Effects of water deprivation on the pharmacokinetics of DA-8159, a new erectogenic, in rats

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ABSTRACT. Purpose: To test the effect of 72 h water deprivation on the non-renal clearance (CL) of DA-8159 in a rat model of dehydration. DA-8159 is mainly metabolized via CYP3A1/2 and the expression and mRNA level of CYP3A1/2 are not affected by dehydration. **Methods:** DA-8159 (30 mg/kg) was administered intravenously or orally to male control Sprague–Dawley rats and rat model of dehydration. **Results:** As expected, after intravenous administration, the CL_{NR} values of DA-8159 were comparable between two groups of rats. This could be supported by comparable intrinsic CL of DA-8159 using hepatic microsomes for both groups of rats. However, the CL was significantly slower in rat model of dehydration due, at least in part, to significantly slower renal CL in rat model of dehydration. The slower CL_R in rat model of dehydration could be due to urine flow rate-dependent renal CL of DA-8159; the less urine output, the less the urinary excretion of unchanged DA-8159. After oral administration, the AUC values of DA-8159 were not significantly different between two groups of rats, although the AUC of DA-8159 in rat model of dehydration was significantly greater than controls after intravenous administration. This could be possibly due to changes in the intestinal first-pass effects in rat model of dehydration. **Conclusions:** After intravenous administration of DA-8159, the non-renal CL values were comparable between two groups of rats due to the lack of effect of dehydration on CYP3A1/2.

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INTRODUCTION

DA-8159 (Udenafil), 5-[2-propyloxy-5-(1-methyl-2-pyrollidinylethylamidosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one, a new inhibitor of cyclic guanosine monophosphate (cGMP)-specific phophodiesterase type V (PDE V), has been synthesized for the treatment of male erectile dysfunction (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, South Korea). DA-8159 is metabolized to 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) in mice, rats, rabbits, dogs, and humans (1). Mechanism (2) and erectogenic effects (3, 4) of DA-8159 have been reported. DA-8159 is a potent, selective, and competitive inhibitor of human PDE V. *In vitro* experiments using a series of PDE isozymes (PDE I, II, III, V, and VI) indicated that DA-8159 is highly selective and potent antagonist of PDE V from human and rabbit platelets producing IC_{50} values of 8.25 and 5.84 nM, respectively (2). Oral DA-8159 is now being evaluated in phase III clinical trial for the treatment of male erectile dysfunction in Korea (Dong-A Pharmaceutical Company, Seoul, South Korea).

Recently, Kim et al. (5) have reported that metabolism of DA-8159 and formation of DA-8164 is mainly catalyzed via the hepatic microsomal cytochrome P450 (CYP) 3A1/2 in male Sprague Dawley rats. For example, pretreated with dexamethasone (a main inducer of CYP3A1/2 in rats), reduced the area under the plasma concentration–time curve (AUC) of DA-8159 after intravenous administration to male Sprague–Dawley rats while increased that of DA-8164 is increased. On the other hand, pretreated with troleandomycin (a main inhibitor of CYP3A1/2 in rats) has opposite effects on the AUC of the drug and its metabolite. However, 3-methylcholanthrene, phenobarbital, and isoniazid (main inducers of CYP1A1/2, 2B1/2, and 2E1, respectively, in rats), and quinine (a main inhibitor of CYP2D1 in rats) appears to have no effect of the AUC of DA-8159.

Dehydration occurs by excessive sweating, polyuria, severe diarrhea, and hyperthermia (6). Water deprivation may cause significant hormonal, physiological, and biochemical changes in the body (7–11 and references therein). For example, kidney and/or liver functions seemed to be impaired in rat model of dehydration based on blood and urine chemistry data and/or microscopic examinations of

the kidney and liver. Therefore, it could be expected that the pharmacokinetics and hence pharmacodynamics of drugs could be altered in water deprivation. Since, the first report on the effects of water deprivation on aspirin disposition kinetics (6), water deprivation has been reported to alter the disposition kinetics of various drugs (7–11 and references therein).

Kim et al. (12) reported that in male Sprague–Dawley rats with 72-h water deprivation (rat model of dehydration), the expressions of CYP1A2, 2B1/2, 2C11, and 3A1/2 were not changed, however, that of CYP2E1 was three-fold induced based on Western blot analysis. The mRNA level of CYP2E1 also increased in rat model of dehydration based on Northern blot analysis (12). Hence, it would be expected that the hepatic metabolism of DA-8159 would not changed in rat model of dehydration. Although the pharmacokinetic changes of many drugs in rat model of dehydration were reported (7–11 and references therein), the changes with respect to CYP isozymes seemed not to be reported except chlorzoxazone (11) and oltipraz (13). In this regard, DA-8159 was chosen in this study, since the drug is metabolized mainly via CYP3A1/2 in rats (5) and CYP3A1/2 was not changed in rat model of dehydration (12). Moreover, DA-8159 could be used in humans with dehydration state. The purpose this study is to find whether the pharmacokinetic parameters of DA-8159 are not changed after intravenous and oral administration of DA-8159 in rat model of dehydration with respect to CYP3A1/2.

