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RESEARCH PAPER

Interaction between udenafil and tamsulosin in rats: non-competitive inhibition of tamsulosin metabolism by udenafil via hepatic CYP3A1/2

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Background and purpose: Orthostatic hypotension has been observed when PDE 5 (cGMP-specific phosphodiesterase type 5) inhibitors are co-administered with α-adrenoceptor antagonists. Here we assessed the pharmacokinetic and haemodynamic interactions between udenafil and tamsulosin in rats, as both drugs are metabolized via rat hepatic cytochrome P450 3A1/2.
Experimental approach: Interactions between the two drugs were evaluated in rats after simultaneous 1 or 15 min i.v. infusion or after p.o. administration of udenafil (30 mg·kg^{-1}) and/or tamsulosin (1 mg·kg^{-1}). *In vitro* metabolism of tamsulosin with udenafil was measured to obtain the inhibition constant (K_i) and [I]/K_i ratio of udenafil.
Key results: The total area under the plasma concentration–time curve from time zero to time infinity (AUC)s (or AUC_{0-4h}) of tamsulosin were significantly greater after 15 min of i.v. infusion or after oral administration with udenafil, compared with tamsulosin alone. The hepatic first-pass metabolism of tamsulosin was inhibited by udenafil, and the inhibition *in vitro* was in a non-competitive mode. The arterial systolic blood pressure was significantly lower at 5, 10 and 60 min after oral co-administration of the drugs.
Conclusions and implications: The significantly greater AUC of tamsulosin after i.v. and p.o. administration of both drugs may be attributable to non-competitive inhibition of cytochrome P450 3A1/2-mediated hepatic tamsulosin metabolism by udenafil. The inhibition was also observed in human liver S9 fractions, suggesting that a reassessment of the oral dosage of tamsulosin is necessary when udenafil and tamsulosin are co-administered to patients with benign prostatic hyperplasia.
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Keywords: pharmacokinetic interaction; udenafil; tamsulosin; hepatic first-pass effect; non-competitive inhibition; hepatic CYP3A1/2; rats

Abbreviations: Ae_{0-24h}, 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; CL_{int}, intrinsic clearance; CL_{NR}, time-averaged non-renal clearance; CL_R, time-averaged renal clearance; C_{max}, peak plasma concentration; CYP, hepatic cytochrome P450; GI_{24h}, percentage of the dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h; I, inhibitor; K_i, inhibition constant; LC-MS/MS, HPLC-tandem mass spectrometry; NADPH, reduced form of β-nicotinamide adenine dinucleotide phosphate; PDE 5, cGMP-specific phosphodiesterase type 5; T_{max}, time to reach C_{max}; V_{ss}, apparent volume of distribution at steady state.

Introduction

A new inhibitor of cGMP-specific phosphodiesterase type 5 (PDE 5), udenafil [3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-*N*-[(2-methyl-pyrrolidin-2-yl)ethyl]-4-propoxy-benzenesulphoamide], has been approved to treat male erectile dysfunction in South Korea. Udenafil (Zydena®) has a unique pharmacokinetic profile; the time to reach maximum plasma concentration is 1.0–1.5 h, and its half-life is 11–13 h after p.o. administration to humans,

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suggesting that the drug has a relatively rapid onset of action, a significantly longer duration than other PDE 5 inhibitors and possible effectiveness for up to 24 h.

Studies in human liver microsomes have shown that hepatic cytochrome P450 (CYP)3A4 is the major enzyme responsible for formation of DA-8164 (*N*-dealkylated udenafil) (Ji *et al.*, 2004), the major circulating active metabolite in humans. Furthermore, Kim *et al.* (2005a) reported that the metabolism of udenafil and formation of DA-8164 are primarily mediated via CYP3A1/2, and not via CYP1A1/2, 2B1/2, 2D1 or 2E1, in male Sprague–Dawley rats. Udenafil is a substrate for P-glycoprotein (Ji *et al.*, 2007) but not for organic cationic transporter 2 (Choi *et al.*, 2008). The pharmacological actions of udenafil have been described (Kim *et al.*, 2005b).

The concurrent use of PDE 5 inhibitors with α-adrenoreceptor antagonists has been reported to have several side effects. Tadalafil increases the hypotensive effect of doxazosin in patients with both hypertension and benign prostatic hyperplasia (BPH) (Kloner, 2005; Kloner *et al.*, 2004). Sildenafil given with doxazosin and vardenafil given with terazosin evoked orthostatic hypotension in some patients (Kloner *et al.*, 2004). Similarly, blood pressure falls when udenafil and terazosin are orally co-administered to rats (Oh *et al.*, 2007).

Tamsulosin is an extremely potent and highly selective α_{1A}-adrenoreceptor antagonist and is commonly used to treat urinary obstruction in patients with BPH. By itself, tamsulosin has several cardiovascular effects, including decreased systolic arterial pressure, reduced systemic vascular resistance via inhibition of α_1-adrenoceptor-mediated vasoconstriction and increased heart rate (Nieminen *et al.*, 2005a,b). Moreover, when vardenafil and tamsulosin were orally co-administered to healthy volunteers, standing systolic blood pressure dropped to <85 mmHg in two (12.5%) of 16 subjects (Kloner, 2005). Kamimura *et al.* (1998) reported that tamsulosin is metabolized via CYP3A4 and 2D6 based on studies in human liver microsomes. In our preliminary study, tamsulosin is metabolized via CYP3A1/2 and 2D subfamily based on studies in rat liver microsomes with chemical inhibitors of specific CYP. Although no studies have been reported, pharmacokinetic and pharmacodynamic interactions between udenafil and tamsulosin are suspected. Because BPH is highly prevalent in men over the age of 50 and is often associated with sexual dysfunction, concomitant use of tamsulosin and udenafil is anticipated. Therefore, it is important to assess the possible interactions between udenafil and tamsulosin. We studied the pharmacokinetic and haemodynamic interactions between udenafil and tamsulosin in rats after simultaneous i.v. or p.o. administration.

Methods

Animals

The protocols for the animal studies were approved by the Institute of Laboratory Animal Resources of Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats (7–9 weeks old, weighing 215–295 g) were purchased from Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea) and maintained in a clean room (Animal Centre for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of 20–23°C with 12 h light (07:00–19:00)/dark (19:00–07:00) cycle and a relative humidity of 50 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered pathogen-free air, with food (Samyang Company, Pyeongtaek, South Korea) and water available *ad libitum*.

