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Pharmacokinetics of DA-8159, a new erectogenic, administered at 10:00 h versus 22:00 h in rats

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Abstract

Little is known about chronopharmacokinetics of PDE V inhibitors in rats as well as in humans. Hence, the pharmacokinetics of DA-8159 and one of its metabolites, DA-8164, were investigated after intravenous and oral administration of DA-8159 at a dose of 30 mg/kg administered at 10:00 h versus 22:00 h in rats. After intravenous administration of DA-8159 at 22:00 h, the AUC of DA-8159 was significantly greater (528 versus 368 μg min/ml) due to significantly slower CL (56.1 versus 79.5 ml/min/kg) in the rats. After intravenous administration of DA-8159 at 22:00 h, the AUC of DA-8164 was also significantly greater (108 versus 66.8 μg min/ml) possibly due to significantly greater exposure of the parent drug (AUC of DA-8159). After intravenous administration of DA-8164 at 22:00 h, the CL of DA-8164 was significantly slower; hence, this factor could also contribute to the greater AUC of DA-8164 after intravenous administration of DA-8159. However, after oral administration of DA-8159, the AUC values of both DA-8159 and DA-8164 were not significantly different between 10:00 h and 22:00 h. This was not due to decrease in gastrointestinal absorption of DA-8159 at 22:00 h and may be due to changes in intestinal first-pass effect at 22:00 h. The above data suggested that modification of dosage regimen of oral DA-8159 is not necessary in humans between 10:00 h and 22:00 h. Further studies are needed in humans.

Keywords: DA-8159; 10:00 h and 22:00 h; Pharmacokinetics; Rats; DA-8164

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

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1. Introduction

Circadian changes in pharmacokinetics (chronopharmacokinetics) have been reported in animals and humans for more than a hundred drugs (Reinberg and Smolensky, 1982; Bruguerolle, 1987; Lévi et al., 1989; Reinberg, 1992; Milano and Chamorey, 2002). Recently, chronopharmacokinetics of acetaminophen (Kolawole et al., 2002), folate (Ahn et al., 2005), desmopressin (Rembratt et al., 2004), cyclosporine A (Reyna Rodriguez et al., 2004), and tacrolimus (Tada et al., 2003) were reported in humans.

A new inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type V (PDE V), DA-8159, 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one, has been synthesized (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, South Korea) for the treatment of male erectile dysfunction. The mechanism (Doh et al., 2002) and erectogenic effects (Ahn et al., 2003; Kang et al., 2003) of DA-8159 in animals were reported.

Based on in vitro metabolism of DA-8159 in liver microsomes containing Baculovirus-expressed rat cytochrome P450 (CYP) isozymes, DA-8159 was metabolized to three metabolites; DA-8164 (*N*-dealkylated DA-8159; 5-[2-propyloxy-5-(aminosulfonyl)-phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo (4,3-d)pyrimidine-7-one) via CYP1A1 and 2C12, M1 (hydroxy DA-8159; 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-1-methyl-3-hydroxypropyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyriidine-7-one) via CYP2D1, and M2 (*N*-demethyl DA-8159;5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one) via CYP1A1 and 2D1 (Choi et al., 2002). Formation of DA-8164 was not a main metabolic pathway for DA-8159 in rats; the intrinsic clearance (CL_{int}) values for the formation of M1, M2, and DA-8164 were 43.0, 0.08, and 16.6 ml/min/mg protein, respectively (Choi et al., 2002). Based on in vivo studies with various enzyme inducers and inhibitors, metabolism of DA-8159 and formation of DA-8164 were mainly mediated via CYP3A1/2 in rats (our unpublished data). For example, in the rats pretreated with dexamethasone (a main inducer of CYP3A1/2 in rats), the total area under the plasma concentration–time curve from time zero to time infinity (AUC) values of DA-8159 and DA-8164 were significantly smaller and greater, respectively, than those in control rats. Reversed results were obtained in rats pretreated with troleandomycin (a main inhibitor of CYP3A1/2 in rats). However, the AUC values of DA-8159 were not significantly different by pretreatment with phenobarbital, isoniazid, 3-methylcholanthrene, and quinine (main inducers of CYP2B1/2, 2E1, and 1A1/2, and a main inhibitor of 2D1, respectively, in rats). The differences between in vitro (Choi et al., 2002) and in vivo (our unpublished data) results on CYP isozymes for the metabolism of DA-8159 and formation of DA-8164 were due to differences in in vitro and in vivo studies (Shim et al., in press). CYP3A4 was the major enzyme for the formation of DA-8164 in human liver microsomes (Ji et al., 2004). Glucuronide- and sulfate-conjugations were not involved in DA-8159 metabolism (Choi et al., 2002). DA-8159 is now being evaluated in phase III clinical trial for the treatment of male erectile dysfunction in Korea.