METHODS AND MATERIALS

Chemicals

DA-8159, DA-8164, and sildenafil [an internal standard of high-performance liquid chromatographic (HPLC) analysis of DA-8159 and DA-8164] were supplied from Research Laboratory of Dong-A Pharmaceutical Company. N,N-dimethylacetamide (DMA), β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), ethylenediamine tetraacetatic acid (EDTA), and tri(hydroxymethyl)aminomethane (Tris)-buffer were products from Sigma–Aldrich Corporation (St. Louis, MO). Polyethylene glycol 400 (PEG 400) was purchased from Duksan Chemical Company (Seoul, South Korea). Other chemicals were of reagent grade or HPLC grade.

Animals

Male Sprague–Dawley rats (weighting 270 to 315 g) were purchased from Charles River Company Korea (Orient, Seoul, South Korea). All rats were maintained in a light-controlled room (light: 0700–1900, dark: 1900–0700) kept at a temperature of 22 ± 2 °C and a relative humidity of 55 ± 5% (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea). The rats were randomly divided into two groups, control rats and rat model of dehydration. For control rats, water and food (Sam Yang Company, Seoul, South Korea) were supplied *ad libitum* for 72-h; for rat model of dehydration, water was deprived for 72-h with free access to food. Food intakes and body weights were recorded daily for four days (before water deprivation and the first, second, and third days after water deprivation) for each group of rats. The study protocol was approved by the Animal Care and Use Committee of the Collage of Pharmacy, Seoul National University.

Measurement of Vmax, Km, and CLint for the Disappearance of DA-8159 and for the Formation of DA-8164 in Hepatic Microsomal Fractions

The procedures were similar to the reported methods (11). The livers of control rats and rat model of dehydration (n = 6; each) were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany). The protein level was measured by the reported method (14). 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 DA-8159 and for the formation of DA-8164 were determined after incubating the above microsomal fraction (equivalent to 1 mg of protein), a 10-μL aliquot of DA-8159 (dissolved in 0.05 M citric acid to have substrate concentrations of 1, 2, 5, 10, 20, 50, and 200 μM), and a 50-μL (1.2 mM) aliquot of NADPH in a total volume of 300 μL by 100 mM phosphate buffer (pH 7.0), in a water-bath shaker kept at 37 ^{o}C and at a rate of 50 oscillations per min (opm) for 10 min. All of the above microsomal incubation conditions were linear. The reaction was terminated by adding a 1-mL aliquot of ethylether after 10-min incubation, and a 100-μL aliquot of 0.1 N Na_2CO_3 containing 3 μg/mL of sildenafil (an internal standard) was added. DA-8159 and DA-8164 were measured by the reported HPLC method (15). The kinetic constants (K_m and V_{max}) for the disappearance of DA-8159 and for the formation of DA-8164 were calculated using the Lineweaver–Burk plot (16) with the method of least

squares. The intrinsic clearance (CL_{int}) values for the disappearance of DA-8159 and for the formation of DA-8164 were calculated by dividing respective V_{max} by respective K_m.

Intravenous Administration of DA-8159 or DA-8164 to Rats

On the fourth day, the carotid artery (for blood sampling) and the jugular vein (for drug administration) of each rat were cannulated under the light ether anesthesia (17,18). And then, 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 the anesthesia before the study began. DA-8159 (dissolved in 0.05 M 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 control rat (n = 9) and rat model of dehydration (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. Heparinized 0.9% NaCl-injectable solution (20 units/mL), approximately 0.3 ml, was used to flush each cannula after each blood sampling to prevent blood clotting. At the end of experiment (24 h), each metabolic cage was rinsed with 15 mL of distilled water and the rinsings were combined with 24-h urine sample. After measuring the exact volume of combined urine sample, a 0.1-mL aliquot of the combined urine sample was stored in a −70°C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until HPLC analysis of DA-8159 (15). At the same time (24 h), as much blood as possible was collected via the carotid artery and each rat was 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 stirring with a glass rod for 1 min, two 0.1-mL aliquots of the supernatant were collected and stored in a –70°C freezer until HPLC analysis of DA-8159 and DA-8164 (15). Similar experiment was also performed with DA-8164. DA-8164 (dissolved in DMA : PEG 400 = 1 : 1; v/v, to produce a concentration of 5 mg/mL) at a dose of 10 mg/kg was infused to control rats (n = 9) and rat model of dehydration (n = 9). Approximately 0.22-mL aliquot of blood sample was collected via the carotid artery at 0, 1, 5, 15, 30, 60, 120, 180, 240, 360, 480, 600, and 720 min after intravenous administration of DA-8164. Other procedures are similar to those of DA-8159 studies.

Oral Administration of DA-8159 in Rats

On the fourth day, the carotid artery of each rat was cannulated under light ether anesthesia (17,18). DA-8159 (the same solution as used in the intravenous study) at a dose of 30 mg/kg was administered orally using a feeding tube in control rats (n = 11) and rat model of dehydration (n = 10). Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600, or 1440 min after oral administration of DA-8159. Other procedures were similar to those in the intravenous studies.

