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RESEARCH PAPER

Pharmacokinetic and pharmacodynamic consequences of inhibition of terazosin metabolism via CYP3A1 and/or 3A2 by DA-8159, an erectogenic, in rats

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Background and purpose: Recently, orthostatic hypotension was observed in patients with benign prostatic hyperplasia who are taking vardenafil (a PDE 5 inhibitor) and terazosin (a long acting alpha blocker). Therefore, this study was performed with DA-8159 (a long acting PDE 5 inhibitor) and terazosin in rats to find whether or not pharmacokinetic and pharmacodynamic interactions between the two drugs were observed.
Experimental approach: Pharmacokinetic and pharmacodynamic (changes in blood pressure) interactions between DA-8159 and terazosin were evaluated after simultaneous i.v. and p.o. administration of DA-8159 (30 mg kg^{-1}) and terazosin (5 mg kg^{-1}) to male Sprague–Dawley rats.
Key results: After simultaneous i.v. and p.o. administration of terazosin and DA-8159, the total area under the plasma concentration–time curve from time zero to time infinity (AUC) of terazosin became significantly greater (57.4 and 75.4% increase for i.v. and p.o. administration, respectively) than those of without DA-8159. The blood pressure dropping effect was considerable after simultaneous p.o. administration of DA-8159 and terazosin compared with each drug alone.
Conclusions and implications: The significantly greater AUC of terazosin after both simultaneous i.v. and p.o. administration of both drugs could be due to the hepatic (both i.v. and p.o.) and intestinal (p.o.) inhibition of the metabolism of terazosin via CYP3A1 and/or 3A2 by DA-8159, since both DA-8159 and terazosin are metabolized via CYP3A1 and/or 3A2 in rats. The blood pressure lowering effect after simultaneous p.o. administration of both drugs could be due to significant increase in plasma concentrations of terazosin.
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Keywords: pharmacokinetic and pharmacodynamic interactions; DA-8159; terazosin; CYP3A1 and/or 3A2; rats

Abbreviations: $Ae_{0-24\,h}$, percentage of dose excreted in 24 h urine; AUC, total area under the plasma concentration–time curve from time zero to time infinity; C_{max}, peak plasma concentration; CL, time-averaged total body clearance; CL_{int}, intrinsic clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged nonrenal clearance; F, extent of absolute oral bioavailability; $GI_{24\,h}$, percentage of dose recovered from the entire gastrointestinal tract (including its contents and faeces) at 24 h; K_m, Michaelis–Menten constant; MRT, mean residence time; T_{max}, time to reach a C_{max}; V_{max}, maximum velocity; V_{ss}, apparent volume of distribution at steady state

Introduction

A new inhibitor of cyclic guanosine monophosphate (cGMP)-specific phophodiesterase type 5 (PDE 5), DA-8159 (Udenafil), 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethyl amidosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo-(4,3-d)pyrimidine-7-one was developed (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, South Korea) for the treatment of male erectile dysfunction. Phase I studies on DA-8159 conducted in both the UK and Korea have shown that the drug has unique pharmacokinetic profiles (T_{max} of 1.0–1.5 h and a half-life of 11–13 h after oral administration of the drug) suggesting that the drug could have a relatively rapid onset of action and sufficiently long duration of action to make it effective for up to 24 h

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compared with other PDE 5 inhibitors (Bahng *et al*., 2002; Padma-Nathan *et al*., 2004). Phase II clinical data of DA-8159 presented at the 11th World Congress of the International Society for the Sexual and Impotence Research showed that in men with mild-to-severe erectile dysfunction, the drug produced a significant improvement in erectile function after 12 weeks of treatment (Salem *et al*., 2006). Prior studies have also demonstrated a selectivity profile of DA-8159 that is similar to sildenafil but unlike tadalafil, the drug does not inhibit the PDE 11 isozyme and thus would not be expected to produce significant myalgia (Salem *et al*., 2006). The hepatic microsomal cytochrome *P*450 (CYP) 3A4 was identified as the major enzyme responsible for the formation of DA-8164 (N-dealkylated DA-8159) based on human liver microsomes (Ji *et al*., 2004). Recently, it was reported that the metabolism of DA-8159 and the formation of DA-8164 were mediated via CYP3A1 and/or 3A2 (not via CYP1A1/2, 2B1/2, 2D1 and 2E1) in male Sprague–Dawley rats (Kim *et al*., 2005b). Based on the *in vivo* studies, DA-8159 was metabolized to DA-8164 in mice, rats, rabbits, dogs and humans (Shim *et al*., 2005). The pharmacological actions, pharmacokinetics and metabolism, and clinical studies on DA-8159 have been reviewed (Kim *et al*., 2005a). DA-8159, a fourth PDE 5 inhibitor, is marketed in Korea under the brand name of Zydena in 100 and 200 mg tablet strengths for the treatment of male erectile dysfunction.

α-Adrenoceptor blockers are commonly used to treat benign prostatic hyperplasia (BPH) and/or hypertension. Evidence is growing that BPH and erectile dysfunction are closely associated, independent of patient age (Giuliano *et al*., 2006). PDE 5 inhibitors, the most effective oral therapies for erectile dysfunction, are mild vasodilators associated with small decreases in blood pressure because the PDE 5 enzyme is found within the vascular smooth muscle cells in the walls of systemic arteries and veins (Haning *et al*., 2003; Kloner, 2005). The concurrent use of an α-adrenergic blocker with a PDE 5 inhibitor may result in an excessive reduction in blood pressure. Recently, it was reported that tadalafil (a long-acting PDE 5 inhibitor) increases the hypotensive effect of doxazosin (a long-acting and nonselective α-blocker) prescribed for both hypertension and BPH (Kloner *et al*., 2004; Kloner, 2005). Also it was shown that sildenafil given with doxazosin, and vardenafil given with terazosin (a long-acting and nonselective α-blocker) evoked orthostatic hypotension in some patients (Kloner *et al*., 2004). Terazosin is one of long-acting α-blockers and commonly used agent for treating BPH and/or hypertension. Therefore, this study was performed with DA-8159 and terazosin in rats to find the reasons for pharmacokinetic and pharmacodynamic (changes in blood pressure) interactions between the two drugs.

This paper reports the reasons for the pharmacokinetic and pharmacodynamic (changes in blood pressure) interactions between DA-8159 and terazosin after simultaneous intravenous (i.v.) and per os (p.o.) administration of the two drugs to male Sprague–Dawley rats. The CYP isozymes responsible for the metabolism of terazosin in rats does not seem to be published to date. Hence, the metabolism of terazosin via CYP3A1 and/or 3A2 in rats is reported.

Methods

Animals

Male Sprague–Dawley rats of 7–9 weeks of age (weighing 220–330 g) purchased from Taconic Farms Inc. (Samtako Bio Korea, O-San, South Korea) were housed in a light-controlled room (light: 0700–1900 h, dark: 1900–0700 h) kept at a temperature of $20 \pm 1^{\circ}C$ and a relative humidity of $50 \pm 5\%$ (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea). The rats were housed in metabolic cages (Tecniplast, Varese, Italy) with a supply of filtered pathogen-free air, and food (Samyang Company, Pyeongtaek, South Korea) and water *ad libitum*. The protocol of this study was approved by Animal Care and Use Committee of College of Pharmacy of Seoul National University.

I.v.study

In the early 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) whereas each rat was under light ether anesthesia (Kim *et al*., 1993). Both cannulas 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. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed for 4–5 h to recover from anesthesia before the study began. They were not restrained during the study.