Although circadian changes in pharmacokinetics of many drugs were reported as mentioned earlier, little is known about chronopharmacokinetics of PDE V inhibitors in rats as well as in humans. The aim of this study was to report the time-dependent pharmacokinetic changes of DA-8159 and DA-8164 after intravenous and oral administration of DA-8159 at a dose of 30 mg/kg at 10:00 h and 22:00 h using rats as an animal model. The time-dependent pharmacokinetic changes of DA-8164 after intravenous administration of DA-8164 at a dose of 10 mg/kg at 10:00 h and 22:00 h were also reported.

2. Materials and methods

2.1. Chemicals

DA-8159, DA-8164, and sildenafil (an internal standard of high-performance liquid chromatographic, HPLC analysis) were supplied from the Research Laboratory of Dong – A Pharmaceutical Company. *N*,*N*-dimethylacetamide (DMA) and polyethylene glycol 400 (PEG 400) were products from Sigma–Aldrich Chemical Company (St. Louis, MO). Other chemicals were of reagent grade or

HPLC grade, and were therefore used without further purification.

2.2. Animals

Male Sprague–Dawley rats of seven weeks of age (weight, 180–200 g) were purchased from Charles River Company, Korea (Biogenomics, Seoul, South Korea). All rats were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea) at a temperature between 20 and 23 °C with 12-h light and dark cycles (light: 07:00 h–19:00 h, dark: 19:00 h–07:00 h) and a relative humidity of 50%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air and with food (Samyang Company, Seoul, South Korea) and water ad libitum. The Animal Care and Use Committee of the College of Pharmacy, Seoul National University, approved the animal study protocol.

2.3. Intravenous infusion study

The procedures for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration; only in the intravenous administration) were reported (Kim et al., 1993). DA-8159 (dissolved in 0.05M citric acid) at a dose of 30 mg/kg was infused (total infusion volume of approximately 0.6 ml) over 1 min via the jugular vein of rats at 10:00 h ($n = 8$) and 22:00 h ($n = 10$). Approximately 0.22-ml 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, and 720 min after intravenous administration of DA-8159. Approximately 0.3-ml aliquot of the heparinized 0.9% NaCl-injectable solution (20 units/ml) was used to flush each cannula immediately after each blood sampling to prevent blood clotting. Blood samples were centrifuged immediately and a 100-μl aliquot of each plasma sample was stored in a −70 °C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until HPLC analysis of DA-8159 and DA-8164 (Shim et al., 2002). At the end of 24 h, the metabolic cage was rinsed twice with 20 ml of distilled water and the rinsings were combined with the 24-h urine. After measuring the exact volume of the combined urine sample, a 100-μl aliquot of the combined urine samples was stored in a −70 °C freezer until HPLC analysis of DA-8159 and DA-8164 (Shim et al., 2002). At the same time (24 h), each rat was exsanguinated via the carotid artery without anesthesia and sacrificed by cervical dislocation, and then the entire gastrointestinal tract (including its contents and feces) was removed, transferred into a beaker containing 100 ml of methanol (to facilitate the extraction of DA-8159 and DA-8164), and cut into small pieces using scissors. After manual shaking and stirring with a glass rod, two 100-μl aliquots of the supernatant were collected from each beaker and stored in a −70 °C freezer until HPLC analysis of DA-8159 and DA-8164 (Shim et al., 2002). Similar experiment was also performed with DA-8164; DA-8164 (dissolved in DMA: PEG 400 = 1:1, v/v) at a dose of 10 mg/kg was administered intravenously (total infusion volume of approximately 0.6 ml) for 1 min to rats at 10:00 h ($n = 8$) and 22:00 h ($n = 12$).