In vitro *studies*

(a) Disappearance (primarily metabolism) of tamsulosin from S9 fractions of rat and human liver, in the presence and absence of udenafil. The procedures used were similar (Yang and Lee, 2008) to a reported method (Litterst *et al.*, 1975). Metabolic activity was initiated by adding 60 μL of distilled water containing a final tamsulosin concentration of 0.2 $\mu mol \cdot L^{-1}$ to an Eppendorf tube containing: 218 μL of 100 $mmol \cdot L^{-1}$ Tris-buffer (pH 7.4); 10 μL of 100 $mmol \cdot L^{-1}$ Tris-buffer (pH 7.4) containing 1 $mmol \cdot L^{-1}$ NADPH (reduced form of β-nicotinamide adenine dinucleotide phosphate); 12 μL of 0.05 $mol \cdot L^{-1}$ citric acid (pH 2.3) containing a final udenafil concentration of 0, 0.1 or 1 $\mu mol \cdot L^{-1}$; and 300 μL of rat or human liver S9 fraction (6.67 mg protein). The mixture was mixed at 600 r.p.m. in a thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany) at 37°C. At 0, 10, 20 and 30 min, a 100 μL aliquot was collected and added to an Eppendorf tube containing 25 μL of methanol containing 200 $ng \cdot mL^{-1}$ terazosin (internal standard) and 1 mL of ether : dichloromethane (70:30; v/v) to terminate the reaction.

(b) Measurement of inhibition constant (K_i) of udenafil and manner of inhibition of tamsulosin metabolism by udenafil in rat hepatic microsomes. The procedures used for the preparation of hepatic microsomes were similar to those previously reported (Oh *et al.*, 2007). The method used to investigate the mode of inhibition of tamsulosin metabolism by udenafil was similar to the method reported by Choi *et al.* (2008). The following components were added to a tube: hepatic microsomes (equivalent to 0.5 mg protein); 50 μL of distilled water containing 0.5, 1, 2 or 5 $\mu mol \cdot L^{-1}$ tamsulosin; 10 μL of 0.05 $mol \cdot L^{-1}$ citrate buffer (pH 2.3) containing udenafil (as an inhibitor) at a concentration of 0, 0.1, 0.2, 0.5, 1 or 1.5 $\mu mol \cdot L^{-1}$; and 50 μL of 0.1 $mol \cdot L^{-1}$ phosphate buffer (pH 7.4) containing 1 $mmol \cdot L^{-1}$ NADPH. The volume was adjusted to 0.5 mL by adding 0.1 $mol \cdot L^{-1}$ phosphate buffer (pH 7.4), and the components were mixed at 37°C by using a thermomixer at 600 r.p.m. All of the microsomal incubation conditions were within the linear range of the reaction rate. After 5 min incubation, the reaction was terminated by the addition of 1 mL of ether : dichloromethane (70:30; v/v).

The apparent K_i of udenafil for the disappearance of tamsulosin in hepatic microsomes was calculated by using Dixon (1953) plot analysis.

In vivo *studies*

(a) Studies of i.v. and p.o. drug administration. There were four experimental groups:

(1) Udenafil 30 $mg \cdot kg^{-1}$, i.v. ± tamsulosin 1 $mg \cdot kg^{-1}$, i.v. (1 min infusion)

(2) Udenafil 30 mg·kg^{-1}, i.v. ± tamsulosin 1 mg·kg^{-1}, i.v. (15 min infusion)
(3) Udenafil 30 mg·kg^{-1}, p.o. ± tamsulosin 1 mg·kg^{-1}, p.o. (single dose in normal rats)
(4) Udenafil 30 mg·kg^{-1}, p.o. ± tamsulosin 1 mg·kg^{-1}, p.o. (single dose after 7 day tamsulosin dosing to normal rats)

The methods used for the preparation of the rats, including cannulation of the carotid artery for blood sampling and the jugular vein for i.v. drug administration, were similar to a previously reported method (Choi *et al.*, 2008).

Udenafil [dissolved in 0.05 mol·L^{-1} citric acid (pH 2.3)] at 30 mg·kg^{-1} (n = 8), tamsulosin (also in 0.05 mol·L^{-1} citric acid) at 1 mg·kg^{-1} (n = 7 and 9 for 1 and 15 min infusions respectively) or both drugs (n = 8 and 9 for 1 and 15 min infusions respectively) were infused for 1 or 15 min via the jugular vein. The total i.v. volume of udenafil, tamsulosin or both drugs was 2 mL·kg^{-1}. A blood sample (~0.22 mL) was collected via the carotid artery at 0 (control), 1 (end of the infusion), 5, 15, 30, 60, 90, 120, 180, 240, 360, 480 and 600 min for the 1 min infusion, and at 0, 7.5, 15 (end of the infusion), 20, 30, 45, 60, 75, 105, 135, 195 and 255 min for the 15 min infusion, after the start of the i.v. drug infusions. The blood samples were immediately centrifuged, and 100 µL aliquots of the plasma samples were stored at –70°C (Revco ULT 1490 D-N-S; Western Mednics, Ashville, NC, USA) until the HPLC-tandem mass spectrometry (LC-MS/MS) analysis of udenafil and tamsulosin. The procedures for the preparation and handling of the 24 h urine ($Ae_{0-24\,h}$) samples and the samples from the gastrointestinal tract (including its contents and faeces) at 24 h ($GI_{24\,h}$) were similar to a reported method (Oh *et al.*, 2007).

Udenafil at 30 mg·kg^{-1} (n = 7), tamsulosin at 1 mg·kg^{-1} (n = 7) or both drugs (n = 8) were administered p.o. to rats by using a feeding tube. The total p.o. volume of udenafil, tamsulosin or both drugs was 3 mL·kg^{-1}. In a similar experiment, the same dose of tamsulosin was administered p.o. daily for 7 days, and then the same doses of udenafil (n = 8), tamsulosin (n = 8) or both drugs (n = 8) were administered on day 8. For both studies, a blood sample (~0.22 mL) was collected via the carotid artery at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600 and 720 min after p.o. administration of the drug(s). Other procedures were similar to those used in the i.v. study.

(b) Measurement of liver concentrations of udenafil after 1 or 15 min i.v. infusion or p.o. administration of both udenafil and tamsulosin. Both udenafil (30 mg·kg^{-1}) and tamsulosin (1 mg·kg^{-1}) were administered, and samples were taken at the indicated times. As much blood as possible was collected via the carotid artery, and the rats were killed by cervical dislocation (n = 3 at each time point for each route of administration). Approximately 1 g of liver tissue was excised and blotted on tissue paper. The liver samples were homogenized with four volumes of 0.9% NaCl injectable solution and centrifuged at 9000× g for 10 min. Two 100 µL aliquots of the supernatant and plasma samples were collected and stored at –70°C until LC-MS/MS analysis.