DA-8159 (dissolved in 0.05 M citric acid to yield $15\,mg\,ml^{-1}$) at a dose of $30\,mg\,kg^{-1}$ ($n=8$), terazosin (dissolved in 0.05 M citric acid to yield $2.5\,mg\,ml^{-1}$) at a dose of $5\,mg\,kg^{-1}$ ($n=8$) and both ($n=10$) were infused for 1 min via the jugular vein of rats. An approximately 0.12 ml (for DA-8159 alone or terazosin alone) or 0.22 ml (for both DA-8159 and terazosin) aliquot of blood sample was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 60, 120, 180, 240, 360, 480, 600 or 720 min after the start of the infusion of the drug(s). Blood samples were centrifuged immediately and a 50 μl aliquot (for DA-8159 alone or terazosin alone) or two 50 μl aliquots (for both DA-8159 and terazosin) of plasma sample were collected in a 1.5 ml polyethylene tube, and was stored in a $-70^{\circ}C$ freezer (Revco ULT 1490 D-N-S; Western Mednics, Ashville, NC, USA) until high performance liquid chromatographic (HPLC) analysis of DA-8159, DA-8164 and terazosin. At the end of the experiment (24 h), each metabolic cage was rinsed with 10 ml of distilled water, and the rinsings were combined with 24-h urine sample. After measuring the exact volume of the combined urine samples, two 50 μl aliquots of the combined urine sample were stored in a $-70^{\circ}C$ freezer until HPLC analysis of DA-8159, DA-8164 and terazosin. At the same time (24 h), each rat was sacrificed through cervical dislocation and exsanguinated. And then its abdomen was opened and the entire gastrointestinal tract (including its contents and feces) of each rat was removed, transferred into a beaker that contained 50 ml of methanol

(to facilitate the extraction of DA-8159, DA-8164 and terazosin) and cut into small pieces with scissors. After it was stirred with a glass rod for 1 min, two 50 μl aliquots of the supernatant were collected from each beaker and stored in a −70°C freezer until HPLC analysis of DA-8159, DA-8164 and terazosin.

Oral study

DA-8159 (the same solution that was used in the i.v. study) at a dose of 30 mg kg^{-1} ($n=8$), terazosin (the same solution that was used in the i.v. study) at a dose of 5 mg kg^{-1} ($n=7$), and both ($n=8$) were orally administered to rats using a feeding tube. The same dose of terazosin (for 7 days) ($n=8$), the same dose of single DA-8159 (just after seventh dose of the same volume of 0.05 M citric acid) ($n=10$), and both (DA-8159 was administered just after seventh dose of terazosin) ($n=9$) were also administered orally to rats. An approximately 0.12 (for DA-8159 alone and terazosin alone) or 0.22 ml (for both DA-8159 and terazosin) aliquot of blood sample was collected via the carotid artery at 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720 or 1440 min after oral administration of the drug(s). Blood samples were collected on day 7 just after administration of the drugs. Other procedures were similar to those in the i.v. study.

Measurement of V_{max}, K_m and CL_{int} for the disappearance of terazosin with and without DA-8159 in hepatic microsomal fractions

The procedures were similar to the reported methods (Shim *et al*., 2004). The livers of control rats ($n=5$) were homogenized in ice-cold buffer (0.154 M KCl/50 mM Tris-HCl in 1 mM ethylenediamine tetraacetic acid (EDTA), pH 7.4). The homogenate was then centrifuged at 10 000 *g* for 30 min and the supernatant fraction was further centrifuged at 1 00 000 *g* for 90 min. Protein content was measured using the reported method (Bradford, 1976). The V_{max} (the maximum velocity) and K_m (the Michaelis–Menten constant; the concentration at which the rate is one-half of V_{max}) for the disappearance of terazosin with and without DA-8159 were determined after incubating the above microsomal fraction (equivalent to 1.0 mg protein), a 50 μl aliquot of distilled water that contained 0.5, 1, 2, 5, 20, 50 and 100 μM of terazosin, with or without a 10 μl aliquot of 0.5 M citrate buffer that contained 100 μg of DA-8159 and a 50 μl aliquot of 0.1 M phosphate buffer that contained 1 mM of nicotinamide adenine dinucleotide phosphate (NADPH) in a final volume of 500 μl by the addition of 0.1 M phosphate buffer, pH 7.4, in a thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany) kept at 37°C and at a rate of 500 oscillations per min (o.p.m). All of the above microsomal incubation conditions were linear. The reaction was terminated by the addition of a 300 μl aliquot of 1 N NaOH after 30 min incubation. Terazosin concentrations were measured by the reported HPLC method. The kinetic constants (K_m and V_{max}) for the disappearance of terazosin with or without DA-8159 were calculated using the nonlinear regression method (Duggleby, 1995). The intrinsic clearance (CL_{int}) for the disappearance of terazosin with and without DA-8159 was calculated by dividing the respective V_{max} by the respective K_m.

I.v. administration of terazosin to rats pretreated with troleandomycin or dexamethasone phosphate

Rats received a single intraperitoneal (i.p.) injection of 500 mg (5 ml) kg^{-1} of troleandomycin (a main inhibitor of CYP3A1 and 3A2 in rats (Correia, 1995); dissolved in 0.9% NaCl-injectable solution acidified to pH 4.0 with 1 N HCl (Sinclair *et al*., 2000)), or three daily i.p. injection of 50 mg (5 ml) kg^{-1} of dexamethasone phosphate (a main inducer of CYP3A1 and 3A2 in rats (Correia, 1995); dissolved in 0.9% NaCl-injectable solution (Roos *et al*., 1993)). An experiment was performed after 2 h for the troleandomycin-pretreated rats (Sinclair *et al*., 2000) and on day 4 for dexamethasone phosphate-pretreated rats (Roos *et al*., 1993). Terazosin (the same solution that was used in the i.v. study) at a dose of 10 mg kg^{-1} was infused for 1 min via the jugular vein of rats pretreated with troleandomycin ($n=7$) or dexamethasone phosphate ($n=7$) and their control rats ($n=8$). Other procedures were similar to those in the i.v. study already described.

Pharmacodynamic (blood pressure) changes after oral administration of DA-8159, terazosin, or both and control rats

The carotid artery was cannulated with polyethylene tubes (Kim *et al*., 1993) to monitor the arterial systolic blood pressure up to 10 h in the rats after p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} ($n=3$), terazosin at a dose of 5 mg kg^{-1} ($n=3$), or both ($n=3$) and control (administration of vehicle, 0.05 M citric acid) rats ($n=3$) without blood sampling. The arterial systolic blood pressure readings were recorded using a pressure transducer and a bridge amplifier connected online to the artery and to a PowerLab system (PowerLab, Version 5; ADI Instruments, Pty Ltd, Castle Hill, NSW, Australia).

HPLC analysis of DA-8159, DA-8164 and terazosin

Concentrations of DA-8159 and DA-8164 in the above samples were analyzed by a slight modification of the reported HPLC method (Shim *et al*., 2002); a biological sample of 50 μl instead of 100 μl was used. To a 50 μl aliquot of biological sample, a 0.1 ml aliquot of 0.1 N Na_2CO_3 that contained 3 μg/ml of sildenafil (an internal standard) and a 1 ml aliquot of ethylether were added. After vortex-centrifugation at 16 000 *g* for 8 min, the ether layer was collected and dried under a gentle stream of nitrogen gas at 30°C. A 100 μl aliquot of the mobile phase was added to reconstitute the residue and a 50 μl aliquot was directly injected onto a reversed-phase (C_{18}) HPLC column. The mobile phase, 20 mM KH_2PO_4:acetonitrile (72:28; v/v), was run at a flow rate of 1.5 ml min^{-1}, and the column effluent was monitored using an ultraviolet detector set at 292 nm at room temperature. The retention times of DA-8159, DA-8164 and the internal standard (sildenafil) were approximately 9.7, 17.1 and 6.9 min, respectively. The detection limits of DA-8159 and DA-8164 in plasma and urine were all 0.05 μg ml^{-1}. The coefficients of variation of the assay (within- and between-days) were below 10.1% for the plasma and 9.91% for the urine samples.