2.4. Oral study

DA-8159 (the same solution as used in the intravenous study) at a dose of 30 mg/kg was administered orally (total oral volume of approximately 1 ml) using a feeding tubing at 10:00 h ($n = 7$) and 22:00 h ($n = 7$) after fasting for 12 h with free access to tap water. Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, and 720 min after oral administration of DA-8159. Other procedures were similar to those in the intravenous study.

2.5. Plasma protein-binding study

Protein bindings of DA-8159 to rat plasma at 10:00 h and 22:00 h were determined using an equilibrium dialysis technique (Shim et al., 2000). One milliliter of plasma was dialyzed against 1 ml of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% dextran (“the buffer”) to minimize volume shift (Boudinot and Jusko, 1984), with 1-ml dialysis cell (Fisher Scientific, Fair Lawn, N.J.) and Spectra/Por 4 membrane (mol. wt. cutoff of 12,000–14,000; Spectrum Medical Industries, Los Angeles, CA). To reduce equilibrium time, DA-8159 was spiked into the plasma side (Øie and Guentert, 1982) at a DA-8159 concentration of 0.2 μg/ml with or without the presence of 0.1 μg/ml of DA-8164. The spiked dialysis cell was

incubated for 24 h in a water-bath shaker kept at 37 °C and at a rate of 50 oscillations per min (opm) (Shim et al., 2000). The binding of DA-8164 at DA-8164 concentration of 10 μg/ml was also determined.

2.6. HPLC analysis of DA-8159 and DA-8164

The concentrations of DA-8159 and DA-8164 in the above biological samples were analyzed by the slight modification of the reported HPLC method developed from our laboratories (Shim et al., 2002). To a 0.1-ml aliquot of biological sample, a 0.1-ml aliquot of 0.1 M Na_2CO_3 containing 3 μg/ml of sildenafil (an internal standard) and a 1-ml aliquot of ethylether were added. After vortex centrifugation at 12,000 rpm for 2 min, the ether layer was collected and dried under a gentle stream of nitrogen gas. A 0.1-ml aliquot of the mobile phase was added to reconstitute the residue and a 0.05-ml aliquot was injected directly onto a reversed-phase column. The mobile phase, 20 mM KH_2PO_4 (pH = 4.7): acetonitrile (72:28, v/v), was run at a flow rate of 1.5 ml/min, and the column effluent was monitored by an UV-detector set at 292 nm at room temperature. The retention times of DA-8159, DA-8164, and 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 20 ng/ml. The coefficients of variation of the assay (within-day and between-day) were generally low below 10.1% for plasma and 9.91% for urine. This HPLC method was validated according to the international guideline (International Conference on Harmonization, 1995).

The HPLC system consisted of Gynkotek autosampler (Gynkotek, Munich, Germany), a model L-6000 pump (Hitachi, Tokyo, Japan), a reversed-phase (C_{18}) column (150 mm, *l.* × 4.6 mm, i.d.; particle size, 5 μm; Hichrom HPRPB, Berkshire, UK), a model UVIS200 UV detector (Linear, Reno, NV), and a model D-2500 integrator (Hitachi).

2.7. Pharmacokinetic analysis

The AUC was calculated by the trapezoidal rule–extrapolation method; this method employed the logarithmic trapezoidal rule for the calculation of the area during the declining level in plasma (Chiou, 1978) and the linear trapezoidal rule during the rising level in plasma. The total area from the last datum point to infinity was estimated by dividing the last measured concentration in plasma by the terminal rate constant.