(c) Measurement of the hepatic first-pass effect of tamsulosin in rats. The procedures used for the cannulation of the carotid artery, jugular vein and vein from the caecum were similar to previously reported methods (Murakami *et al.*, 2003, Choi *et al.*, 2006). Tamsulosin (1 mg·kg^{-1}) without or with udenafil (30 mg·kg^{-1}) was infused (2 mL·kg^{-1}) for 15 min into the jugular and portal veins for i.v. (n = 8 without and n = 7 with udenafil) and intraportal (n = 8 for both groups) administration respectively, with the assistance of an infusion pump (Model 2400-006; Harvard Instrument, South Natick, MA, USA). At the same time, an equal volume of 0.05 mol·L^{-1} citric acid (pH 2.3) was also infused for 15 min into the portal and jugular veins for i.v. and intraportal administration respectively. Blood samples were collected via the carotid artery at the same time points as in 15 min i.v. infusion.

(d) Pharmacodynamic (blood pressure) changes in rats after p.o. administration of udenafil, tamsulosin, both drugs or 0.05 mol·L^{-1} citric acid (control). The carotid artery was cannulated with a polyethylene tube (Choi *et al.*, 2008) in order to monitor the arterial systolic blood pressure without blood sampling, for up to 12 h after p.o. administration of udenafil, tamsulosin, both drugs or 0.05 mol·L^{-1} citric acid (control rats; n = 4 each). Arterial systolic blood pressure readings were recorded by using a pressure transducer and a bridge amplifier connected online to the artery and to a PowerLab system (Version 5; ADI Instruments; Pty Ltd., Castle Hill, NSW, Australia).

LC-MS/MS analysis of udenafil and tamsulosin

The concentrations of udenafil and tamsulosin in the samples were simultaneously determined by a modification of a reported LC-MS/MS method for the analysis of tamsulosin (Ramakrishna *et al.*, 2005); 1 mol·L^{-1} sodium hydroxide solution was not added before the extraction procedures, and a different internal standard and mobile phase composition were used. In brief, 1 mL of ether : dichloromethane (70 : 30; v/v) and 25 µL of methanol containing 200 ng·mL^{-1} of terazosin (internal standard) were added to 100 µL of each sample. After mixing and centrifugation (16 000× g, 8 min), the organic layer was collected and dried (Dry Thermobath; Eyela, Tokyo, Japan) under a gentle stream of nitrogen gas at 50°C. Then, 100 µL of the mobile phase was added to reconstitute the residue, and 20 µL was injected directly onto a reversed-phase HPLC column (Symmetry Shield™ RP_8; l.50 × 2.1 mm i.d.; particle size, 3.5 µm; Waters Corporation, Milford, MA, USA). The mobile phase, 0.1% formic acid : methanol : acetonitrile (20 : 35 : 45, v/v/v), was passed through the column at a flow rate of 0.37 mL·min^{-1}. The eluent was monitored by using a 1200 L quadrupole tandem mass spectrometer (Varian, Palo Alto, CA, USA) equipped with an electrospray ionization (ESI) source, using a turbo ion spray interface in the positive ion (ESI$^+$) mode, with multiple reaction monitoring. All instrumentation and data processing were managed by using Varian software 6.5. The precursor to product ion transitions for udenafil, tamsulosin and terazosin (internal standard) were 517.7 → 283.0, 409.3 → 228.0 and 388.2 → 290.0 respectively. The retention times of udenafil, tamsulosin and terazosin were approximately 0.290, 0.295 and 0.285 min respectively. The detection limit of udenafil in the rat plasma and urine samples was 0.02 µg·mL^{-1}. The detection limit of tamsulosin was 0.5 ng·mL^{-1}.

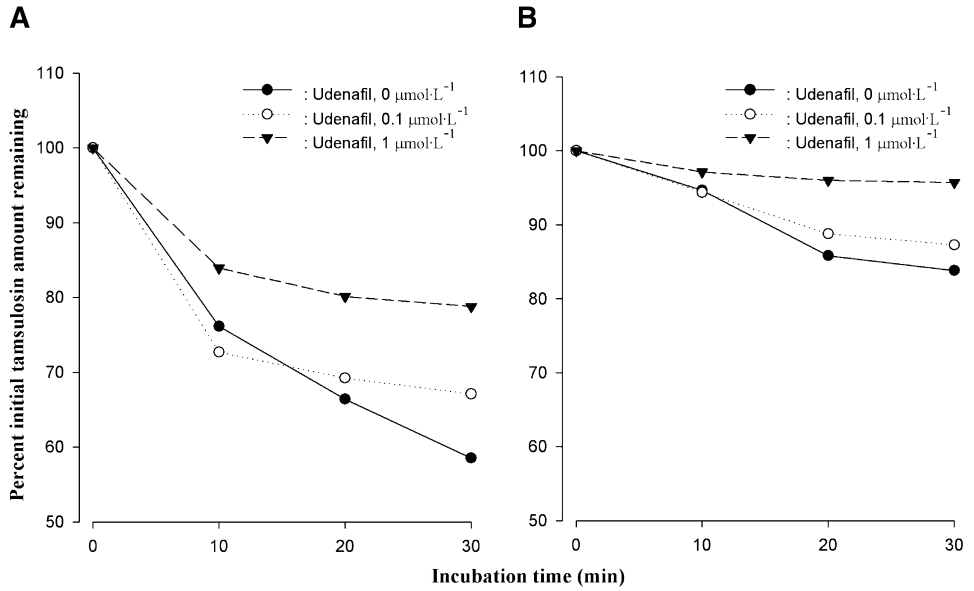

Figure 1 Disappearance (shown as % remaining) of tamsulosin in the liver S9 fractions of the rat (A) and human (B) after 0, 10, 20 and 30 min incubation with 0, 0.1 and 1 μmol·L^{-1} of udenafil.