Concentrations of terazosin in the above samples were analyzed by a slight modification of the reported HPLC method (Cheah *et al*., 2000); a biological sample of 50 μl instead of 500 μl, and column oven of 50°C instead of room temperature were used. To a 50-μl aliquot of biological sample, a 25-μl aliquot of 1 N NaOH, a 50-μl aliquot of distilled water that contained 0.6 μg ml^{-1} of the internal standard (prazosin hydrochloride) and a 1-ml aliquot of dichloromethane were added. After vortex-centrifugation at 16 000 *g* for 8 min, the organic layer was collected and dried under a gentle stream of nitrogen gas at room temperature. A 150-μl aliquot of the mobile phase was added to reconstitute the residue and a 50-μl aliquot was directly injected onto a reversed-phase (C_{18}) HPLC column. The mobile phase, 10 mM Na_2HPO_4:acetonitrile:tetrahydrofuran (76:22:2; v/v/v) adjusted to pH 6.5 using 85% phosphoric acid, was run at a flow rate of 1.3 ml min^{-1}, and the column effluent was monitored using a fluorescence detector set at an excitation wavelength of 250 nm and an emission wavelength of 370 nm at 50°C using column oven (Eldex CH-150, Eldex Laboratories Inc, CA, USA). The retention times of terazosin and the internal standard (prazosin hydrochloride) were approximately 6.1 and 11.6 min, respectively. The detection limits of terazosin in plasma and urine were all 5 ng ml^{-1}. The coefficients of variation of the assay (within- and between-days) were below 7.41% for the plasma and 9.38% for the urine samples.

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. This method uses the logarithmic trapezoidal rule for the calculation of the area during the declining plasma-level phase (Chiou, 1978), and the linear trapezoidal rule for the rising plasma-level phase. 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 and Perrier, 1982) were used to calculate the following pharmacokinetic parameters using the noncompartmental analysis (WinNonlin; Pharsight Corporation, Mountain View, CA, USA); the time-averaged total body (CL), renal (CL_R) and nonrenal (CL_{NR}) clearances, terminal half-life ($t_{1/2}$), first moment of AUC (AUMC), mean residence time (MRT), apparent volume of distribution at a steady state (V_{ss}) and extent of absolute oral bioavailability (*F*) (Kim *et al*., 1993). The peak plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were read directly from the experimental data.

The harmonic mean method was used to calculate the mean values of V_{ss} (Chiou, 1979), terminal $t_{1/2}$ (Eatman *et al*., 1977) and each clearance (Chiou, 1980).

Statistical analysis

A *P*-value of <0.05 was considered to be statistically significant using a *t*-test between the two means for the unpaired data. All results are expressed as mean $\pm$ s.d., except median (ranges) for T_{max}.

Chemicals

DA-8159, DA-8164 and sildenafil citrate (an internal standard of HPLC analysis of DA-8159 and DA-8164) were supplied from Research Laboratory of Dong-A Pharmaceutical Company. Tris(hydroxymethyl) aminomethane (Tris)-buffer, terazosin, dexamethasone phosphate, troleandomycin, reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt) and EDTA were purchased from Sigma–Aldrich Corporation (St Louis, MO, USA). Prazosin hydrochloride (an internal standard of HPLC analysis of terazosin) was purchased from Tokyo Chemical Industry Corporation (Tokyo, Japan). Other chemicals were of reagent grade or HPLC grade.

Results

Pharmacokinetics of DA-8159 and DA-8164 after single i.v. administration of DA-8159 with or without simultaneous single i.v. administration of terazosin to rats

After i.v. administration of DA-8159 with or without i.v. terazosin, the mean arterial plasma concentration–time profiles of DA-8159 and DA-8164 are shown in Figure 1a and b, respectively, and some relevant pharmacokinetic parameters are listed in Table 1. The pharmacokinetic parameters of DA-8159 listed in Table 1 were not significantly different between two groups of rats, except significantly slow CL_R (73.7% decrease) and significantly smaller percentages of i.v. dose of DA-8159 excreted in 24 h urine ($Ae_{0-24\ h}$; 62.3% decrease) and recovered from the gastrointestinal tract (including its contents and feces) at 24 h ($GI_{24\,h}$; 55.5% decrease) as unchanged DA-8159 in rats with terazosin.

After i.v. administration of DA-8159 with or without terazosin, the formation of DA-8164 became rapid; DA-8164 was detected in plasma from the first blood sampling time (1 min) for both groups of rats (Figure 1b), and reached T_{max} values of DA-8164 rapidly; the values were 45 and 30 min for with and without terazosin, respectively (they were not significantly different). The pharmacokinetic parameters of DA-8164 listed in Table 1 were also not significantly different between the two groups of rats, except significantly slower CL_R (65.3% decrease) and significantly smaller $Ae_{0-24\,h}$ (48.1% decrease; expressed in terms of the i.v. dose of DA-8159) in rats with terazosin.

Pharmacokinetics of terazosin after single i.v. administration of terazosin with or without simultaneous single i.v. administration of DA-8159 to rats

After i.v. administration of terazosin with and without i.v. DA-8159, the mean arterial plasma concentration–time profiles of terazosin are shown in Figure 1c, and some relevant pharmacokinetic parameters are also listed in Table 1. After simultaneous i.v. administration with DA-8159, the differences in the pharmacokinetic parameters of terazosin are as follows: the AUC became significantly greater (57.4% increase), MRT became significantly longer (86.2% increase), and CL, CL_R and CL_{NR} became significantly slower (37.2, 60.8 and 32.4% decrease, respectively) than those after terazosin alone.

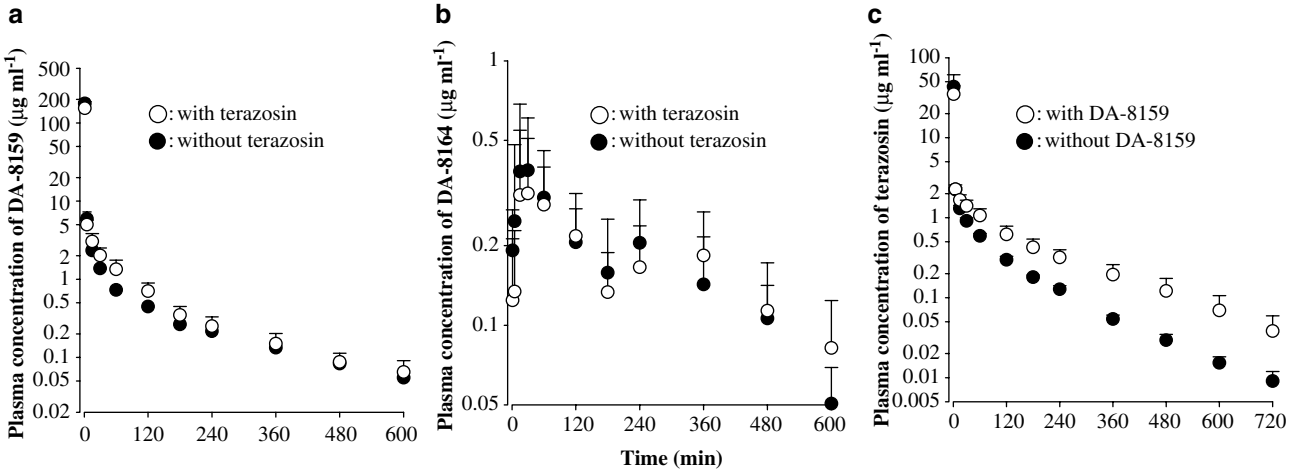

Figure 1 Arterial plasma concentration–time profiles of DA-8159 (**a**) and DA-8164 (**b**) after single i.v. administration of DA-8159 at a dose of 30 mg kg^{-1} with ($n=10$) or without ($n=8$) simultaneous single i.v. administration of terazosin at a dose of 5 mg kg^{-1} to rats, and of terazosin (**c**) after single i.v. administration of terazosin at a dose of 5 mg kg^{-1} with ($n=10$) or without ($n=8$) simultaneous single i.v. administration of DA-8159 at a dose of 30 mg kg^{-1} to rats. Data are presented as mean$\pm$s.d.