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the following pharmacokinetic parameters: the terminal half-life, mean residence time (MRT), apparent volume of distribution at steady state (V_{ss}), and time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances (Kim et al., 1993). The maximum plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were directly read from the experimental data. The extent of absolute oral bioavailability (F) was calculated by dividing the AUC after oral administration by the AUC after intravenous administration.

The harmonic mean method was used to calculate the mean values of V_{ss} (Chiou, 1979), terminal half-life (Eatman et al., 1977), and each clearance (Chiou, 1980) and the geometric mean method for AUC (Türck et al., 2000).

2.8. Statistical analysis

A $P < 0.05$ was considered to be statistically significant using the *t*-test between two means for unpaired data. All data are expressed as mean (coefficients of variation, %).

3. Results

3.1. Pharmacokinetics of DA-8159 and DA-8164 after intravenous administration of DA-8159 at a dose of 30 mg/kg to rats at 10:00 h and 22:00 h

After intravenous administration of DA-8159, the mean arterial plasma concentrations of DA-8159 declined in a polyexponential fashion for both groups of rats (Fig. 1A). At 22:00 h, the AUC of DA-8159 was significantly greater (43.5% increase; $P < 0.01$), CL (29.4% decrease; $P < 0.01$), CL_R (25.6% decrease; $P < 0.05$), and CL_{NR} (28.1% decrease; $P < 0.01$) of DA-8159 were significantly slower, and V_{ss} of DA-8159 was significantly smaller (44.4% decrease; $P < 0.01$) than those at 10:00 h (Table 1). Other pharmacokinetic parameters of DA-8159 listed in Table 1 were not significantly different between 10:00 h and 22:00 h. After intravenous administration of DA-8159 at 22:00 h, the C_{max} of DA-8164 was significantly higher (25.7% increase; $P < 0.05$) and

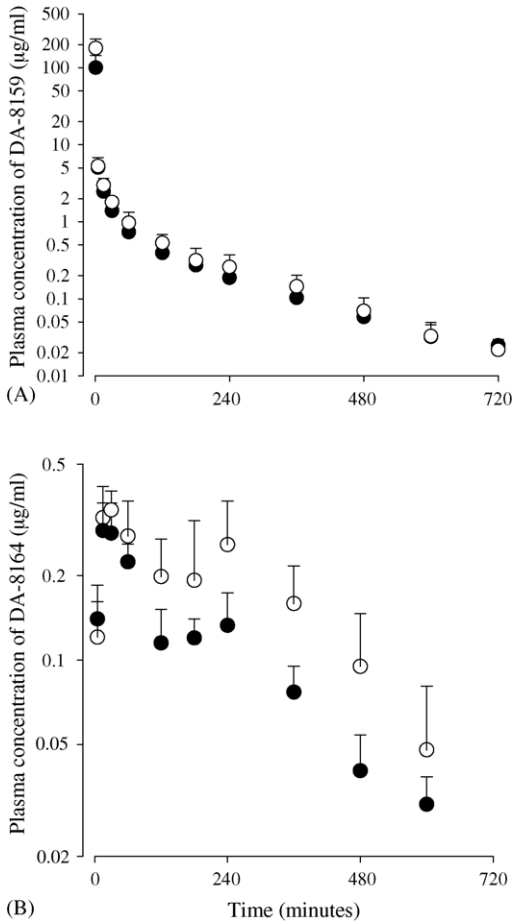

Fig. 1. Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after 1-min intravenous infusion of DA-8159 at a dose of 30 mg/kg to rats at 10:00 h (●; $n = 8$) and 22:00 h (○; $n = 10$). Vertical bars represent standard deviation.

AUC of DA-8164 was significantly greater (61.7% increase; $P < 0.05$) (Table 1). Other pharmacokinetic parameters of DA-8164 listed in Table 1 were not significantly different between 10:00 h and 22:00 h.