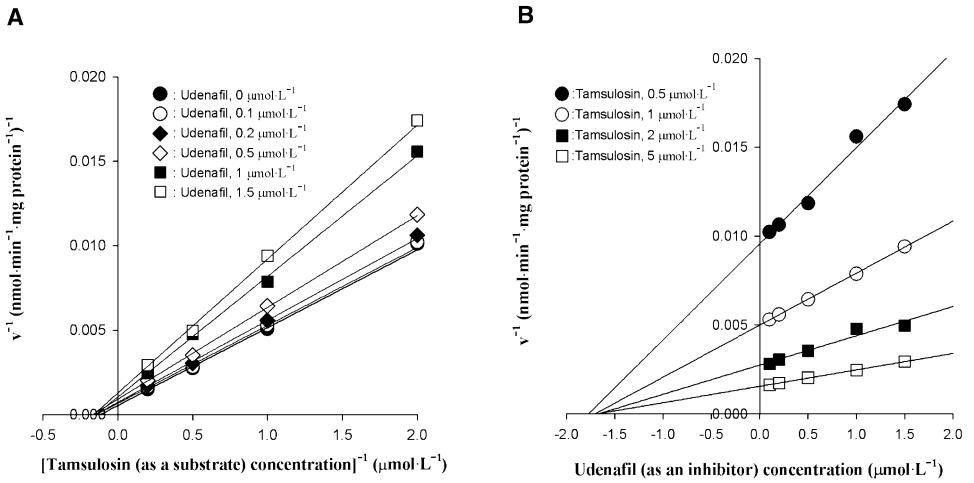

Figure 2 Lineweaver and Burke (A) and Dixon (B) plots showing inhibition of the disappearance of tamsulosin by udenafil.

Pharmacokinetic analysis

Using methods similar to previously reported ones (Gibaldi and Perrier, 1982), we calculated the total area under the plasma concentration–time curve from time zero to time infinity (AUC) or up to the last measured time t in the plasma ($AUC_{0\text{-}t}$) (Chiou, 1978); the time-averaged total body, renal and non-renal clearances (CL, CL_R, and CL_{NR} respectively); 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 directly read from the experimental data.

Statistical analysis

A P-value < 0.05 was considered statistically significant for the unpaired t-test or Duncan's multiple range test. The Statistical Package for the Social Sciences (SPSS) ANOVA programme was used to calculate P-values among the four means for unpaired data. All results, except the median (range) for T_{max}, are expressed as means ± SD

Chemicals

Udenafil and tamsulosin hydrochloride were obtained from Dong-A Pharmaceutical Company, Ltd. and Hetero Drug, Ltd. (Hyderabad, India) respectively. Terazosin (internal standard for LC-MS/MS analysis of udenafil and tamsulosin), the NADPH (tetrasodium salt), Tris-buffer, EDTA disodium salt were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). The liver S9 (9000× g supernatant) fractions from rat and human were purchased from XenoTech (Lenexa, KS, USA). Other chemicals were of reagent or HPLC grade.

Results

In vitro *studies*

(a) Disappearance of tamsulosin from the liver S9 fractions of rat and human in the presence and absence of udenafil. This experiment was performed in human and rat liver S9 fractions to determine whether udenafil can inhibit the metabolism of tamsulosin *in vitro*. The percentages of tamsulosin remaining in the liver S9 fractions of the rat and human are shown in Figure 1. The amount of tamsulosin that disappeared from the liver S9 fractions decreased as the concentrations of udenafil increased.

(b) Non-competitive inhibition of tamsulosin metabolism by udenafil in rat hepatic microsomes. To investigate the kinetics of the inhibitory effects of udenafil on tamsulosin metabolism, tamsulosin disappearance in rat hepatic microsomes was examined in the absence and presence of various concentrations of udenafil. The Lineweaver and Burke (1934) and Dixon (1953) plots for the disappearance of tamsulosin are shown in Figure 2. The Lineweaver and Burke plots (Figure 2A) revealed a linear relationship between the inverse of the substrate (tamsulosin) concentration and the inverse of tamsulosin disappearance, indicating non-competitive inhibition of tamsulosin metabolism by udenafil. The Dixon plots (Figure 2B) for the various substrate concentrations are linear and converge on the *x*-axis, which further suggests non-competitive inhibition of tamsulosin metabolism by udenafil. From these data, the K_i value was approximately 1.7 μmol·L^{-1}.

In vivo *studies*

(a) Pharmacokinetics of udenafil and tamsulosin after i.v. and p.o. drug administration. The relevant pharmacokinetic parameters of udenafil in rats after a 1 min i.v. infusion of udenafil alone or with co-infusion of tamsulosin (Table 1) and after a single p.o. administration of udenafil alone or with simultaneous p.o. administration of tamsulosin (Table 2) did not change significantly with the co-administration of tamsulosin. To investigate the effect of multiple p.o. administrations of tamsulosin on the pharmacokinetics of orally administered udenafil, tamsulosin was first administered orally to the rats for 7 days. The relevant pharmacokinetic parameters of udenafil administered after tamsulosin pretreatment (Table 2) did not change significantly when tamsulosin was co-administered with udenafil.

The relevant pharmacokinetic parameters of tamsulosin in rats after a 1 or 15 min i.v. infusion of tamsulosin alone or with co-infusion of udenafil are listed in Table 1. For the 1 min infusion, the pharmacokinetic parameters of tamsulosin were comparable between with and without udenafil, except for a significantly larger V_{ss} (132% increase) with udenafil co-infusion. For the 15 min infusion (Figure 3), in the presence of udenafil, the AUC was significantly greater (191% increase); the CL, CL_R and CL_{NR} were significantly slower (66.1, 75.2 and 65.1% decrease respectively); and both the V_{ss} (37.5% decrease) and the percentage of the i.v. dose of tamsulosin excreted as unchanged drug in the 24 h urine ($Ae_{0\text{–}24h}$) (28.6% decrease) were significantly smaller in comparison with the respective values for tamsulosin alone.