Table 1 Pharmacokinetic parameters of DA-8159 and DA-8164 after single i.v. administration of DA-8159 at a dose of 30 mg kg^{-1} without and with simultaneous single i.v. administration of terazosin at a dose of 5 mg kg^{-1}, and of terazosin after single i.v. administration of terazosin at a dose of 5 mg kg^{-1} without and with simultaneous single i.v. administration of DA-8159 at a dose of 30 mg kg^{-1} to rats

Parameter	*DA-8159*		*Parameter*	*Terazosin*	
	Without terazosin	*With terazosin*		*Without DA-8159*	*With DA-8159*
DA-8159			*Terazosin*		
AUC (μg min ml^{-1})	514$\pm$56.0	553$\pm$77.3	AUC (μg min ml^{-1})	202$\pm$27.4	318$\pm$70.3[a]
Terminal $t_{1/2}$ (min)	192$\pm$70.4	212$\pm$69.2	Terminal $t_{1/2}$ (min)	141$\pm$28.2	143$\pm$73.0
MRT (min)	85.3$\pm$22.5	101$\pm$32.0	MRT (min)	84.3$\pm$11.2	157$\pm$39.3[a]
V_{ss} (ml kg^{-1})	4630$\pm$1520	5160$\pm$1430	V_{ss} (ml kg^{-1})	2020$\pm$488	2420$\pm$404
CL (ml min^{-1} kg^{-1})	58.4$\pm$6.26	54.3$\pm$8.61	CL (ml min^{-1} kg^{-1})	24.7$\pm$3.41	15.5$\pm$3.87[a]
CL_R (ml min^{-1} kg^{-1})	3.21$\pm$1.27	0.844$\pm$1.11[b]	CL_R (ml min^{-1} kg^{-1})	6.69$\pm$1.65	2.62$\pm$2.40[c]
CL_{NR} (ml min^{-1} kg^{-1})	54.7$\pm$4.86	53.0$\pm$8.23	CL_{NR} (ml min^{-1} kg^{-1})	17.9$\pm$2.26	12.1$\pm$2.99[a]
$Ae_{0-24\,h}$ (% of DA-8159 dose)	6.29$\pm$1.68	2.37$\pm$1.75[b]	$Ae_{0-24\,h}$ (% of terazosin dose)	28.6$\pm$2.73	22.7$\pm$11.5
$GI_{24\,h}$ (% of DA-8159 dose)	1.46$\pm$0.693	0.650$\pm$0.418[d]	$GI_{24\,h}$ (% of terazosin dose)	0.245$\pm$0.238	0.308$\pm$0.242
DA-8164					
AUC (μg min ml^{-1})	113$\pm$32.5	115$\pm$40.0			
Terminal $t_{1/2}$ (min)	134$\pm$75.4	124$\pm$68.0			
CL_R (ml min^{-1} kg^{-1})	0.386$\pm$0.0741	0.134$\pm$0.117[b]			
C_{max} (μg ml^{-1})	0.411$\pm$0.294	0.382$\pm$0.260			
T_{max} (min)	30 (15–30)	45 (15–120)			
$Ae_{0-24\,h}$ (% of DA-8159 dose)	0.150$\pm$0.0534	0.0778$\pm$0.0317[d]			
$GI_{24\,h}$ (% of DA-8159 dose)	0.325$\pm$0.255	0.314$\pm$0.260			

Abbreviations: $Ae_{0-24\,h}$, percentage of dose excreted in 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_R, time-averaged renal clearance; CL_{NR}, time-averaged nonrenal clearance; C_{max}, peak plasma concentration; $GI_{24\,h}$, percentage of dose recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h; i.v., intravenous; MRT, mean residence time; T_{max}, time to reach a C_{max}; $t_{1/2}$, half-life; V_{ss}, apparent volume of distribution at steady state.
Data are expressed as mean$\pm$s.d. (without terazosin, $n=8$; with terazosin, $n=10$ for DA-8159, without DA-8159, $n=8$; with DA-8159, $n=10$ for terazosin). Significant difference from without DA-8159, [a]$P<0.001$ and [c]$P<0.01$. Significantly different from without terazosin, [b]$P<0.001$ and [d]$P<0.01$.

Pharmacokinetics of DA-8159 and DA-8164 after oral administration of DA-8159 with or without simultaneous oral administration of terazosin to rats

After single p.o. administration of DA-8159 with and without single p.o. terazosin, the mean arterial plasma concentration–time profiles of DA-8159 and DA-8164 are shown in Figure 2a and b, respectively, and some relevant pharmacokinetic parameters are listed in Table 2. The pharmacokinetic parameters of both DA-8159 and DA-8164 listed in Table 2 were not changed significantly by terazosin.

After p.o. administration of single DA-8159 with and without daily p.o. administration of terazosin for 7 days, the mean arterial plasma concentration–time profiles of DA-8159 and DA-8164 are shown in Figure 3a and b, respectively, and some relevant pharmacokinetic parameters are listed in Table 3. The pharmacokinetic parameters of both

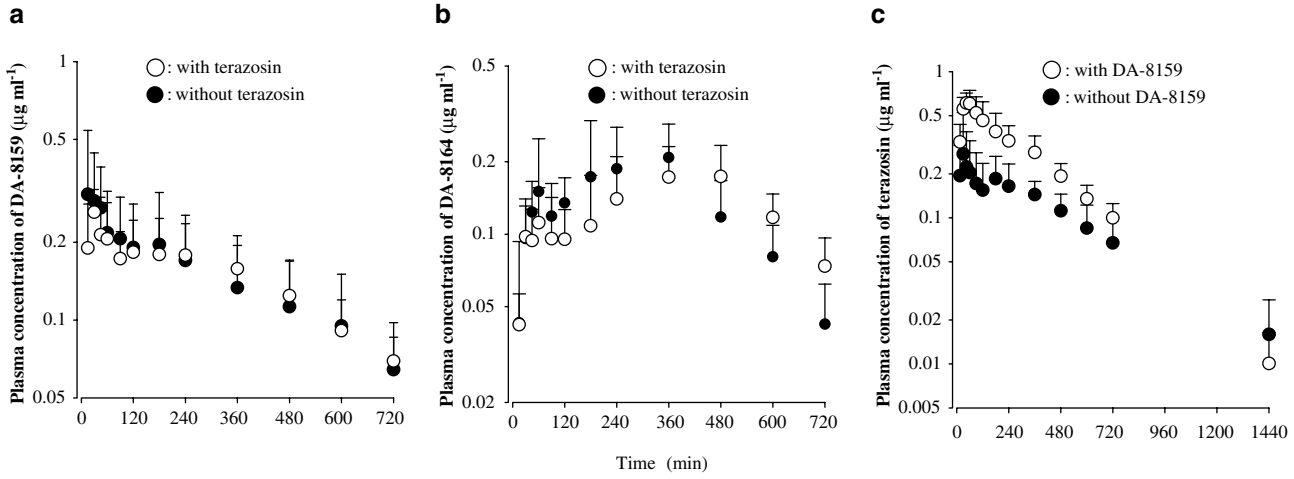

Figure 2 Arterial plasma concentration–time profiles of DA-8159 (**a**) and DA-8164 (**b**) after single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} with ($n=8$) or without ($n=8$) simultaneous single p.o. administration of terazosin at a dose of 5 mg kg^{-1} to rats, and of terazosin (**c**) after single p.o. administration of terazosin at a dose of 5 mg kg^{-1} with ($n=8$) or without ($n=7$) simultaneous single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} to rats. Data are presented as mean$\pm$s.d.