Protein Binding of DA-8159 to Rat Plasma Using an Equilibrium Dialysis Technique

Plasma protein binding of DA-8159 to additional control rats and rat model of dehydration (n = 5; each) was determined at a DA-8159 plasma concentration of 5 μg/mL using an equilibrium dialysis technique (19). One mL of fresh plasma was dialyzed against 1 mL of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% dextran, with 1 mL dialysis cell (Fisher Scientific, Fair Lawn, NJ) and Spectra/Por 4 membrane (mol. wt. cutoff of 12,000–14,000; Spectrum Medical Industries, Los Angeles, CA). The spiked dialysis cell was incubated for 24 h in a water-bath shaker kept at 37 ^{o}C and at a rate of 50 opm (19).

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 (15). To a 0.1-mL aliquot of biological sample, a 0.1-mL aliquot of 0.1 N 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 16,000 *g* 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 (C_{18}) 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 0.02 μg/mL. The coefficients of variation were below 9.4%.

Table 1: Mean (± standard deviation) V_{max}, K_m, and CL_{int} values for the disappearance of DA-8159 and for the formation of DA-8164 in liver microsomes of control rats and rat model of dehydration (n = 6; each).

Parameter	Disappearance of DA-8159		Formation of DA-8164	
	Control	Dehydration	Control	Dehydration
V_{max} (nmol/min/mg protein)	0.293±0.129	0.219±0.101	0.0547±0.0461	0.0925±0.0550
K_m (μM)	18.6±8.93	15.8±6.09	61.1±56.0	81.0±52.1
CL_{int} (μL/min/mg protein)	15.8±2.26	13.4±2.74	0.966±0.167	1.15±0.0707

Pharmacokinetic Analysis

AUC was calculated by the trapezoidal rule–extrapolation method; this method employs the logarithmic trapezoidal rule for the calculation of the area during the phase of a declining level in plasma (20) and the linear trapezoidal rule for the phase of a rising level in plasma. The area from the last datum point to time infinity was estimated by dividing the last measured concentration in plasma by the terminal rate constant.

Compartment and model independent methods (21) were used to calculate the following pharmacokinetic parameters; the time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, terminal half-life, total area under the first moment of the plasma concentration–time curve from time zero to time infinity (AUMC), mean residence time (MRT), apparent volume of distribution at steady state (Vd_{ss}), and extent of absolute oral bioavailability (F) (17). The maximum 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 Vd_{ss} (22), terminal half-life (23), and clearance values (24).

Statistical Analysis

A $p < 0.05$ was considered to be statistically significant using the *t*-test between the two means for the unpaired data. All data are expressed as mean ± standard deviation.

RESULTS

Measurement of V_{max}, K_m, and CL_{int} for the Disappearance of DA-8159 and for the Formation of DA-8164 in Hepatic Microsomal Fractions

V_{max}, K_m, and CL_{int} for the disappearance of DA-8159 were comparable between control rats and rat model of dehydration (Table 1), suggesting that the maximum velocity for the disappearance (mainly due to metabolism) of DA-8159, affinity of DA-8159 to the enzyme(s), and disappearance of DA-8159 were not affected considerably by dehydration. Similar trends were also obtained for the formation of DA-8164 (Table 1). Protein contents were comparable between control rats and rat model of dehydration (18.7 ± 1.50 and 19.5 ± 2.00 mg/g liver).

Pharmacokinetics of DA-8159 and DA-8164 after Intravenous Administration of DA-8159 in Rats

After intravenous administration of DA-8159 at a dose of 30 mg/kg in control rats and rat model of dehydration, the mean arterial plasma concentration–time profiles of DA-8159 and DA-8164 are shown in Figures 1A and 1B, respectively, and some relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration of DA-8159 in rat model of dehydration, the changes in pharmacokinetic parameters of DA-8159 are as follows; the AUC was significantly greater (33.9% increase), MRT was significantly longer (95.0% increase), Vd_{ss} was significantly larger (32.7% increase), CL (18.6% decrease) and CL_R (62.3% decrease) were significantly slower, and percentages of intravenous dose of DA-8159 excreted in 24-h urine ($Ae_{0–24\ h,\ DA\text{-}8159}$) and recovered from the entire gastrointestinal tract at 24 h ($GI_{24\ h,\ DA\text{-}8159}$) as unchanged drug were significantly smaller (37.1% decrease) and greater (105% increase), respectively, than controls. After intravenous administration of DA-8159 in rat model of dehydration, the changes in pharmacokinetic parameter of DA-8164 are as follows; the AUC was significantly greater (108% increase), CL_R was significantly slower (80.7% decrease), and $Ae_{0–24\ h,\ DA\text{-}8164}$ and $GI_{24\ h,\ DA\text{-}8164}$ were significantly smaller (75.2% decrease) and greater (313% increase), respectively, than controls.