Table 1 Pharmacokinetic parameters of udenafil after a 1 min i.v. infusion of udenafil (30 mg·kg^{-1}) alone and with co-infusion of tamsulosin (1 mg·kg^{-1}) and of tamsulosin after a 1 or 15 min i.v. infusion of tamsulosin (1 mg·kg^{-1}) alone and with co-infusion of udenafil (30 mg·kg^{-1}) to rats

Parameter	*Udenafil*		*Tamsulosin*			
	1 min infusion		*1 min infusion*		*15 min infusion*	
	Without tamsulosin (n = 8)	*With tamsulosin* (n = 8)	*Without udenafil* (n = 7)	*With udenafil* (n = 8)	*Without udenafil* (n = 9)	*With udenafil* (n = 9)
AUC (μg·min·mL^{-1})	561 ± 88.6	529 ± 97.5	33.8 ± 6.03	32.6 ± 6.52	10.6 ± 2.56†	30.8 ± 6.35***
CL (mL·min^{-1}·kg^{-1})	54.4 ± 8.67	58.6 ± 11.6	30.4 ± 5.27	31.8 ± 6.51	99.8 ± 24.0†	33.8 ± 7.23***
CL_R (mL·min^{-1}·kg^{-1})	2.13 ± 0.698	2.73 ± 1.17	1.32 ± 0.412	1.47 ± 0.571	9.89 ± 3.67†	2.45 ± 1.10***,‡
CL_{NR} (mL·min^{-1}·kg^{-1})	52.2 ± 8.17	55.9 ± 10.7	29.1 ± 5.11	30.4 ± 6.20	89.9 ± 21.6†	31.4 ± 6.39***
V_{ss} (mL·kg^{-1})	4220 ± 1510	6130 ± 3690	509 ± 239	1180 ± 420**	3310 ± 984†	2070 ± 277**,‡‡
$Ae_{0\text{–}24h}$ (% of dose)	3.83 ± 1.04	4.53 ± 1.35	4.36 ± 1.33	4.64 ± 1.29	9.92 ± 2.29†	7.08 ± 2.17*,‡
GI_{24h} (% of dose)	0.306 ± 0.241	0.333 ± 0.127	0.0949 ± 0.0784	0.0585 ± 0.0342	0.566 ± 0.809	0.243 ± 0.200‡

Data are expressed as mean ± SD.
$Ae_{0\text{–}24h}$, 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; CL_{NR}, time-averaged non-renal clearance; CL_R, time-averaged renal clearance; GI_{24h}, percentage of the dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h; V_{ss}, apparent volume of distribution at steady state.
Significantly different from without udenafil, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.
Significantly different from 1 min i.v. infusion without udenafil, $^{\dagger}P < 0.001$.
Significantly different from 1 min i.v. infusion with udenafil, $^{\ddagger}P < 0.05$ and $^{\ddagger\ddagger}P < 0.001$.

Table 2 Pharmacokinetic parameters of udenafil and tamsulosin after a single p.o. administration of udenafil (30 mg·kg^{-1}), tamsulosin (1 mg·kg^{-1}), or both drugs to normal rats and to rats pretreated with daily p.o. administration of tamsulosin (1 mg·kg^{-1}) for 7 days

Parameter	*Udenafil*		*Parameter*	*Tamsulosin*	
	Without tamsulosin	*With tamsulosin*		*Without udenafil*	*With udenafil*
In normal rats	$n = 7$	$n = 8$	In normal rats	$n = 7$	$n = 8$
AUC (μg·min·mL^{-1})	146 ± 32.5	133 ± 19.6	AUC_{0-4h} (μg·min·mL^{-1})	2.39 ± 0.486	11.2 ± 4.25***
C_{max} (μg·mL^{-1})	0.482 ± 0.239	0.488 ± 0.180	C_{max} (μg·mL^{-1})	0.0312 ± 0.0139	0.145 ± 0.0424***
T_{max}[a] (min)	30 (15–120)	30 (15–45)	T_{max} (min)	15 (5–180)	22.5 (15–45)
CL_R (mL·min^{-1}·kg^{-1})	2.05 ± 1.34	2.90 ± 1.23	Ae_{0-24h} (% of tamsulosin dose)	0.873 ± 0.255	1.82 ± 0.489***
Ae_{0-24h} (% of udenafil dose)	1.02 ± 0.743	1.25 ± 0.466	GI_{24h} (% of tamsulosin dose)	0.632 ± 0.427	0.497 ± 1.00
GI_{24h} (% of udenafil dose)	3.68 ± 3.97	1.41 ± 1.11			
In rats pretreated with tamsulosin	$n = 8$	$n = 8$	In rats pretreated with tamsulosin	$n = 8$	$n = 8$
AUC_{0-12h} (μg·min·mL^{-1})	114 ± 22.0	133 ± 23.1	AUC_{0-4h} (μg·min·mL^{-1})	5.75 ± 1.46	27.2 ± 7.39***
C_{max} (μg·mL^{-1})	0.593 ± 0.364	0.629 ± 0.227	C_{max} (μg·mL^{-1})	0.103 ± 0.0846	0.348 ± 0.106***
T_{max}[a] (min)	30 (15–480)	52.5 (15–180)	T_{max} (min)	22.5 (5–60)	52.5 (15–90)
Ae_{0-24h} (% of udenafil dose)	1.22 ± 0.335	1.48 ± 0.908	Ae_{0-24h} (% of tamsulosin dose)	1.35 ± 0.370	4.46 ± 2.11**
GI_{24h} (% of udenafil dose)	2.48 ± 1.75	1.12 ± 0.795	GI_{24h} (% of tamsulosin dose)	0.325 ± 0.372	0.467 ± 0.411

Data are expressed as mean ± SD except for T_{max} values [a] that are median (ranges).
Ae_{0-24h}, 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_R, time-averaged renal clearance; C_{max}, peak plasma concentration; GI_{24h}, percentage of the dose recovered from the gastrointestinal tract (including its contents and faeces) at 24 h; T_{max}, time to reach C_{max}.
Significantly different from without udenafil, $^{**}P < 0.01$ and $^{***}P < 0.001$.

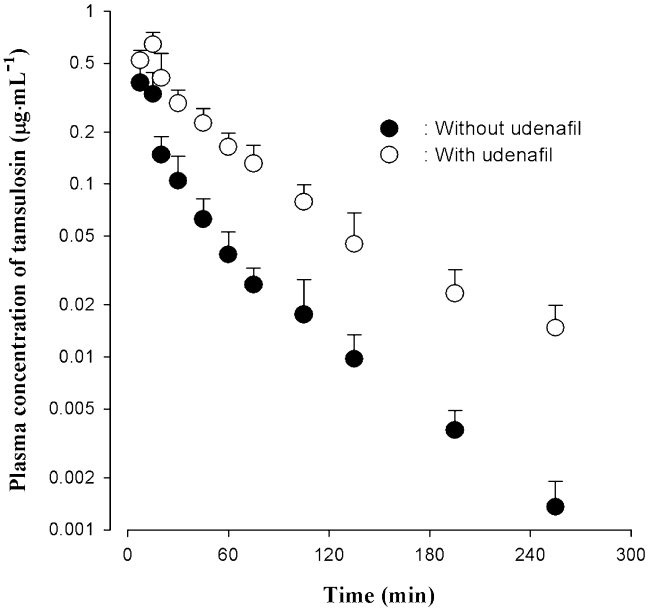

Figure 3 Mean arterial plasma concentration–time profiles of tamsulosin after a 15 min i.v. infusion of tamsulosin at a dose of 1 mg·kg^{-1} with or without co-infusion of udenafil at a dose of 30 mg·kg^{-1} to rats ($n = 9$ for both the without and with simultaneous infusion of udenafil). Data are presented as mean ± SD.