Table 2 Pharmacokinetic parameters of DA-8159 and DA-8164 after single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} without and with simultaneous single p.o. administration of terazosin at a dose of 5 mg kg^{-1}, and of terazosin after single p.o. administration of terazosin at a dose of 5 mg kg^{-1} without and with simultaneous single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} to rats

Parameter	*DA-8159*		*Parameter*	*Terazosin*	
	Without terazosin	*With terazosin*		*Without DA-8159*	*With DA-8159*
DA-8159			*Terazosin*		
AUC (μg min ml^{-1})	133$\pm$62.4	137$\pm$30.3	AUC (μg min ml^{-1})	134$\pm$33.5	235$\pm$49.7[a]
Terminal $t_{1/2}$ (min)	298$\pm$50.8	306$\pm$139	Terminal $t_{1/2}$ (min)	303$\pm$119	208$\pm$53.6
CL_R (ml min^{-1} kg^{-1})	1.91$\pm$1.21	1.89$\pm$1.18	CL_R (ml min^{-1} kg^{-1})	4.64$\pm$0.824	5.97$\pm$1.77[b]
C_{max} (μg ml^{-1})	0.305$\pm$0.0883	0.274$\pm$0.0775	C_{max} (μg ml^{-1})	0.303$\pm$0.242	0.631$\pm$0.118[c]
T_{max} (min)	30 (15–90)	30 (15–45)	T_{max} (min)	30 (15–360)	45 (30–60)
$Ae_{0-24\,h}$ (% of DA-8159 dose)	0.878$\pm$0.259	1.09$\pm$0.480	$Ae_{0-24\,h}$ (% of terazosin dose)	12.4$\pm$2.28	29.5$\pm$7.31[a]
$GI_{24\,h}$ (% of DA-8159 dose)	2.10$\pm$3.20	1.52$\pm$0.956	$GI_{24\,h}$ (% of terazosin dose)	7.04$\pm$3.38	1.48$\pm$1.06[a]
F (%)	25.9	24.8	F (%)	67.8	73.9
DA-8164					
AUC (μg min ml^{-1})	104$\pm$35.4	114$\pm$34.0			
Terminal $t_{1/2}$ (min)	153$\pm$47.0	194$\pm$71.0			
CL_R (ml min^{-1} kg^{-1})	0.428$\pm$0.224	0.227$\pm$0.345			
C_{max} (μg ml^{-1})	0.272$\pm$0.106	0.201$\pm$0.0583			
T_{max} (min)	180 (60–360)	45 (30–480)			
$Ae_{0-24\,h}$ (% of DA-8159 dose)	0.176$\pm$0.113	0.108$\pm$0.0492			
$GI_{24\,h}$ (% of DA-8159 dose)	0.654$\pm$0.286	0.621$\pm$0.258			

Abbreviations: 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; $t_{1/2}$, half-life; T_{max}, time to reach a C_{max}; $Ae_{0-24\,h}$, percentage of dose excreted in 24 h urine; *F*, extent of absolute oral bioavailability; $GI_{24\,h}$, percentage of dose recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h; p.o., per os.
Data are expressed as mean$\pm$s.d., (without terazosin, $n=8$; with terazosin, $n=8$ for DA-8159, without DA-8159, $n=7$; with DA-8159, $n=8$ for terazosin). Significant difference from without DA-8159, [a]$P<0.001$, [b]$P<0.05$ and [c]$P<0.01$.

DA-8159 and DA-8164 listed in Table 3 were also not changed significantly by terazosin.

Pharmacokinetics of terazosin after oral administration of terazosin with or without simultaneous oral administration of DA-8159 to rats

After single p.o. administration of terazosin with and without single p.o. administration of DA-8159, the mean arterial plasma concentration–time profiles of terazosin are shown in Figure 2c, and some relevant pharmacokinetic parameters are also listed in Table 2. After simultaneous administration with DA-8159, the changes in the pharmacokinetic parameters of terazosin are as follows: the AUC became significantly greater (75.4% increase), CL_R became significantly faster (28.7% increase), C_{max} became significantly higher (108% increase), and $Ae_{0-24\,h}$ (138% increase) and $GI_{24\,h}$ (79.0% decrease) became significantly greater and smaller, respectively, than those after terazosin alone.

After 7 days p.o. administration of terazosin with single p.o. administration of DA-8159, the mean arterial plasma concentration–time profiles of terazosin are shown in Figure 3c, and some relevant pharmacokinetic parameters are also listed in Table 3. After single p.o. administration of DA-8159, the changes in the pharmacokinetic parameters of terazosin are as follows: the AUC became significantly greater (138% increase), CL_R became significantly slower (61.2% decrease),

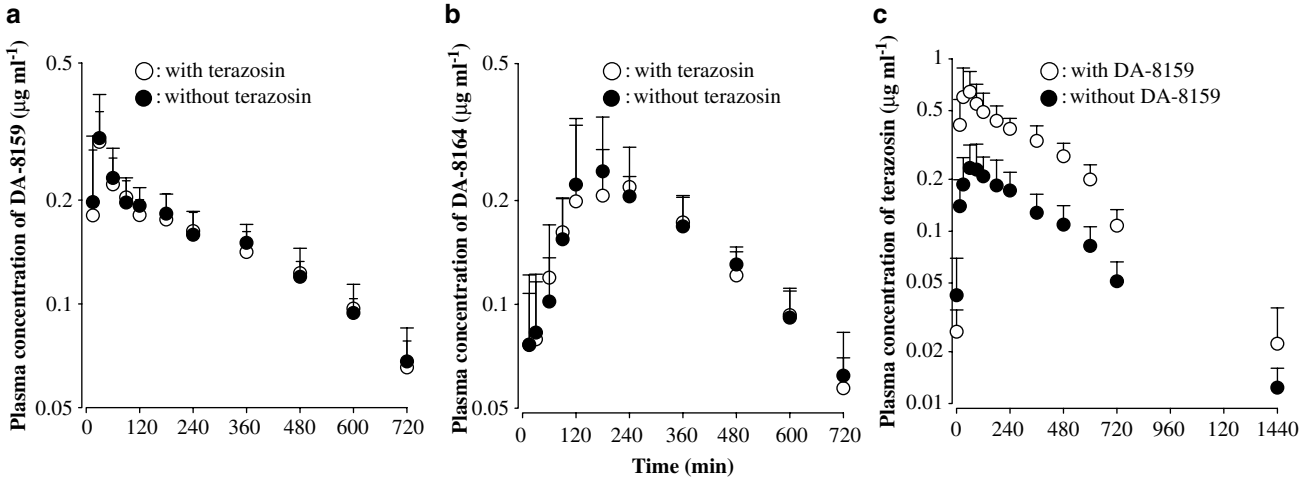

Figure 3 Arterial plasma concentration–time profiles of DA-8159 (**a**) and DA-8164 (**b**) after single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} with ($n=9$) or without ($n=10$) simultaneous p.o. administration of terazosin at a dose of 5 mg/kg/day for 7 days to rats, and of terazosin (**c**) after p.o. administration of terazosin at a dose of 5 mg/kg/day for 7 days with ($n=9$) or without ($n=8$) simultaneous single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} to rats. Data are presented as mean ± s.d.