Pharmacokinetics of DA-8164 after Intravenous Administration of DA-8164 in Rats

After intravenous administration of DA-8164 at a dose of 10 mg/kg in control rats and rat model of dehydration, the mean arterial plasma concentration–time profiles of DA-8164 are shown in Figure 2, and some relevant pharmacokinetic parameters are listed in Table 3.

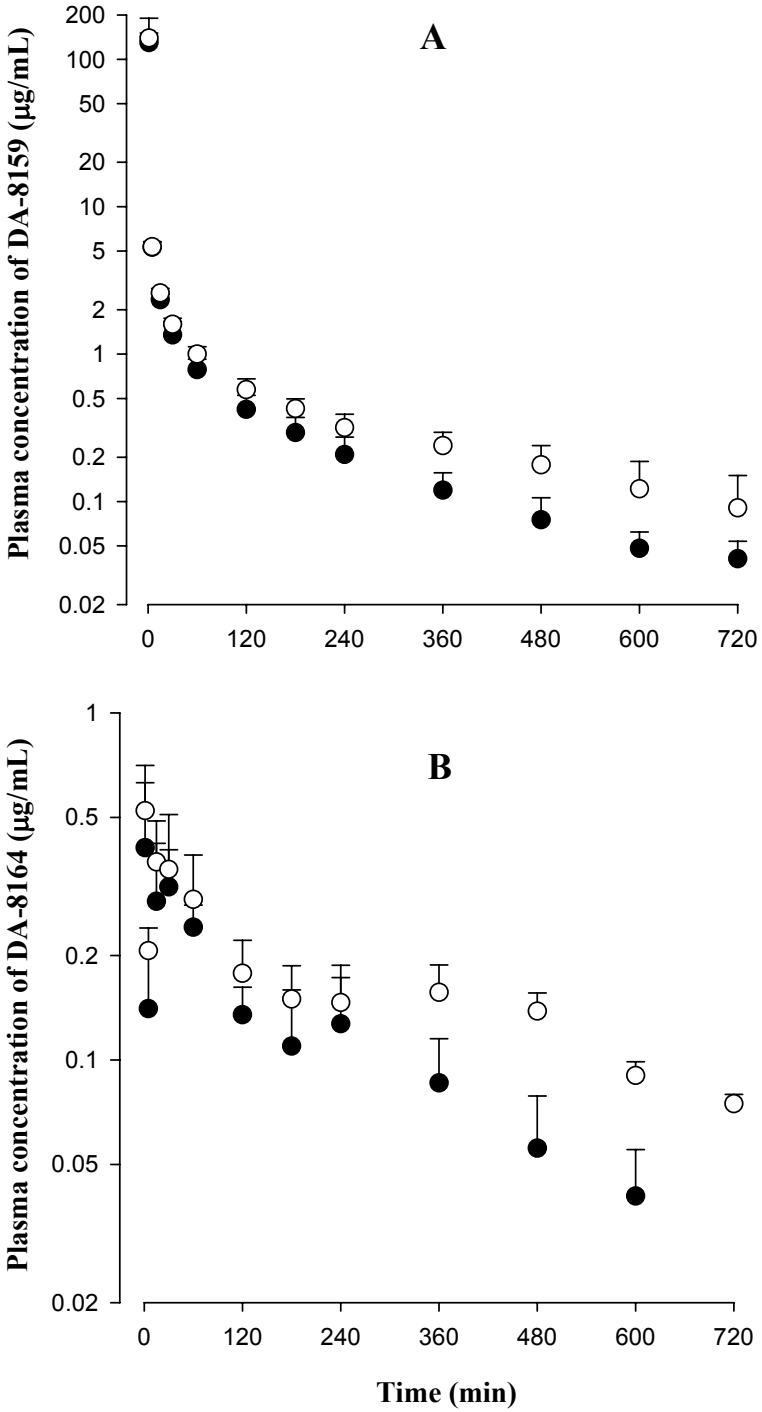

Figure 1: Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after 1-min intravenous infusion of DA-8159 at a dose of 30 mg/kg to control rats (●; n = 9) and rat model of dehydration (○; n = 10). Bars represent standard deviation.

Table 2: Mean (± standard deviatition) pharmacokinetic parameters of DA-8159 and DA-8164 after intravenous administration of DA-8159 at a dose of 30 mg/kg to control rats and rat model of dehydration.