Figure 4 shows the mean arterial plasma concentration–time profiles of tamsulosin for a single p.o. administration of tamsulosin alone or with udenafil in normal rats and in rats pretreated with tamsulosin daily (for 7 days p.o.), and Table 2 presents the relevant pharmacokinetic parameters. With udenafil co-administration, the AUC_{0-4h}, C_{max} and Ae_{0-24h} for tamsulosin were significantly increased, by 369%, 365% and 108% respectively (in normal rats) and by 373%, 238% and 230% respectively (in rats pretreated with tamsulosin daily for 7 days) compared with the values for tamsulosin alone.

(b) Liver concentrations and [I]/K_i ratios of udenafil after i.v. infusion (1 or 15 min) or p.o. administration of both udenafil and tamsulosin. These experiments examined whether the inhibitory effect of udenafil on tamsulosin metabolism observed *in vitro* (K_i values) is consistent with the *in vivo* liver concentrations of udenafil. After 1 and 15 min i.v. infusions and p.o. administration of both udenafil and tamsulosin, the drug concentrations and the ratios of udenafil in the liver to udenafil in the plasma were measured (Table 3). Rat liver showed a high affinity for udenafil, with liver-to-plasma ratios greater than unity. The [I]/K_i ratios of udenafil for the inhibition of tamsulosin metabolism (Table 3) were maintained above 2 for up to 4 h after the 1 and 15 min i.v. infusions and after p.o. administration.

(c) Hepatic first-pass effect of tamsulosin. Tamsulosin, either alone or with udenafil, was administered i.v. or intraportally in rats to determine the hepatic first-pass effect of tamsulosin and the effect of udenafil on the hepatic clearance of tamsulosin. In the absence of udenafil, the AUC of tamsulosin was significantly smaller (75.4% decrease) following intraportal administration (AUC = 5.60 ± 2.51 μg·min·mL^{-1}) than after i.v. administration of tamsulosin (AUC = 22.8 ± 4.72 μg·min·mL^{-1}; $P < 0.001$). However, in the presence of udenafil, the AUC of tamsulosin was not significantly different between the routes of administration (36.2 ± 13.1 vs. 43.0 ± 15.7 μg·min·mL^{-1} for i.v. vs. intraportal administration respectively; $P > 0.05$). The AUC of tamsulosin was significantly greater with udenafil than without udenafil for both the i.v. infusion (58.8%; $P < 0.05$) and intraportal administration (668%; $P < 0.001$) of tamsulosin.

(d) Pharmacodynamic (blood pressure) changes in rats after p.o. administration of udenafil, tamsulosin, both drugs or 0.05 mol·L^{-1} citric acid (control). Table 4 shows the changes in arterial systolic blood pressure in rats after a single p.o. administration of udenafil, tamsulosin, both drugs or 0.05 mol·L^{-1} citric acid

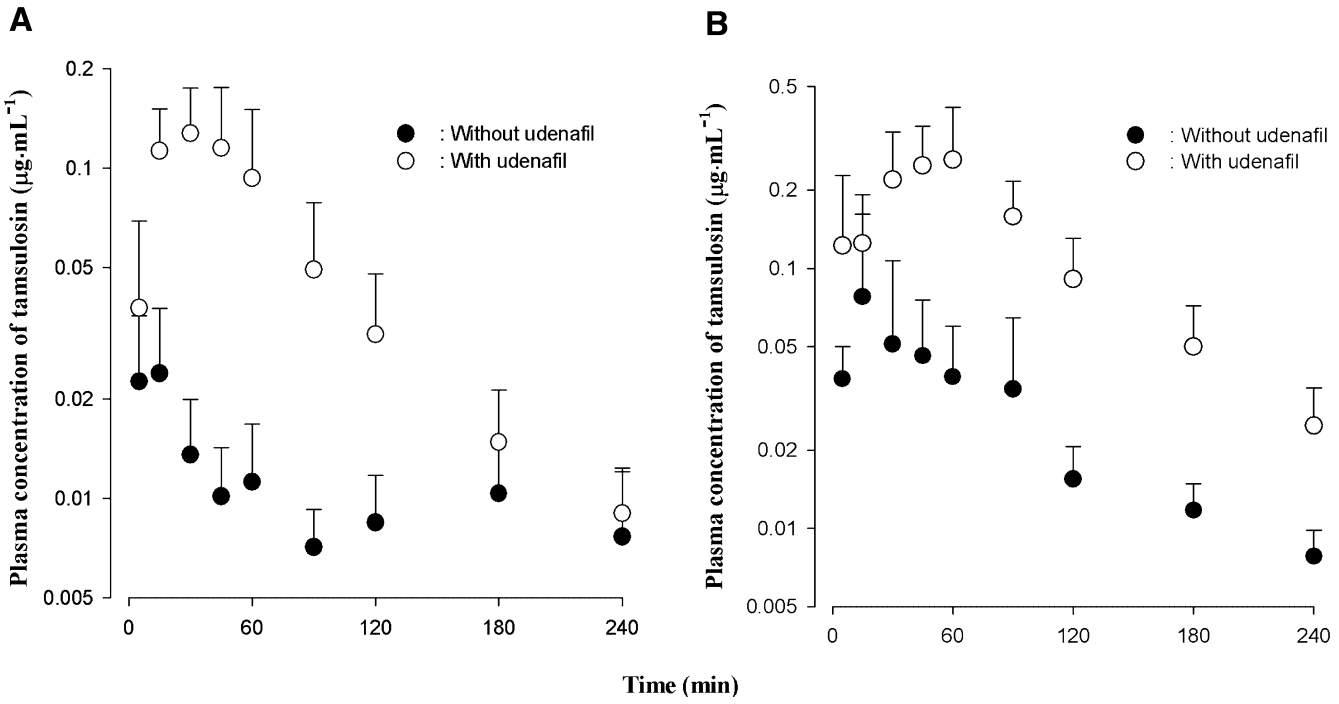

Figure 4 Mean arterial plasma concentration–time profiles of tamsulosin after a single p.o. administration of tamsulosin at a dose of 1 mg·kg^{-1} with or without simultaneous p.o. administration of udenafil at a dose of 30 mg·kg^{-1} to rats (n = 8 and 7 for with and without udenafil, respectively; A) and to rats pretreated with daily p.o. administration of tamsulosin at a dose of 1 mg·kg^{-1} for 7 days (n = 8 for both with and without udenafil; B). Data are presented as mean ± SD.