Table 3 Pharmacokinetic parameters of DA-8159 and DA-8164 after single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} without and with simultaneous p.o. administration of terazosin at a dose of 5 mg kg^{-1} per day for 7 days, and of terazosin after p.o. administration of terazosin at a dose of 5 mg kg^{-1} per day for 7 days without and with simultaneous single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} to rats

Parameter	*DA-8159*		*Parameter*	*Terazosin*	
	Without terazosin	*With terazosin*		*Without DA-8159*	*With DA-8159*
DA-8159			*Terazosin*		
AUC (μg min ml^{-1})	137 ± 12.5	134 ± 20.7	AUC (μg min ml^{-1})	124 ± 16.7	295 ± 38.7[a]
Terminal $t_{1/2}$ (min)	310 ± 78.1	289 ± 98.0	Terminal $t_{1/2}$ (min)	305 ± 45.2	254 ± 63.5
CL_R (ml min^{-1} kg^{-1})	2.25 ± 2.73	1.57 ± 2.05	CL_R (ml min kg^{-1})	8.63 ± 1.60	3.35 ± 2.19[a]
C_{max} (μg ml^{-1})	0.336 ± 0.0913	0.314 ± 0.0753	C_{max} (μg ml^{-1})	0.292 ± 0.0713	0.722 ± 0.193[a]
T_{max} (min)	45 (15–90)	30 (15–60)	T_{max} (min)	120 (60–240)	45 (15–180)[b]
$Ae_{0-24\,h}$ (% of DA-8159 dose)	1.46 ± 0.735	1.10 ± 1.07	$Ae_{0-24\,h}$ (% of terazosin dose)	22.2 ± 5.38	24.7 ± 10.3
$GI_{24\,h}$ (% of DA-8159 dose)	5.06 ± 5.14	3.72 ± 3.52	$GI_{24\,h}$ (% of terazosin dose)	3.11 ± 3.27	2.37 ± 2.25
DA-8164					
AUC (μg min ml^{-1})	132 ± 26.0	123 ± 20.7			
Termianal $t_{1/2}$ (min)	245 ± 115	230 ± 88.2			
CL_R (ml min^{-1} kg^{-1})	0.151 ± 0.069	0.131 ± 0.167			
C_{max} (μg ml^{-1})	0.294 ± 0.101	0.307 ± 0.100			
T_{max} (min)	210 (120–360)	180 (120–240)			
$Ae_{0-24\,h}$ (% of DA-8159 dose)	0.0730 ± 0.0259	0.0754 ± 0.0463			
$GI_{24\,h}$ (% of DA-8159 dose)	0.539 ± 0.221	0.458 ± 0.258			

Abbreviations: $Ae_{0-24\,h}$, percentage of dose excreted in 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; $GI_{24\,h}$, percentage of dose recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h; $t_{1/2}$, half-life; T_{max}, time to reach a C_{max}.
Data are expressed as mean ± s.d., (without terazosin, $n=10$; with terazosin, $n=9$ for DA-8159, without DA-8159, $n=8$; with DA-8159, $n=9$ for terazosin). Significant difference from without DA-8159, [a]$P<0.001$ and [b]$P<0.05$.

C_{max} became significantly higher (147% increase) and T_{max} became significantly shorter (62.5% decrease) than those without DA-8159.

Measurement of V_{max}, K_m and CL_{int} for the disappearance of terazosin with and without DA-8159 in hepatic microsomal fractions

The V_{max}, K_m and CL_{int} for the disappearance of terazosin with or without DA-8159 are listed in Table 4. The V_{max} and K_m for the disappearance of terazosin became significantly slower (13.8% decrease) and higher (123% increase), respectively, in the presence of DA-8159, suggesting that the maximum velocity for the disappearance (mainly due to metabolism) of terazosin was slower and affinity of terazosin to enzymes (mainly to CYP3A1 and/or 3A2) decreased by DA-8159. Hence, the CL_{int} for the disappearance of terazosin became significantly slower (62.0% decrease) in the presence of DA-8159, suggesting that the hepatic metabolism of terazosin was inhibited by DA-8159.

Pharmacokinetics of i.v. terazosin in rats pretreated with dexamethasone phosphate or troleandomycin

After i.v. administration of terazosin in rats pretreated with dexamethasone phosphate or troleandomycin, the mean

Table 4 V_{max}, K_m and CL_{int} for the disappearance of terazosin with or without DA-8159

Parameter	*Without DA-8159*	*With DA-8159*
V_{max} (nmol min^{-1} mg protein^{-1})	0.311 ± 0.0303	0.268 ± 0.0244[a]
K_m (μM)	22.1 ± 3.42	49.3 ± 2.41[b]
CL_{int} (ml min^{-1} mg protein^{-1})	0.0142 ± 0.00134	0.00539 ± 0.00102[b]

Abbreviations: V_{max}, maximum velocity; K_m, Michaelis–Menten constant; CL_{int}, intrinsic clearance.
Data are expressed as mean ± s.d. ($n = 5$; each). Significant difference from without DA-8159, [a]$P < 0.05$ and [b]$P < 0.001$.

arterial plasma concentration–time profiles of terazosin are shown in Figure 4a or b, respectively, and some relevant pharmacokinetic parameters are listed in Table 5. After i.v. administration of terazosin in rats pretreated with dexamethasone phosphate (a major inducer of CYP3A1 and 3A2 in rats), the changes in the pharmacokinetic parameters of terazosin are as follows: the AUC became significantly smaller (17.9% decrease), CL and CL_{NR} became significantly faster (22.4 and 29.8% increase, respectively), CL_R became significantly slower (54.0% decrease) and $Ae_{0-24\,h}$ became significantly smaller (52.5% decrease) than those without dexamethasone phosphate. After i.v. administration of terazosin in rats pretreated with troleandomycin (a major inhibitor of CYP3A1 and 3A2 in rats), the changes in the pharmacokinetic parameters of terazosin are as follows: the AUC became significantly greater (74.2% increase), MRT became significantly longer (106% increase), CL, CL_R and CL_{NR} became significantly slower (42.2, 59.4 and 41.9% decrease, respectively), and $GI_{24\,h}$ became significantly greater (582% increase) than those of without troleandomycin. The aforementioned data suggest that terazosin is metabolized via CYP3A1 and/or 3A2 in rats.

Pharmacodynamic (blood pressure) changes in control rats and after oral administration of DA-8159, terazosin or both to rats
As DA-8159 and terazosin are available for orally administration, the pharmacodynamic effects (lowering blood pressure) were monitored after p.o. administration of the drugs. Pharmacodynamic changes after p.o. administration of DA-8159, terazosin, or both in rats and control rats are shown in Figure 6. After p.o. administration of DA-8159 or terazosin alone, the blood pressure changes were not considerable compared with the controls. However, with simultaneous administration of terazosin and DA-8159, the blood pressure was clearly lowered, for up to 7 h, compared with the blood pressures observed with each drug given alone. A positive correlation was found (Figure 6) between the log increase in plasma concentrations of terazosin (the concentrations with DA-8159 minus the concentrations without DA-8159) and difference in blood pressure (the blood pressure with DA-8159 minus the blood pressure with DA-8159; the values were expressed in terms of + instead of − from 15 to 600 min).