Parameter	Control (n = 9)			Dehydration (n = 10)		
Body weight (g)						
Initial	303	±	28.9	310	±	28.7
Final	320	±	14.6	253	±	24.2[a]
Hematocrit (%)	50.6	±	6.62	64.4	±	5.74[a]
Urine output (mL/24-h)	24.9	±	6.90	1.71	±	0.756[a]
DA-8159						
AUC (μg * min/mL)	436	±	45.7	584	±	120[b]
Terminal half-life (min)	177	±	68.7	187	±	102
MRT (min)	84.6	±	21.5	165	±	85.1[c]
Vd_{ss} (L/kg)	5.50	±	1.18	7.30	±	3.45[c]
CL (mL/min/kg)	69.9	±	8.16	56.9	±	9.74[c]
CL_R (mL/min/kg)	5.28	±	1.28	1.99	±	1.02[a]
CL_{NR} (mL/min/kg)	64.4	±	7.71	54.7	±	9.36
$Ae_{0–24\ h,\ DA\text{-}8159}$ (% of dose of DA-8159)	7.79	±	1.44	4.90	±	2.30[c]
$GI_{24\ h,\ DA\text{-}8159}$ (% of dose of DA-8159)	0.963	±	0.317	1.97	±	1.10[c]
DA-8164						
AUC (μg * min/mL)	78.8	±	23.2	164	±	68.0[b]
CL_R (mL/min/kg)	0.358	±	0.295	0.0692	±	0.0565[a]
Terminal half-life (min)	197	±	61.0	219	±	154
C_{max} (μg/mL)	0.390	±	0.146	0.500	±	0.154
T_{max} (min)	21.9	±	18.3	9.60	±	12.2
$Ae_{0–24\ h,\ DA\text{-}8164}$ (% of dose of DA-8159)	0.173	±	0.121	0.0412	±	0.0312[c]
$GI_{24\ h,\ DA\text{-}8164}$ (% of dose of DA-8159)	0.136	±	0.0583	0.562	±	0.446[c]

[a] *Significantly different ($p < 0.001$) from control.*
[b] *Significantly different ($p < 0.01$) from control.*
[c] *Significantly different ($p < 0.05$) from control.*

Table 3: Mean (± standard deviation) pharmacokinetic parameters of DA-8164 after intravenous administration of DA-8164 at a dose of 10 mg/kg to control rats and rat model of dehydration.

Parameter	Control (n = 9)			Dehydration (n = 9)		
Body weight (g)						
Initial	288	±	14.7	294	±	10.1
Final	299	±	14.5	243	±	5.65[a]
Urine output (mL/24-h)	19.7	±	5.94	2.14	±	1.18[a]
AUC (μg * min/mL)	1710	±	279	2170	±	265[b]
Terminal half-life (min)	132	±	21.7	128	±	26.2
MRT (min)	101	±	17.2	114	±	32.6
CL (mL/min/kg)	5.83	±	0.985	4.60	±	0.602[b]
CL_R (mL/min/kg)	0.0479	±	0.0155	0.00672	±	0.0200[d]
CL_{NR} (mL/min/kg)	5.78	±	0.985	4.57	±	0.595[b]
Vd_{ss} (L/kg)	0.585	±	0.0759	0.495	±	0.116
$Ae_{0–24\ h,\ DA\text{-}8164}$ (% of dose of DA-8164)	0.903	±	0.284	0.461	±	0.223[b]
$GI_{24\ h,\ DA\text{-}8164}$ (% of dose of DA-8164)	1.15	±	0.834	1.63	±	2.19

[a] *Significantly different ($p < 0.001$) from control.*
[b] *Significantly different ($p < 0.01$) from control.*

After intravenous administration of DA-8164 in rat model of dehydration, the changes in pharmacokinetic parameters of DA-8164 are as follows; the AUC was significantly greater (26.9% increase), CL (21.1% decrease), CL_R (86.0% decrease), and CL_{NR} (20.9% decrease) were significantly slower, and $Ae_{0–24\ h,\ DA\text{-}8164}$ was significantly smaller (48.9% decrease) than controls.

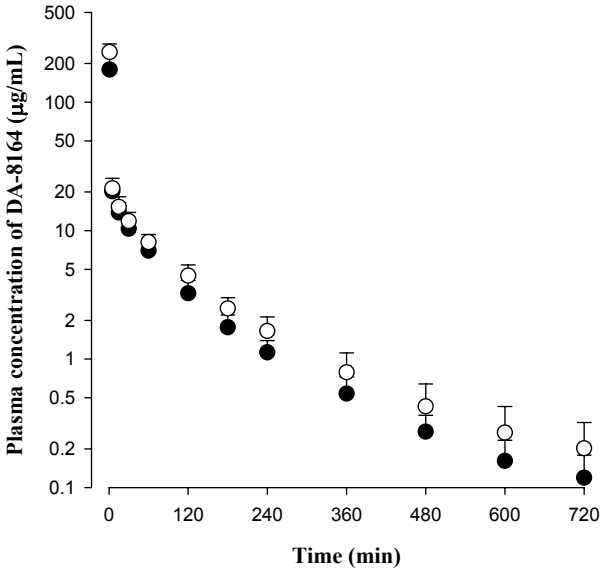

Figure 2: Mean arterial plasma concentration–time profiles of DA-8164 after 1-min intravenous infusion of DA-8164 at a dose of 10 mg/kg to control rats (●; n = 9) and rat model of dehydration (○; n = 9). Bars represent standard deviation.

Pharmacokinetics of DA-8159 and DA-8164 after Oral Administration of DA-8159 in Rats

After oral administration of DA-8159 at a dose of 30 mg/kg in control rats and rat model of dehydration, the mean arterial plasma concentration–time profiles of DA-8159 and DA-8164 are shown in Figures 3A and 3B, respectively, and some relevant pharmacokinetic parameters are listed in Table 4.