(control). Arterial systolic blood pressure did not differ significantly between rats treated with udenafil and those treated with tamsulosin alone, whereas simultaneous p.o. administration of tamsulosin and udenafil significantly lowered arterial systolic blood pressure as compared with the values in rats treated with each drug alone at 5, 10 and 60 min after drug co-administration.

Discussion

The doses of udenafil (30 mg·kg^{-1}) and tamsulosin (1 mg·kg^{-1}) chosen for this study were based on the results of previous studies. Although the absolute dosages of the drugs used in rats in the present study differ from the dosages used clinically in humans (udenafil 100 mg and tamsulosin 0.4 mg), the plasma concentration–time profiles and AUC values for the two drugs at these doses are comparable between humans and rats (Kim *et al.*, 2008 for udenafil; van Hoogdalem *et al.* 1997 for tamsulosin).

As the CL_{NR} and AUC for udenafil were comparable after i.v. infusion with and without tamsulosin (Table 1), Udenafil metabolism was not inhibited by tamsulosin. Udenafil has a low hepatic extraction ratio in rats, with a hepatic first-pass effect of 23% (Shim *et al.*, 2003); thus, its hepatic clearance depends more on the hepatic CL_{int} (intrinsic clearance) than on the hepatic blood flow rate. In the present study, the CL_{int} values for the disappearance of udenafil from hepatic microsomes in the absence and presence of tamsulosin were comparable (data not shown). Furthermore, in a preliminary study by using equilibrium dialysis (Shim *et al.*, 2000), the percentages of udenafil (at 0.5 μg·mL^{-1}) that bound to plasma proteins of the rat were 70.3 ± 7.98% and 63.5 ± 4.90% with and without tamsulosin (at 0.1 μg·mL^{-1}) respectively, demonstrating that the amount of udenafil bound to plasma proteins was not affected by tamsulosin treatment.

In the 15 min i.v. infusion protocol, the AUC and CL_{NR} of tamsulosin were significantly greater and slower respectively, with udenafil co-infusion than without udenafil (Table 1), suggesting that udenafil inhibited CYP3A1/2-mediated tamsulosin metabolism. The significantly slower CL_{NR} of tamsulosin with udenafil treatment may have several explanations. Tamsulosin could almost be considered as having an intermediate hepatic extraction ratio in rats; its hepatic clearance depends on the hepatic CL_{int} for the disappearance of the drug, the free (unbound) fraction of the drug in plasma and the flow rate of hepatic blood. The CL_{int} for the disappearance of tamsulosin in rat hepatic microsomes was significantly slower (62.4% decrease) in the presence of udenafil (data not shown), and tamsulosin metabolism was inhibited by udenafil in a non-competitive mode (Figure 2). Moreover, the $[I]/K_i$ ratios (>2) of udenafil for the inhibition of tamsulosin metabolism in the liver with i.v. infusion of both drugs for 15 min (Table 3) indicate that udenafil indeed inhibits CYP-mediated tamsulosin metabolism (Bachmann and Lewis, 2005). The free fraction of tamsulosin in plasma was comparable with and without udenafil, as reflected in the plasma protein binding values of tamsulosin: 47.0 ± 9.62% and 45.5 ± 7.93% without and with udenafil respectively. Udenafil had no effect on the hepatic blood flow rate.

The results from the measurement of hepatic first-pass effect of tamsulosin suggested that the hepatic first-pass effect

Table 3 Concentrations (μmol·L^{-1}) of udenafil in the liver and [udenafil; I]/K_i (inhibition constant) ratios for the inhibition of tamsulosin metabolism after simultaneous single i.v. infusion (for 1 and 15 min) and p.o. administration of udenafil (30 mg·kg^{-1}) and tamsulosin (1 mg·kg^{-1}) to rats

Time (min)	*1 min i.v. infusion*		*Time (min)*	*15 min i.v. infusion*		*Time (min)*	*p.o.*	
	Liver concentration (μmol·L^{-1})	*[I]/Ki*		*Liver concentration (μmol·L^{-1})*	*[I]/Ki*		*Liver concentration (μmol·L^{-1})*	*[I]/Ki*
1	92.5 ± 8.14 (2.10 ± 1.03)	49.1–58.2				5	32.1 ± 13.3 (288 ± 156)	12.2–27.5
15	67.1 ± 3.86 (15.7 ± 1.75)	37.9–42.0	15	109 ± 68.7 (6.73 ± 1.78)	35.8–110	15	54.3 ± 2.29 (138 ± 91.4)	30.6–33.2
30	35.8 ± 13.6 (12.9 ± 5.78)	13.9–29.6	45	58.1 ± 22.4 (17.6 ± 2.34)	19.6–45.3	30	86.3 ± 34.1 (163 ± 106)	33.2–72.6
60	23.4 ± 11.7 (12.1 ± 6.00)	8.78–21.6	75	52.3 ± 24.3 (17.8 ± 5.53)	17.0–45.5	60	35.2 ± 16.4 (51.1 ± 14.7)	10.9–30.2
120	5.52 ± 2.10 (5.72 ± 2.50)	1.99–4.46	135	13.0 ± 4.46 (9.83 ± 1.69)	6.08–10.7	120	10.0 ± 5.46 (28.6 ± 8.83)	2.44–8.79
240	7.53 ± 1.61 (13.3 ± 2.97)	3.34–5.13	255	9.86 ± 8.49 (16.0 ± 11.3)	2.68–11.6	240	6.16 ± 2.56 (21.8 ± 12.5)	2.54–5.34

Data are expressed as mean ± SD (concentrations and liver/plasma ratios) or range ([I]/*Ki*) (n = 3, each). The values in parentheses represent the liver/plasma ratios.

of tamsulosin after absorption into the portal vein was 75.4% without udenafil; however, that was almost negligible in rats treated with udenafil. Collectively, these data suggest that the hepatic first-pass metabolism of tamsulosin was inhibited by udenafil.