Discussion

DA-8164 is a main metabolite of DA-8159 in humans and the pharmacological effect of DA-8164 in terms of its PDE 5 inhibitory activity is half that of DA-8159 (Kim *et al*., 2005a). Hence, the pharmacokinetics of DA-8164 were evaluated in this study. It was reported (Shim *et al*., 2003) that the AUC values of DA-8159 were dose-proportional after i.v. administration of the drug at doses of 5–30 mg kg^{-1} and p.o. administration of the drug at doses of 20–30 mg kg^{-1} to male Sprague–Dawley rats. Hence, the 30 mg kg^{-1} of both i.v. and p.o. dose of DA-8159 was arbitrarily chosen in this study. Pharmacokinetic studies on terazosin in rats are scarce. It was reported (Fort *et al*., 1984) that LD50s of terazosin were 277 and 293 mg kg^{-1} for male and female rats, respectively, after i.v. administration of the drug. Hence, the i.v. and p.o. dose of 5 mg kg^{-1} was arbitrarily chosen in this study.

After i.v. administration of DA-8159 or terazosin alone, the contribution of the CL_R to CL of DA-8159 or terazosin did not turn out to be considerable: the $Ae_{0-24\,h}$ values of DA-8159 or terazosin were 6.29 or 28.6% of i.v. dose, respectively (Table 1). This suggests that most of the i.v. administered DA-8159 or terazosin are eliminated via the nonrenal route

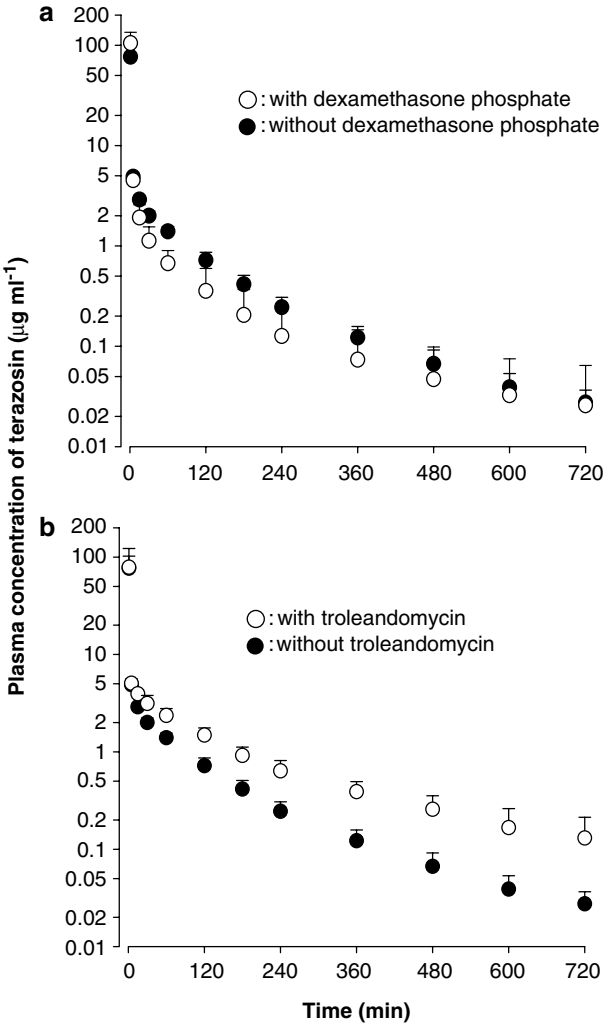

Figure 4 Arterial plasma concentration–time profiles of terazosin after i.v. administration of terazosin at a dose of 10 mg kg^{-1} to rats with ($n = 7$) or without ($n = 8$) pretreatment with dexamethasone phosphate (**a**) and with ($n = 7$) or without ($n = 8$) pretreatment with troleandomycin (**b**). Data are presented as mean ± s.d.

Table 5 Pharmacokinetic parameters of terazosin after i.v. administration of terazosin at a dose of 10 mg kg^{-1} to rats without (control) and with pretreatment with dexamethasone phosphate or troleandomycin

Parameter	*Control*	*With dexamethasone phosphate*	*With troleandomycin*
AUC (μg min ml^{-1})	430 ± 54.6	353 ± 78.6[a]	749 ± 177[b]
Terminal $t_{1/2}$ (min)	186 ± 31.6	191 ± 91.3	219 ± 96.3
MRT (min)	97.5 ± 17.9	87.0 ± 88.1	201 ± 81.1[c]
V_{ss} (ml kg^{-1})	2180 ± 507	1600 ± 1960	2510 ± 792
CL (ml min^{-1} kg^{-1})	23.2 ± 2.84	28.4 ± 6.42[a]	13.4 ± 3.26[b]
CL_R (ml min^{-1} kg^{-1})	4.63 ± 1.13	2.13 ± 1.68[a]	1.88 ± 1.45[c]
CL_{NR} (ml min^{-1} kg^{-1})	19.8 ± 2.85	25.7 ± 5.20[c]	11.5 ± 2.45[b]
$Ae_{0-24\,h}$ (% of terazosin dose)	20.4 ± 3.11	9.70 ± 3.41[b]	16.6 ± 7.53
$GI_{24\,h}$ (% of terazosin dose)	0.148 ± 0.114	0.208 ± 0.289	1.01 ± 1.05[a]

Abbreviations: $Ae_{0-24\,h}$, percentage of dose excreted in 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_R, time-averaged renal clearance; CL_{NR}, time-averaged nonrenal clearance; $GI_{24\,h}$, percentage of dose recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h; MRT, mean residence time; $t_{1/2}$, half-life; V_{ss}, apparent volume of distribution at steady state. Data are expressed as mean ± s.d. (control, $n=8$; with dexamethasome phosphate, $n=7$; with troleandomycin, $n=7$). Significant difference from control, [a]$P<0.05$, [b]$P<0.001$ and [c]$P<0.01$.

(CL_{NR}). The contribution of gastrointestinal (including biliary) excretion of unchanged DA-8159 or terazosin to the CL_{NR} of DA-8159 or terazosin seemed almost negligible; the $GI_{24\,h}$ values of DA-8159 or terazosin were 1.46 or 0.245% of the i.v. dose, respectively (Table 1). Moreover, the percentages of DA-8159 at a p.o. dose of 10 mg kg^{-1} or terazosin at an i.v. dose of 5 mg kg^{-1} excreted in 24 h bile as an unchanged drug were <0.1% in rats ($n=5$) (Shim *et al*., 2003) or 1.96%, respectively, after bile duct cannulation. Note that the almost negligible values of $GI_{24\,h}$ of DA-8159 or terazosin (Table 1) were not due to degradation of DA-8159 or terazosin in the rat's gastric fluid; DA-8159 and terazosin were stable in five rat gastric juices (pHs of 1.5, 1.0, 2.0, 1.5 and 3.0, respectively) using the reported method (Yu *et al*., 2003). The above data suggest that the CL_{NR} of DA-8159 or terazosin listed in Table 1 could represent the metabolic clearance of DA-8159 or terazosin. Thus, the changes in the CL_{NR} of DA-8159 or terazosin could represent the metabolic changes of DA-8159 or terazosin in rats.

