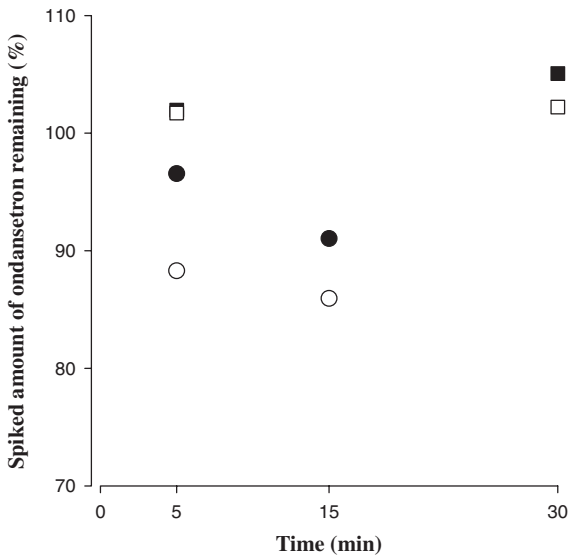

Figure 2. Percentages of the spiked amount of ondansetron remaining in the human liver (●), rat liver (○), human intestinal (■) and rat intestinal (□) S9 fraction at 5, 15 or 30 min incubation

nate are listed in Table 3. Rat liver and small intestine showed some metabolic activities for ondansetron; approximately 67.0% and 18.5% of the spiked amount of ondansetron disappeared (primarily metabolism) for the rat liver and small intestinal homogenates, respectively. However, other tissues (organs) had almost negligible metabolic activities for ondansetron.

Disappearance of ondansetron in the liver and intestinal S9 fraction of rat and human

The percentages of the spiked amount of ondansetron remaining in the liver and intestinal S9 fraction of rat and human are shown in Figure 2. The rat liver had a faster disappearance rate (primarily metabolism) of ondansetron than that in human liver; 244% and 148% faster for 5 and 15 min, respectively. However, the disappearance of ondansetron was almost negligible in both rat and human intestine.

Tissue distribution of ondansetron after intravenous infusion

Tissue distribution of liver, kidney, heart, stomach, lung, small intestine, large intestine, muscle and brain of ondansetron at a dose of 8 mg/kg is listed in Table 4. Rat tissues had good affinity to ondansetron; the tissue/plasma ratios of ondansetron at both 5 and 60 min were greater-than-unity except for the brain (Table 5).

Stability of ondansetron

Ondansetron was stable for up to 24 h incubation in various buffer solutions having pHs ranging from 1 to 13 and for up to 4 h incubation in five rat gastric juices; more than 92.7%, 94.3%, 93.8% and 95.6% of the spiked amounts of ondansetron were recovered in various buffer solutions, rat plasma, urine and gastric juices, respectively. The above data suggest that chemical and enzymatic

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Table 3. Percentages of spiked amount of ondansetron remaining in rat tissue homogenate

Liver	Kidney	Stomach	Lung	Heart	Small intestine	Large intestine	Muscle	Brain
33.0	106	108	96.8	101	81.5	96.0	100	97.8

Table 4. Plasma concentrations (μg/ml) and amount (μg/g tissue) of ondansetron recovered from tissue (or organ) 5 and 60 min after intravenous administration of ondansetron at a dose of 8 mg/kg. T/P represents to tissue/plasma ratio

Plasma	Liver	Kidney	Stomach	Lung	Heart	Small intestine	Large intestine	Muscle	Brain
5 min 5.48	26.4	70.6	17.4	55.4	7.62	40.0	24.8	6.50	4.71
T/P	4.86	12.8	3.21	10.0	1.41	7.33	4.53	1.20	0.862
60 min 0.493	1.57	5.56	6.01	5.20	0.711	3.74	4.17	2.38	0.444
T/P	3.17	11.4	12.7	8.53	1.46	7.65	8.73	4.84	0.899

Table 5. Mean ($\pm$ SD) protein binding value (%) of ondansetron (n=3, each)