3.2. Pharmacokinetics of DA-8164 after intravenous administration of DA-8164 at a dose of 10 mg/kg to rats at 10:00 h and 22:00 h

After intravenous administration of DA-8164, the mean arterial plasma concentrations of DA-8164 declined in a polyexponential fashion for both groups of rats (Fig. 2). At 22:00 h, the AUC of DA-8164 was

Table 1
Pharmacokinetic parameters of DA-8159 and DA-8164 after intravenous administration of DA-8159 at a dose of 30 mg/kg to rats at 10:00 h and 22:00 h[a]

Parameter	10:00 h ($n = 8$)	22:00 h ($n = 10$)
DA-8159		
AUC (μg min/ml)	368 (23.2)	528 (18.2)[b]
Terminal half-life (min)	140 (60.7)	122 (67.7)
MRT (min)	82.2 (19.1)	66.0 (27.0)
CL (ml/min/kg)	79.5 (29.0)	56.1 (16.1)[b]
CL_R (ml/min/kg)	5.44 (56.2)	4.05 (25.0)[c]
CL_{NR} (ml/min/kg)	72.2 (33.7)	51.9 (16.2)[b]
*V*ss (l/kg)	6.24 (46.2)	3.47 (34.2)[b]
$Ae_{0-24\,h}$ (% of dose of DA-8159)	8.51 (49.3)	7.45 (17.1)
$GI_{24\,h}$ (% of dose of DA-8159)	0.656 (98.0)	0.747 (38.2)
Plasma protein-binding (%)		
Without DA-8164	49.6 (34.1)	48.1 (26.8)
With DA-8164	36.9 (30.1)	45.6 (19.1)
DA-8164		
AUC (μg min/ml)	66.8 (24.4)	108 (40.1)[c]
Terminal half-life (min)	162 (36.3)	126 (45.9)
C_{max} (μg/ml)	0.300 (23.8)	0.377 (18.2)[c]
T_{max} (min)	28.1 (52.9)	72.0 (124)
$Ae_{0-24\,h}$ (% of dose of DA-8159)	0.325 (114)	0.0891 (20.4)
$GI_{24\,h}$ (% of dose of DA-8159)	0.139 (108)	0.130 (59.4)

[a] Each value represents the mean (coefficients of variation, %).
[b] 22:00 h was significantly different ($P < 0.01$) from 10:00 h.
[c] 22:00 h was significantly different ($P < 0.05$) from 10:00 h.

significantly greater (34.6% increase; $P < 0.001$), CL (26.3% decrease; $P < 0.001$), CL_R (67.8% decrease; $P < 0.001$), and CL_{NR} (27.7% decrease; $P < 0.001$) of DA-8164 were significantly slower, and total amount of unchanged DA-8164 excreted in 24-h urine ($Ae_{0-24\,h}$; expressed in terms of intravenous dose of DA-8164; 53.4% decrease; $P < 0.001$) and V_{ss} of DA-8164 (23.8% decrease; $P < 0.05$) were significantly smaller than those at 10:00 h (Table 2).

3.3. Pharmacokinetics of DA-8159 and DA-8164 after oral administration of DA-8159 at a dose of 30 mg/kg to rats at 10:00 h and 22:00 h

After oral administration of DA-8159, the mean arterial plasma concentrations of DA-8159 (Fig. 3A) and DA-8164 (Fig. 3B) were similar for both groups of rats. Pharmacokinetic parameters of DA-8159 and DA-