After simultaneous single i.v. administration of terazosin and DA-8159, the slower CL_{NR} of terazosin with DA-8159 could be due to the inhibition of the metabolism of terazosin by DA-8159 via CYP3A1 and/or 3A2. DA-8159 is metabolized mainly via CYP3A1 and/or 3A2 in rats (Kim *et al*., 2005a), and terazosin is also metabolized via CYP3A1 and/or 3A2 in rats (Table 5). The hepatic first-pass effect of terazosin was estimated (Lee and Chiou, 1983) by dividing the CL_{NR} of terazosin (Table 1) by the reported hepatic blood flow rate of 55.2 ml min^{-1} kg^{-1} (Davies and Morris, 1993) and hematocrit of approximately 45% (Mitruka and Rawnsley, 1981) in rats. The hepatic first-pass effect of terazosin thus estimated was 59.0%. As, terazosin is an intermediate hepatic extraction ratio drug, its hepatic clearance depends on the intrinsic clearance (CL_{int}), free (unbound to plasma proteins) fractions of terazosin in plasma and hepatic blood flow rate in rats (Wilkinson and Shand, 1975). The significantly slower CL_{NR} of terazosin by DA-8159 (Table 1) could be supported by the significantly slower CL_{int} for the disappearance of terazosin with DA-8159 (Table 4). The contribution of free fractions of terazosin in plasma and hepatic blood flow rate to the significantly slower CL_{NR} of terazosin with DA-8159 seemed to be almost negligible; the free fractions of terazosin were comparable between with and without DA-8159. The plasma protein binding values of terazosin at a 0.5 μg ml^{-1} were 41.3 ± 2.79 and 47.5 ± 8.89% for with and without DA-8159 at a 0.5 μg ml^{-1} in control rats ($n=5$), respectively, using the equilibrium dialysis technique (Shim *et al*., 2000); they were not significantly different. DA-8159 had no effect on hepatic blood flow rate in rats (an internal report). The plasma protein binding values of DA-8159 at 0.5 μg ml^{-1} were 65.3 ± 2.35 and 68.4 ± 7.47% for with and without terazosin at 0.5 μg ml^{-1}, respectively; they were also comparable between two groups.

After simultaneous p.o. administration of terazosin and DA-8159, the AUC of terazosin was also significantly greater than that after terazosin alone (Tables 2 and 3). This could be for the same reasons as already proposed for the i.v. studies. However, this was not due to the increase in the gastrointestinal absorption of terazosin after simultaneous administration of DA-8159 because the fractions of terazosin dose remaining in gastrointestinal tract at 24 h were less than 7.04%. For comparison, the mean 'true' fractions of p.o. dose of terazosin unabsorbed (F_{unabs}) from the gastrointestinal tract in this study could be estimated using the reported equations (Lee and Chiou, 1983). The F_{unabs} values thus estimated were 6.87 and 1.25% for terazosin alone and with DA-8159, respectively. Thus, more than 93% of p.o. dose of terazosin was absorbed in both groups of rats. The significantly greater AUC of oral terazosin with DA-8159 (Tables 2 and 3) could also be due to inhibition of intestinal metabolism of terazosin by DA-8159 via CYP3A1 and/or 3A2. This could be supported by rat tissue homogenates study. After 30 min incubation of 0.1 μg of terazosin with 5 μg of DA-8159 with rat small intestinal homogenates in a water-bath shaker kept at 37°C and 500 o.p.m., the amount of terazosin remaining in small intestine was significantly greater (11.8% increase) than that of without DA-8159. Note that the $GI_{24\,h}$ values for both drugs were <7.04% of dose (Tables 1–3) which could not affect considerably the overall pharmacokinetics of the two drugs.

The blood pressure lowering effect was considerable after simultaneous p.o. administration of both drugs than that after each drug alone (Figure 5), and this could be due to elevation of plasma terazosin concentration, because of the

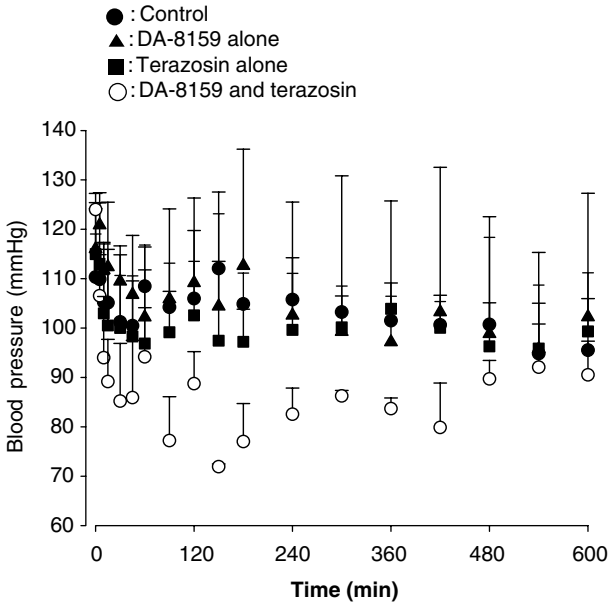

Figure 5 Arterial systolic blood pressure (mmHg) in control rats ($n=3$), and after single p.o. administration of DA-8159 at a dose of 30 mg kg^{-1} ($n=3$), terazosin at a dose of 5 mg kg^{-1} ($n=3$), or both ($n=3$) to rats. Data are presented as mean ± s.d.

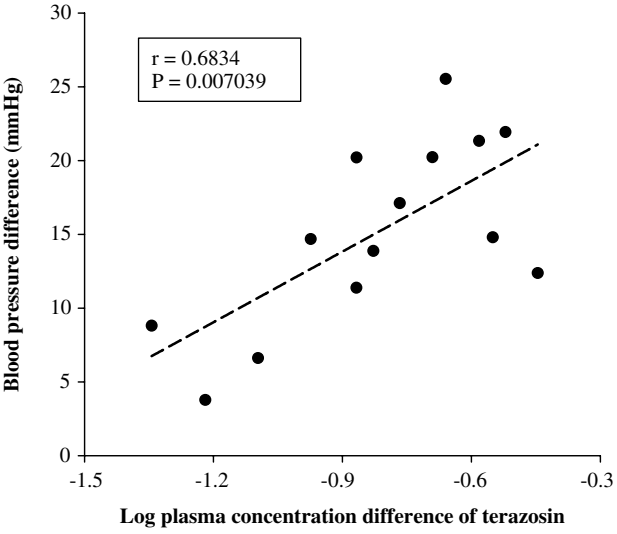

Figure 6 Relationship between log mean plasma concentration difference of terazosin (the concentrations with DA-8159 minus the concentrations without DA-8159) and mean changes in systolic blood pressure (the blood pressure with DA-8159 minus the blood pressure without DA-8159; the values were expressed in terms of + instead of −) after oral administration of both terazosin and DA-8159.

inhibition of terazosin metabolism by DA-8159 via CYP3A1 and/or 3A2 (Table 4). A positive correlation was found between the log increase terazosin plasma concentration and blood pressure changes (Figure 6). A possible correlation was also found between log plasma concentrations of prazosin and blood pressure differences in healthy male volunteers (Bateman *et al*., 1979). All these results suggest that p.o. DA-8159 and terazosin should not be given simultaneously. Human studies are required to determine the time interval needed to avoid these interactions between p.o. DA-8159 and terazosin.

Conclusions

After simultaneous i.v. and p.o. administration of terazosin and DA-8159 to rats, the AUC values of terazosin were significantly greater than those after terazosin alone (Tables 1, 2 and 3). This could be due to hepatic (both i.v. and p.o.) and intestinal (p.o.) inhibition of metabolism of terazosin via CYP3A1 and/or 3A2 by DA-8159 in rats. The blood pressure lowering effects after simultaneous p.o. administration of both drugs were considerable compared with those of each drug alone (Figure 5), and this may be due to significantly higher plasma concentration of terazosin after simultaneous p.o. administration of both drugs (Figure 6).

Acknowledgements

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Conflict of interest

The authors state no conflict of interest.

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