HSA concentration	1%	2%	3%	4%	5%	6%
	22.3 $\pm$ 1.82	20.9 $\pm$ 1.24	26.8 $\pm$ 8.35	44.5 $\pm$ 1.27	53.2 $\pm$ 7.62	55.6 $\pm$ 2.47
Buffer pH	5.8	6.4	7.0	7.4	8.0	
	30.6 $\pm$ 1.28	41.6 $\pm$ 3.22	41.1 $\pm$ 5.16	45.5 $\pm$ 1.27	47.1 $\pm$ 1.54	
AAG concentration	0.08%	0.16%	0.32%			
	64.6 $\pm$ 2.04	75.3 $\pm$ 1.88	87.6 $\pm$ 0.605			

degradation of ondansetron in rat gastric juices is almost negligible, if any, in rats.

Factor affecting binding of ondansetron to 4% HSA using equilibrium dialysis

The equilibrium of ondansetron between 4% HSA and the 'buffer compartments' was reached after 4 h incubation, and the protein binding values were not affected for up to 24 h incubation (data not shown). Protein binding values of ondansetron to 4% HSA were independent of ondansetron concentrations; the mean binding value was 45.6% at ondansetron concentrations ranging from 0.2 to 5 μg/ml. Thus, an ondansetron concentration of 0.5 μg/ml was arbitrarily chosen for this plasma protein binding study. Binding values of ondansetron seemed to be dependent on HSA concentrations, buffer pHs and AAG concentrations. Protein binding values of ondansetron to fresh rat and human plasma were 53.2 $\pm$ 4.23% and 58.2 $\pm$ 6.08%, respectively.

In vitro distribution kinetics of ondansetron between plasma and blood cells of rat blood

The whole blood and plasma concentrations of ondansetron were almost constant for up to 2 h incubation for initial blood ondansetron concentrations of 0.5–5 μg/ml (data not shown). This suggests that ondansetron is stable in rat blood and reached equilibrium rapidly (within 30 s mixing manually) between plasma and blood cells of rat blood. The equilibrium plasma-to-blood cells partition ratios of ondansetron were independent of the initial blood ondansetron concentrations of 0.5 and 1 μg/ml; the mean values were 1.74 and 1.97, respectively. However, the value at 5 μg/ml was 5.31, possibly due to limited binding sites in blood cells. Similar results were also reported with gentamycin [12].

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Discussion

In pharmacokinetic studies, accurately measured plasma drug concentrations are usually assumed to be equal to their *in vivo* plasma concentrations. Such an assumption may be valid for drugs that have a very rapid or instantaneous rate of equilibration between plasma and blood cells [12]. If this equilibration process is slow or irregular, then the length of time elapsed between the collection and centrifugation of a blood sample might have a profound effect on the measured drug concentration (the 'blood storage effect'); the sooner the blood sample is centrifuged, the higher the plasma concentration that will be measured [17]. This factor might have contributed in part to the 'reported' inconsistencies in the time to achieve the peak plasma level after intravenous administration, to the 'calculated' time-dependent renal clearances, and to the 'unsmooth' plasma level profiles reported in the literature [17]. Moreover, it has been reported that the red blood cells act as barriers for the elimination of adriamycin [18] and propranolol [19]. Thus, the experiments on the distribution kinetics of ondansetron between plasma and blood cells of rat blood were performed. The mean equilibrium plasma-to-blood cells partition ratios of ondansetron was 1.74–5.31, suggesting that the binding of ondansetron to rat blood cells was not considerable.

For the exact measurement of the first-pass effects of ondansetron in rats, the mean 'true' fraction of oral dose unabsorbed (F_{unabs}) from the gastrointestinal tract for up to 24 h is required. Therefore, amount of ondansetron remaining in the whole gastrointestinal tract (including its contents and feces) at 24 h as unchanged drug ($GI_{24\,h}$) after its intravenous (Table 1) and oral (Table 2) administration were measured. For the measurement of the exact amount of $GI_{24\,h}$, a stability test of ondansetron was performed in rat gastric juices.