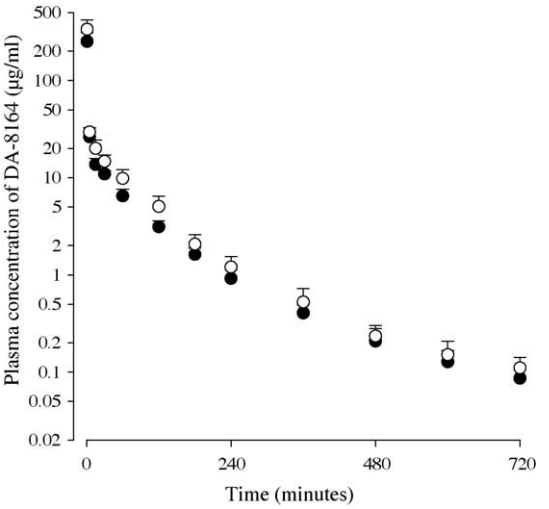

Fig. 2. Mean arterial plasma concentration–time profiles of DA-8164 after 1-min intravenous infusion of DA-8164 at a dose of 10 mg/kg to rats at 10:00 h (●; $n = 8$) and 22:00 h (○; $n = 12$). Vertical bars represent standard deviation.

8164 listed in Table 3 were not significantly different between two groups of rats except significantly smaller total amount of unchanged DA-8159 (81.4% decrease; $P < 0.01$) and DA-8164 (44.0% decrease; $P < 0.05$) recovered from the entire gastrointestinal tract at 24 h ($GI_{24\,h}$; expressed in terms of oral dose of DA-8159) at 22:00 h.

Table 2
Pharmacokinetic parameters of DA-8164 after intravenous administration of DA-8164 at a dose of 10 mg/kg to rats at 10:00 h and 22:00 h[a]

Parameter	10:00 h ($n = 8$)	22:00 h ($n = 12$)
AUC (μg min/ml)	1820 (8.54)	2450 (10.9)[b]
Terminal half-life (min)	145 (30.9)	160 (23.8)
MRT (min)	81.8 (14.7)	85.0 (19.5)
CL (ml/min/kg)	5.47 (8.70)	4.03 (11.0)[b]
CL_R (ml/min/kg)	0.0438 (30.2)	0.0141 (175)[b]
CL_{NR} (ml/min/kg)	5.45 (9.72)	3.94 (34.0)[b]
*V*ss (l/kg)	0.442 (13.3)	0.337 (22.4)[c]
$Ae_{0-24\,h}$ (% of dose of DA-8164)	0.824 (19.5)	0.384 (24.8)[b]
$GI_{24\,h}$ (% of dose of DA-8164)	2.37 (75.8)	1.61 (51.3)
Plasma protein-binding (%)	73.9 (4.24)	79.4 (3.06)[c]

[a] Each value represents the mean (coefficients of variation, %).
[b] 22:00 h was significantly different ($P < 0.001$) from 10:00 h.
[c] 22:00 h was significantly different ($P < 0.05$) from 10:00 h.

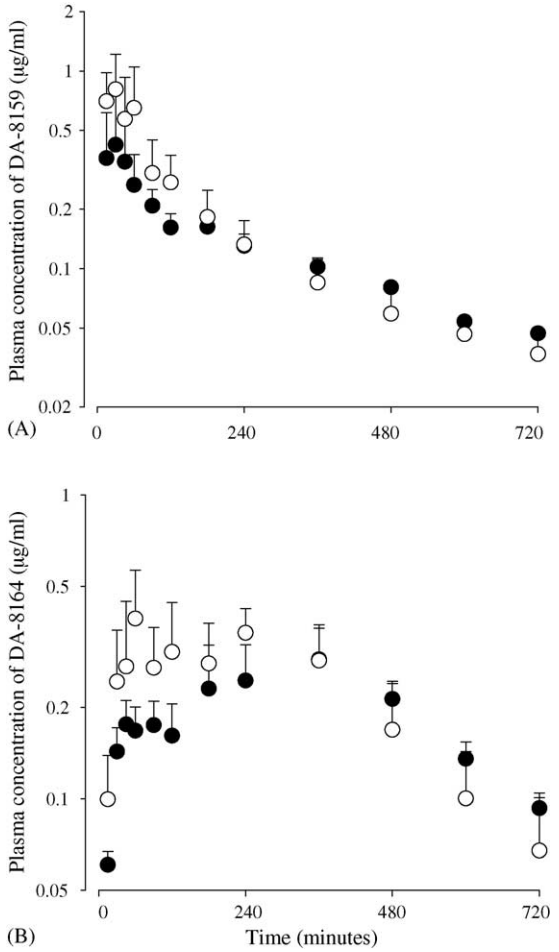

Fig. 3. Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after oral administration of DA-8159 at a dose of 30 mg/kg to rats at 10:00 h (●; $n = 7$) and 22:00 h (○; $n = 7$). Vertical bars represent standard deviation.