After oral administration of DA-8159, DA-8159 was absorbed rapidly and almost completely from rat gastrointestinal tract; DA-8159 was detected in plasma from the first blood sampling time, 15 min, for both groups of rats, and rapidly reached T_{max} at 23.2 and 33.0 min for control rats and rat model of dehydration, respectively. Moreover, the $GI_{24\ h,\ DA\text{-}8159}$ values were 1.60 and 1.53% of oral dose of DA-8159 for control rats and rat model of dehydration, respectively. After oral administration of DA-8159 in rat model of dehydration, the changes in pharmacokinetic parameters of DA-8159 are as follows; the CL_R was significantly slower (61.8% decrease), T_{max} was significantly longer (42.2% increase), and $Ae_{0–24\ h,\ DA\text{-}8159}$ was significantly smaller (55.7% decrease) than controls. After oral administration of DA-8159 in rat model of dehydration, CL_R of DA-8164 was significantly slower (39.0% decrease) than controls.

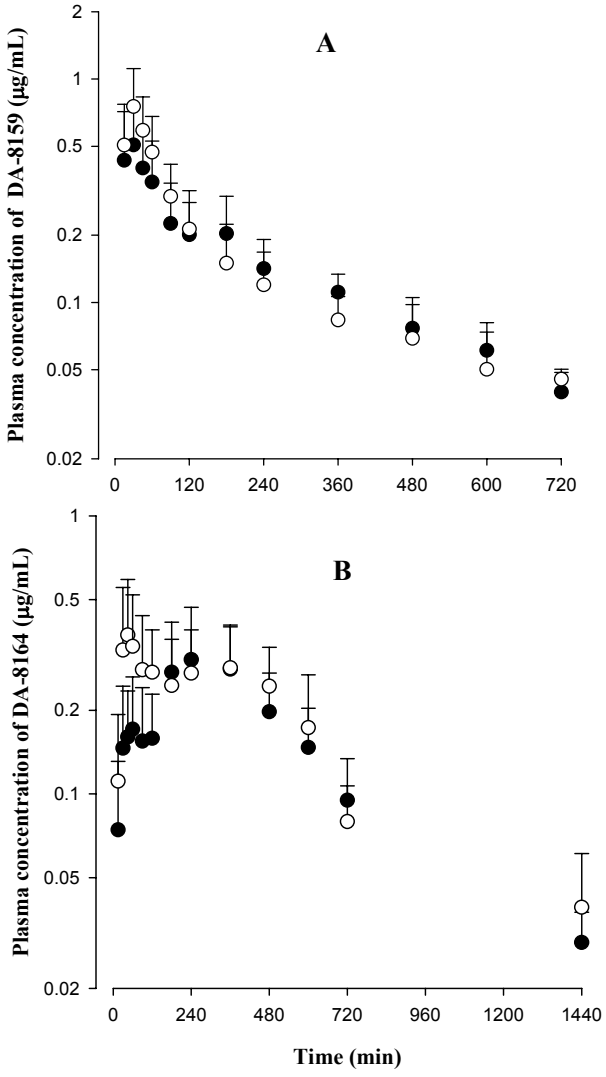

Figure 3: Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after oral administration of DA-8159 at a dose of 30 mg/kg to control rats (●; n = 11) and rat model of dehydration (○; n = 10). Bars represent standard deviations.

Table 4: Mean (± standard deviation) pharmacokinetic parameters of DA-8159 and DA-8164 after oral administration of DA-8159 at a dose of 30 mg/kg to control rats and rat model of dehydration.

PARAMETER	Control (n = 11)			Dehydration (n = 10)		
Body weight (g)						
Initial	295	±	20.3	295	±	20.3
Final	296	±	10.7	252	±	15.5[a]
Hematocrit (%)	46.6	±	2.59	56.3	±	3.65[a]
Urine output (mL/24-h)	15.6	±	10.3	4.63	±	1.19[b]
DA-8159						
AUC (μg * min/mL)	111	±	32.2	118	±	21.8
Terminal half-life (min)	218	±	45.7	243	±	112
CL_R (mL/min/kg)	9.55	±	1.73	3.65	±	1.39[a]
C_{max} (μg/mL)	0.537	±	0.246	0.792	±	0.352
T_{max} (min)	23.2	±	7.83	33.0	±	9.49[c]
$Ae_{0–24\ h,\ DA\text{-}8159}$ (% of dose of DA-8159)	3.66	±	1.26	1.62	±	0.823[a]
$GI_{24\ h,\ DA\text{-}8159}$ (% of dose of DA-8159)	1.60	±	1.59	1.53	±	0.818
F (%)		25.5			20.2	
DA-8164						
AUC (μg * min/mL)	192	±	66.9	243	±	66.4
CL_R (mL/min/kg)	0.134	±	0.0594	0.0818	±	0.0150[b]
Terminal half-life (min)	245	±	107	294	±	172
C_{max} (μg/mL)	0.362	±	0.129	0.450	±	0.174
T_{max} (min)	276	±	75.9	200	±	178
$Ae_{0–24\ h,\ DA\text{-}8164}$ (% of dose of DA-8159)	0.0954	±	0.0406	0.0726	±	0.0201
$GI_{24\ h,\ DA\text{-}8164}$ (% of dose of DA-8159)	0.159	±	0.0984	0.199	±	0.0743