After intravenous administration of ondansetron at doses of 1–20 mg/kg to rats, the $Ae_{0-24\,h}$ value was less than 2.97% of the intravenous doses (Table 1), suggesting that almost all the intravenous ondansetron is eliminated via a non-renal (Cl_{nr}) route. The contribution of the gastrointestinal (including the biliary) excretion to the Cl_{nr} of ondansetron did not seem to be considerable; the $GI_{24\,h}$ value of ondansetron was almost negligible for all four intravenous doses studied, less than 0.422% of the doses (Table 1). Moreover, the 24 h biliary excretion of ondansetron was less than 0.231% of the intravenous dose at 8 mg/kg. Thus, the Cl_{nr} of ondansetron listed in Table 1 could represent the metabolic clearance of ondansetron.

The *AUC* values of ondansetron were dose-proportional after its intravenous administration at doses of 1–20 mg/kg (Table 1) and oral administration at doses of 4–20 mg/kg (Table 2) to rats. Thus, a dose of 8 mg/kg was arbitrarily chosen to measure the first-pass effects of ondansetron in rats.

The *F* value of ondansetron was low; 0.0407 at an oral dose of 8 mg/kg (Table 2). The mean 'true' fraction of the oral dose unabsorbed (F_{unabs}) at an oral dose of 8 mg/kg was 0.0158, indicating that ondansetron was absorbed essentially complete from the entire gastrointestinal tract for up to 24 h following its oral administration. Saynor and Dixon [3] also reported that the absorption of ondansetron from the rat gastrointestinal tract is rapid and extensive. Therefore, approximately 0.940 (1.00–0.0407–0.0158) of the orally administered ondansetron at a dose of 8 mg/kg could be eliminated by the first-pass effects.

Following the intravenous administration of ondansetron, the *Cl* range, 39.7–49.6 ml/min/kg, based on the plasma data (Table 1) was considerably smaller than the reported cardiac output of 295 ml/min/kg based on the blood data [20] and a hematocrit of approximately 45% [21] in rats. This suggests that the first-pass effects of ondansetron in the lung and heart could be almost negligible, if any, in rats.

Following the intraportal and intraduodenal administration of ondansetron at a dose of 8 mg/kg to rats, the *AUC* following its intraduodenal administration was significantly smaller (34.2% decrease) than that following its intraportal administration. Therefore, the intestinal extraction ratio of ondansetron could be 0.342 in rats. An intestinal first-pass effect of ondansetron could be expected based on the *in vitro* studies; an approximately 18.5% of the spiked amount of the drug disappeared after 30 min incubation

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with a homogenate of small intestine as mentioned earlier. Considering the large surface area of small intestine, the value of 0.342 could be expected. However, the *AUC* values of ondansetron following its intragastric and intraduodenal administration were comparable (not significantly different), suggesting that the gastric extraction ratio of ondansetron could be almost negligible, if any, in rats. This could also be expected based on the gastric homogenate studies. Thus, it could be concluded that approximately 0.620 (1.00–0.342–0.0407) of the orally administered ondansetron at a dose of 8 mg/kg could be absorbed into the portal vein.

Following the intraportal and intravenous administration of ondansetron at a dose of 8 mg/kg to rats, the *AUC* of ondansetron following its intraportal administration was significantly smaller (64.8% decrease) than that following its intravenous administration, suggesting that the hepatic extraction ratio of ondansetron after absorption into the portal vein is 0.648 in rats. The considerable hepatic first-pass effect of ondansetron could be expected based on the liver homogenate studies. Since approximately 0.620 of the oral dose of ondansetron at 8 mg/kg was absorbed into the portal vein, the 0.648 of the hepatic extraction ratio is equivalent to 0.400 of the oral dose of 8 mg/kg.

The hepatic microsomal cytochrome P450 (CYP) isozymes responsible for the metabolism of ondansetron were different in humans and rats. Saynor and Dixon [3] reported that ondansetron was metabolized to 6-,7-,8-hydroxyondansetron and their glucuronide and sulfate conjugates, and demethylondansetron following its intravenous and/or oral administration of [^{14}C]ondansetron to rats and humans. Based on the human CYP isozymes (CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1 and 3A4), the CYP1A1, 1A2 and 2D6 (major enzyme for the hydroxylation of ondansetron) and 3A4 interacted for the metabolism of ondansetron [22,23]. It has also been reported that, in humans, the hepatic CYP1A1/2 plays the most important role, whereas CYP2D6 plays a relatively minor role, and involvement of CYP3A seems important only at relatively high concentrations of ondansetron for the metabolism of ondansetron [24]. However, Yang *et al*. [25] reported that hepatic