3.4. Plasma protein-binding study

After both intravenous and oral administration of DA-8159 to rats at both 10:00 h and 22:00 h, the plasma concentrations of both DA-8159 and DA-8164 were maintained for a long period of time in the range of 0.05–0.5 μg/ml (Figs. 1 and 3). Hence, the DA-8159 and DA-8164 concentrations of 0.2 and 0.1 μg/ml, respectively, were arbitrarily chosen in the plasma protein-binding studies. At 22:00 h, the plasma protein-binding of DA-8159 was considerably greater (23.6% increase; $P < 0.0824$) in the presence of DA-8164

Table 3
Pharmacokinetic parameters of DA-8159 and DA-8164 after oral administration of DA-8159 at a dose of 30 mg/kg to rats at 10:00 h and 22:00 h[a]

Parameter	10:00 h ($n = 7$)	22:00 h ($n = 7$)
DA-8159		
AUC (μg min/ml)	106 (23.2)	125 (29.7)
Terminal half-life (min)	232 (64.3)	307 (42.1)
CL_R (ml/min/kg)	8.60 (90.0)	7.51 (47.9)
C_{max} (μg/ml)	0.469 (55.9)	1.02 (37.0)[b]
T_{max} (min)	40.7 (59.1)	30.0 (50.0)
$Ae_{0–24\,h}$ (% of dose of DA-8159)	3.52 (34.3)	3.64 (36.4)
$GI_{24\,h}$ (% of dose of DA-8159)	1.81 (59.5)	0.337 (63.2)[b]
F (%)	28.9	24.1
DA-8164		
AUC (μg min/ml)	164 (33.1)	178 (25.6)
Terminal half-life (min)	174 (89.7)	204 (33.8)
C_{max} (μg/ml)	0.319 (33.9)	0.445 (32.1)
T_{max} (min)	291 (38.4)	178 (25.6)
$Ae_{0–24\,h}$ (% of dose of DA-8159)	0.0876 (31.8)	0.111 (51.9)
$GI_{24\,h}$ (% of dose of DA-8159)	0.243 (42.6)	0.136 (45.2)[c]

[a] Each value represents the mean (coefficients of variation, %).
[b] 22:00 h was significantly different ($P < 0.01$) from 10:00 h.
[c] 22:00 h was significantly different ($P < 0.05$) from 10:00 h.

(Table 1) and the value of DA-8164 was significantly greater (7.44% increase; $P < 0.05$) (Table 2).

4. Discussion

DA-8164 was a main metabolite in humans and pharmacological effect of DA-8164 in terms of PDE V inhibitory activity was half of that of DA-8159 (an internal report). Hence, the pharmacokinetics of DA-8164 were evaluated in this study. It was reported that the AUC values of DA-8159 were dose-proportional after intravenous administration at doses of 5–30 mg/kg and oral administration at doses of 20–30 mg/kg in rats (Shim et al., 2003). Hence, the 30 mg/kg of DA-8159 was arbitrarily chosen in this study.