On the other hand, the CL_{NR} of tamsulosin in the 1 min infusion protocol was comparable with and without a 1 min infusion of udenafil (Table 1). These data suggest that udenafil minimally inhibited tamsulosin metabolism after infusion of the drugs for 1 min. The CL_{NR} of tamsulosin without udenafil was significantly greater (209% increase) in the 15 min infusion protocol than in the 1 min infusion protocol (Table 1). This might have resulted from the saturation of the hepatic first-pass metabolism of tamsulosin. The plasma concentration of tamsulosin without udenafil was lower after the 15 min infusion than after the 1 min infusion. Thus, less tamsulosin might have entered the liver during the 15 min infusion than during the 1 min infusion. The preceding data indicate infusion time-dependent pharmacokinetics of tamsulosin in rats.

After p.o. administration, the AUC of udenafil was not significantly different between with and without tamsulosin (Table 2). This is consistent with the negligible inhibition of hepatic metabolism of udenafil by tamsulosin. The intestinal metabolism of udenafil was almost negligible; only 7.70 and 5.60%, respectively, of the udenafil disappeared after a 30 min incubation of 1 μg of udenafil with the S9 fractions of the small and large intestines from male Sprague–Dawley rats (Shim *et al.*, 2003).

However, the AUC_{0-4h} of tamsulosin was significantly greater after p.o. administration of both drugs, compared with treatment without udenafil (Table 2). This is probably not due to the result of a udenafil-induced increase in gastrointestinal absorption of tamsulosin (almost negligible GI_{24h}; Table 2), decrease in biliary excretion of tamsulosin (almost negligible; Soeishi *et al.*, 1996), decrease in metabolism of tamsulosin in rat kidney and plasma [tamsulosin was shown to be minimally metabolized in the kidney and plasma in male Fisher rats (Soeishi *et al.*, 1996)] or inhibition of gastrointestinal tamsulosin metabolism [tamsulosin was minimally metabolized in the S9 fractions of rat small and large intestines (Soeishi *et al.*, 1996)]. The greater AUC_{0-4h} of tamsulosin in the presence of udenafil appears to be due to the inhibition of CYP3A1/2, which mediates the hepatic metabolism of tamsulosin. The [I]/K_i ratios (>2) of udenafil after p.o. administration (Table 3) further indicate that udenafil inhibits CYP-mediated metabolism of tamsulosin in the liver (Bachmann and Lewis, 2005).

With p.o. administration, the increases in AUC_{0-4h} of tamsulosin co-administered with udenafil [369% and 373% (Table 2) increases compared with no udenafil] were considerably greater than the 191% increase after i.v. administration of the drugs (Table 1). This might have occurred due to a greater inhibition of hepatic metabolism of tamsulosin by udenafil after p.o. administration. The plasma concentrations of tamsulosin after p.o. administration of both drugs (Figure 4) were considerably lower than those after i.v. infusion of both drugs for 1 or 15 min (Figure 3). Thus, hepatic tamsulosin metabolism appeared to be inhibited to a greater extent by orally administered udenafil compared with i.v.

Table 4 Mean arterial systolic blood pressure (mmHg) in rats after a single p.o. administration of udenafil (30 mg·kg^{-1}), tamsulosin (1 mg·kg^{-1}), both drugs or 0.05 mol·L^{-1} citric acid (control)

Time (min)	*Control*	*Udenafil*	*Tamsulosin*	*Udenafil and tamsulosin*
0	113 ± 10.9	108 ± 10.7	110 ± 10.3	107 ± 4.33
5	116 ± 15.9	107 ± 10.7	108 ± 10.5	89.4 ± 4.73*
10	113 ± 15.1	106 ± 12.9	107 ± 9.05	81.1 ± 7.69*
15	114 ± 17.1	106 ± 11.4	98.6 ± 6.42	81.6 ± 4.72
30	111 ± 14.0	100 ± 9.34	99.3 ± 6.04	85.3 ± 6.25
45	109 ± 11.5	99.3 ± 6.85	102 ± 9.60	89.8 ± 7.93
60	110 ± 13.4	101 ± 8.90	104 ± 6.71	84.7 ± 6.99*
90	110 ± 14.5	103 ± 6.26	101 ± 11.3	90.1 ± 6.85
120	111 ± 12.0	96.3 ± 5.84	98.8 ± 12.7	90.1 ± 6.89
150	111 ± 13.1	97.5 ± 8.83	94.2 ± 14.5	91.9 ± 5.22
180	109 ± 8.75	92.9 ± 16.7	96.4 ± 15.0	90.6 ± 8.34
240	105 ± 15.0	88.7 ± 15.2	102 ± 6.89	91.5 ± 7.83
300	103 ± 13.2	91.6 ± 18.7	99.4 ± 9.43	93.5 ± 9.37
360	107 ± 16.4	89.1 ± 24.8	101 ± 9.48	92.6 ± 3.84
420	107 ± 15.8	87.5 ± 21.4	101 ± 14.8	90.1 ± 8.75
480	109 ± 16.1	93.8 ± 18.1	98.8 ± 17.2	91.9 ± 18.6
540	107 ± 17.8	92.9 ± 17.2	94.2 ± 13.5	94.8 ± 19.6
600	106 ± 20.9	94.7 ± 18.5	98.3 ± 11.6	99.9 ± 24.9
720	101 ± 22.4	97.2 ± 8.77	90.2 ± 10.0	87.6 ± 21.3

Data are expressed as mean ± SD (n = 4, each).
Values in the (udenafil + tamsulosin) group significantly different from udenafil alone and tamsulosin alone groups, are shown thus *$P < 0.05$.

administered udenafil. Inhibition of hepatic first-pass tamsulosin metabolism by udenafil could underlie this phenomenon.

In conclusion, after i.v. infusion for 15 min or p.o. administration of both tamsulosin and udenafil, the AUC of tamsulosin was significantly greater than with tamsulosin alone. This appears to be attributable to non-competitive inhibition of CYP3A1/2-mediated hepatic tamsulosin metabolism by udenafil. It is difficult to extrapolate these animal data to humans because the relationships between animals and humans are not easily correlated, which is a limitation of this study. Nonetheless, both udenafil and tamsulosin are metabolized via the CYP3A subfamily in both humans and rats, and the metabolism of tamsulosin was inhibited by udenafil in the human liver S9 fraction. The results of the present study justify a reassessment of the oral dosage of tamsulosin when udenafil and tamsulosin are co-administered to patients with BPH.

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Conflict of interest

The authors state no conflict of interest.

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