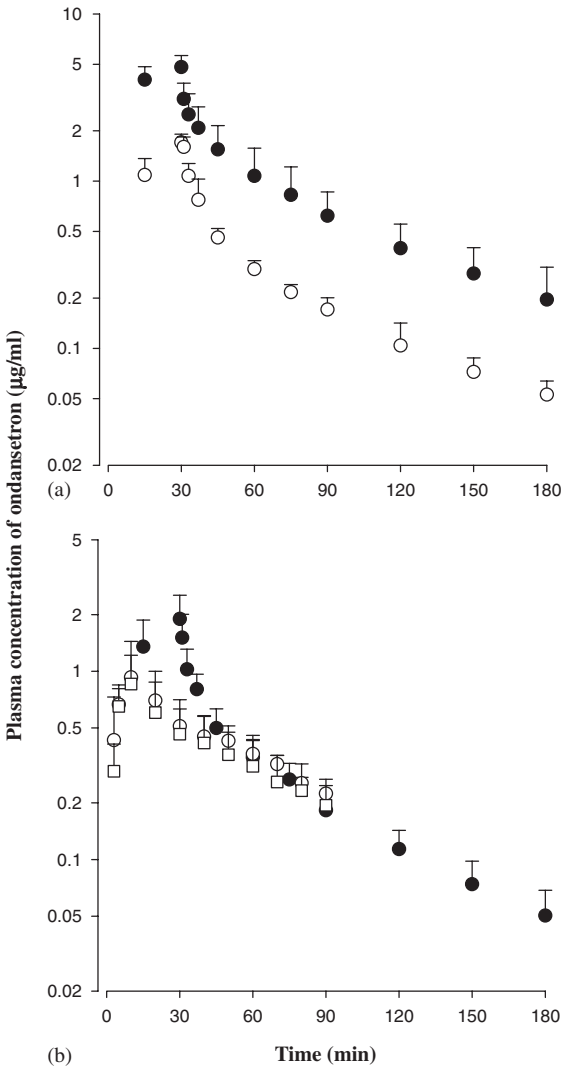

Figure 3. Mean arterial plasma concentration–time profiles of ondansetron after its intravenous (●; *n*=5) and intraportal (○; *n*=6) administration (a), and its intraportal (●; *n*=4), intragastric (○; *n*=3) and intraduodenal (□; *n*=5) administration (b) at a dose of 8 mg/kg to rats. Bars represent standard deviation

CYP2D and 3A1/2 (not CYP1A1/2, 2B1/2, 2C11 and 2E1) are responsible for metabolism of ondansetron in male Sprague–Dawley rats.

The Cl_r of ondansetron was estimated from the free (unbound to plasma proteins) fraction of the drug in plasma based on the Cl_r (Table 1) and rat plasma protein binding value of the drug. The values thus estimated were 2.33, 1.99, 1.97

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and 1.31 ml/min/kg for the intravenous doses of 1, 4, 8 and 20 mg/kg, respectively. The 1.31–2.33 ml/min/kg range was slower than the reported glomerular filtration rate of 5.24 ml/min/kg in rats [20]. The above data indicate that ondansetron is mainly reabsorbed in the renal tubules in rats.

In conclusion, after the oral administration of ondansetron at a dose of 8 mg/kg to rats, the unabsorbed fraction was 0.0158 of the dose, the *F* value was 0.0407, and the hepatic and intestinal extraction ratio were approximately 0.400 and 0.342 of the dose, respectively. The above data suggest that approximately 0.200 (1.00−0.0158−0.0407−0.400−0.342) of the oral dose of ondansetron disappeared in rat organs (tissues) except in the liver and intestine. The low *F* of ondansetron in rats was mainly due to considerable hepatic and intestinal first-pass effects. The lower *F* of ondansetron in rats (4.07%) than that in humans (62 ± 15%) [2] was mainly due to greater metabolism of ondansetron in rat liver (Figures 2 and 3).

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