After intravenous infusion of DA-8159 at 22:00 h, the AUC of DA-8159 was significantly greater due to significantly slower CL of DA-8159 (Table 1). The slower CL of DA-8159 at 22:00 h was due to significantly slower both CL_R and CL_{NR} of DA-8159 (Table 1). Contribution of CL_R to CL of DA-8159 was not considerable; the values were 6.84 and 7.22% for 10:00 h and 22:00 h, respectively (Table 1). Contribution of gastrointestinal (including biliary) excretion of unchanged DA-8159 to CL_{NR} of DA-8159 was almost negligible; the $GI_{24\,h}$ values were 0.656 and 0.747% of intravenous dose of DA-8159 for 10:00 h and 22:00 h, respectively (Table 1). Moreover, the percentages of oral dose of DA-8159 (10 mg/kg) excreted in 24-h bile as unchanged drug were less than 0.1% in four rats (Shim et al., 2003). The above data indicated that CL_{NR} values of DA-8159 listed in Table 1 could represent metabolic clearance values of DA-8159. Hence, the slower CL_{NR} of DA-8159 at 22:00 h (Table 1) was due to slower metabolism of DA-8159. The $Ae_{0–24\,h}$ values of DA-8159 were comparable (not significantly different) between two groups of rats (Table 1). Hence, the significantly slower CL_R of DA-8159 at 22:00 h could be mainly due to significantly greater AUC of DA-8159 (Table 1). The V_{ss} of DA-8159 at 22:00 h was significantly smaller and this may be due to considerably smaller free (unbound to plasma proteins) fraction of DA-8159; the free fractions were 63.1 and 54.4% for 10:00 h and 22:00 h, respectively, in the presence of DA-8164 (Table 1). After intravenous infusion of DA-8164 at 22:00 h, the V_{ss} of DA-8164 was significantly smaller due to significantly smaller free fraction of DA-8164 in the rats; the free fractions were 26.1 and 20.6% for 10:00 h and 22:00 h, respectively (Table 2). Changes in volume of distribution of drugs due to plasma protein-binding changes with circadian time were also reported (Guentert, 1984).

After intravenous infusion of DA-8159 at 22:00 h, the AUC of DA-8164 was significantly greater (Table 1). This could be due to significantly greater exposure of the parent drug (the AUC of DA-8159) at 22:00 h. The $AUC_{DA\text{-}8164}/AUC_{DA\text{-}8159}$ ratios were similar for both groups of rats; the values were 18.2 and 20.5% for 10:00 h and 22:00 h, respectively (Table 1). After intravenous administration of DA-8164 at 22:00 h, the AUC of DA-8164 was significantly greater and the CL of DA-8164 was significantly slower (Table 2). Hence, this factor could also contribute to the significantly greater AUC of DA-8164 after intravenous administration of DA-8159 at 22:00 h (Table 1).

After oral administration of DA-8159, the AUC values of both DA-8159 and DA-8164 were not significantly different between two groups of rats (Table 3), although the values were significantly greater after

intravenous administration (Table 1). This was not due to decreased gastrointestinal absorption of DA-8159 at 22:00 h as estimated by the reported equations (Lee and Chiou, 1983); more than 98% of oral dose of DA-8159 was absorbed from gastrointestinal tract for both groups of rats. Although the exact reason is not known, comparable AUC values of both DA-8159 and DA-8164 after oral administration of DA-8159 could be due to changes in the intestinal first-pass effect at 22:00 h; the intestinal first-pass effect of DA-8159 at a dose of 30 mg/kg was approximately 59% of oral dose in rats (Shim et al., 2003). After oral administration of DA-8159, formation of DA-8164 was considerably greater than that after intravenous infusion of DA-8159; the $AUC_{DA\text{-}8164}/AUC_{DA\text{-}8159}$ ratios were 18.2 and 20.5% after intravenous infusion for 10:00 h and 22:00 h, respectively (Table 1). However, the corresponding values after oral administration of DA-8159 were 155 and 142% (Table 3). This suggested that intestinal first-pass effect of DA-8159 to form DA-8164 was considerable after oral administration in rats.

5. Conclusions

After oral administration of DA-8159, the AUC values of both DA-8159 and DA-8164 were comparable between two groups of rats (Table 3). This may be due to changes in intestinal first-pass effect of DA-8159 at 22:00 h. DA-8159 was developed for oral administration. Although the efficacy and toxicity studies were not performed in the present study, the modification of dosage regimen of DA-8159 did not seem to be necessary between oral administration at 10:00 h and 22:00 h, if the present rat data could be extrapolated to humans.

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