[a] *Significantly different ($p < 0.001$) from control.*
[b] *Significantly different ($p < 0.01$) from control.*
[c] *Significantly different ($p < 0.05$) from control.*

DISCUSSION

Induction of dehydration was evident in rat model of dehydration; body weight gain and 24-h urine output were significantly smaller and hematocrit was significantly greater than controls (Tables 2–4). After intravenous administration in control rats, body weight gain increased with days; the mean body weights were 303 ± 28.9, 316 ± 18.9, 319 ± 17.0, and 320 ± 14.6 g for before and the first, second, and third days, respectively. However, in rat model of dehydration, dehydration caused significant decrease in body weight gain; the corresponding values were 310 ± 28.7, 287 ± 22.6, 274 ± 23.1, and 253 ± 24.2 g. In control rats, daily food intakes were almost constant; the mean values were 22.1 ± 4.40, 26.1 ± 4.79, and 25.1 ± 1.08 g for the first, second, and third days, respectively. However, in rat model of dehydration, food intakes decreased with days; the corresponding values were 15.7 ± 5.07, 8.21 ± 2.61, and 5.36 ± 2.01 g. Similar results were also obtained after oral administration (data not shown). The above

data indicated that significant decrease in body weight gain in rat model of dehydration was due to less food consumption in addition to water deprivation. Similar results were also reported from other rat studies (11,12).

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 was evaluated in this study. Shim et al. (18) 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. Hence, the 30 mg/kg of DA-8159 was arbitrarily chosen in this study.

After intravenous administration of DA-8159, contribution of CL_R to CL of DA-8159 was not considerable; the $Ae_{0-24\ h,\ DA-8159}$ values were less than 7.79% of intravenous dose of DA-8159 for both groups of rats (Table 2), indicating that most of the intravenously administered DA-8159 are eliminated via the nonrenal route (CL_{NR}). The contribution of gastrointestinal (including biliary) excretion of unchanged DA-8159 to CL_{NR} of DA-8159 seemed also not to be considerable; the $GI_{24\ h,\ DA-8159}$ values were less than 1.97% of intravenous dose of DA-8159 for both groups of rats (Table 2). The small value in $GI_{24\ h,\ DA-8159}$, less than 1.97%, could not be due to chemical degradation of DA-8159 in gastrointestinal tract; DA-8159 was stable in various pH solutions (25). Moreover, the percentages of oral dose of DA-8159 (30 mg/kg) excreted in 24-h bile as unchanged drug were < 0.1% in 4 rats (18). The above data indicated that the CL_{NR} values of DA-8159 listed in Table 2 could represent metabolic clearances of DA-8159 in rats.

After intravenous administration of DA-8159, the CL_{NR} values of DA-8159 were comparable between two groups of rats (Table 2), and this could be expected because DA-8159 was mainly metabolized via CYP3A1/2 in rats (5) and CYP3A1/2 was not changed in rat model of dehydration (12). This could be supported by comparable *in vitro* CL_{int} values for the disappearance of DA-8159 for both groups of rats (Table 1). After intravenous administration of DA-8159 in rat model of dehydration, the significantly slower CL of DA-8159 was at least partly due to significantly slower CL_R of DA-8159 in rat model of dehydration, although the contribution of CL_R of DA-8159 were not considerable as mentioned earlier (Table 2). The significantly longer MRT of DA-8159 in rat model of dehydration could support the slower CL of DA-8159 in rat model of dehydration (Table 2). The slower CL_R of DA-8159 in rat model of dehydration could be mainly resulted from significantly smaller $Ae_{0-24\ h,\ DA-8159}$ and significantly greater AUC of DA-8159 in rat model of dehydration (Table 2). The smaller $Ae_{0-24\ h,\ DA-8159}$ in rat model of dehydration could be due to urine flow rate-dependent CL_R of DA-8159 in rats. Recently, Kim et al. (26) reported that CL_R of DA-8159 was dependent on urine flow rate in rats; the less urine output, the less $Ae_{0-24\ h,\ DA-8159}$. The 24-h urine output was significantly smaller in rat model of dehydration than controls (93.1, 89.1, and 70.3% decrease; Tables 2–4). The smaller $Ae_{0-24\ h}$ of DA-8159 in rat model of dehydration may also be due to impaired kidney function in rat model of dehydration. Impaired kidney function in rat model of dehydration was also reported from other rat studies (7–11 and references therein).

After intravenous administration of DA-8159 in rat model of dehydration, the Vd_{ss} of DA-8159 was significantly larger than controls (Table 2). However, this could not be due to increase in free (unbound to plasma proteins) fractions of DA-8159 in plasma in rat model of dehydration. The plasma protein binding values of DA-8159 were 67.2 ± 1.57 and 70.2 ± 3.82% for control rats and rat model of dehydration, respectively; they were not significantly different.

After intravenous administration of DA-8159 in rat model of dehydration, the AUC of DA-8164 was significantly greater than controls (Table 2). However, this was not due to increase in expression of CYP3A1/2 in rat model of dehydration since CYP3A1/2 was not changed in rat model of dehydration (12). This could be supported by comparable *in vitro* CL_{int} values for the formation of DA-8164 for both groups of rats (Table 1). The greater AUC of DA-8164 could be due to greater exposure of the parent drug (the significantly greater AUC of DA-8159) in rat model of dehydration (Table 2). In order to explain the significantly greater AUC of DA-8164 after intravenous administration of DA-8159 in rat model of dehydration, DA-8164 was administered intravenously in both groups of rats. After intravenous administration of DA-8164 in rat model of dehydration, the AUC of DA-8164 was significantly greater than controls (Table 3). Moreover, the CL of DA-8164 (Table 3) was significantly slower than that of DA-8159 (Table 2). This factor could also contribute to the significantly greater AUC of DA-8164 after intravenous administration of DA-8159 in rat model of dehydration (Table 3).

After intravenous administration of DA-8159 in rat model of dehydration, the AUC of DA-8159 was significantly greater than controls (Table 2). However, after oral administration of DA-8159, the AUC of DA-8159 was comparable between two groups of rats (Table 4). However, this was not due to decrease in gastrointestinal absorption of DA-8159 in rat model of dehydration. Based on the linear pharmacokinetics (18), the mean "true" fractions of oral dose unabsorbed (F_{unabs}) in this study were estimated based on the reported equation (27). The estimated F_{unabs} values were 1.35 and 1.13% for control rats and rat model of dehydration, respectively. Hence, more than 98% of oral dose of DA-8159 were absorbed from the gastrointestinal tract for both groups of rats. The comparable AUC values of DA-8159 after oral administration of DA-8159 could be due to changes in first-pass effect in rat model of dehydration; the intestinal first-pass effect of DA-8159 at a dose of 30 mg/kg was approximately 59% of oral dose in rats (18). After oral administration of DA-8159, formation of DA-8164 increased compared with that after intravenous administration; the $AUC_{DA\text{-}8164}/AUC_{DA\text{-}8159}$ ratios after intravenous administration of DA-8159 were 18.1 and 28.1% for control rats and rat model of dehydration, respectively, however, the corresponding values after oral administration of DA-8159 were 173 and 206%. This could be due to considerable intestinal first-pass effect of DA-8159 in rats (18).

In the rat model of dehydration, hematocrit was significantly greater than controls after both intravenous and oral administration of DA-8159 (Tables 2 and 4). Similar results were also reported from other rat studies (7,11,28–30). The binding of DA-8159 to blood cells was considerable; the mean plasma-to-blood cells concentration ratios of DA-8159 in three rabbit blood at initial DA-8159 blood concentrations of 1–10 μg/ml were 0.662–0.812 (25). The bound fractions of adriamycin (31) and propranolol (32) to red blood cells were reported to act as barriers for elimination. Hence, the significantly greater hematocrit value in rat model of dehydration (Table 2 and 4) could influence at least partly to the slower CL of DA-8159 in rat model of dehydration (Table 2).

CONCLUSIONS

After intravenous administration of DA-8159 in rat model of dehydration, the CL_{NR} of DA-8159 was comparable to controls (Table 2), since DA-8159 was metabolized mainly via CYP3A1/2 (5) and CYP3A1/2 was not changed in rat model of dehydration (12). However, the CL of DA-8159 was significantly slower than controls, and this could be at least partly due to significantly slower CL_R of DA-8159 in rat model of dehydration (Table 2). The slower CL_R could be due to significantly smaller $Ae_{0–24\ h,\ DA\text{-}8159}$ in rat model of dehydration (Table 2), and this could be due to urine flow rate-dependent CL_R of DA-8159 in rats (26) and impaired kidney function in rat model of dehydration. After intravenous administration of DA-8159 in rat model of dehydration, the significantly greater AUC of DA-8164 was possibly due to significantly greater exposure of the parent drug (the significantly greater AUC of DA-8159), and significantly greater AUC of DA-8164 in rat model of dehydration (Table 3). After oral administration of DA-8159, the AUC values of both DA-8159 and DA-8164 were comparable between two groups of rats and this could be due to changes in intestinal first-pass effects in rat model of dehydration.

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Nonstandard abbreviations:

HPLC, high-performance liquid chromatography; V_{max}, maximum velocity; K_m, Michaelis–Menten constant; CL_{int}, intrinsic clearance; AUC, total area under the plasma concentration–time curve from time zero to time infinity; MRT, mean residence time; Vd_{ss}, apparent volume of distribution at steady state; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged nonrenal clearance; $Ae_{0–24\ h}$, 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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