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Pharmacokinetics of intravenous and oral DA-8159, a new erectogenic, in rats with protein–calorie malnutrition

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Abstract

Influence of dietary protein deficiency on the pharmacokinetics of DA-8159 and one of its metabolites, DA-8164, was investigated after intravenous and oral administration of DA-8159 at a dose of 30 mg kg^{-1} to male Sprague–Dawley rats allowed free access to a 23% (control) or 5% (protein–calorie malnutrition, PCM) casein diet for 4 weeks. The total area under the plasma concentration–time curve from time zero to time infinity (AUC) values of DA-8164 were significantly smaller after both intravenous (87.0 vs 162 μg min mL^{-1}) and oral (144 vs 319 μg min mL^{-1}) administration of DA-8159 to PCM rats. This could be due to the decrease in CYP3A1/2 (50–60%) in the rats because DA-8164 was mainly formed via CYP3A1/2 in rats. This could be supported by significantly slower in-vitro CL_{int} (2.04 ± 0.646 vs 3.15 ± 0.693 μL min^{-1} (mg protein)$^{-1}$) for the formation of DA-8164 in hepatic microsomal fraction of PCM rats. After intravenous administration of DA-8159, the AUC values of DA-8159 were not significantly different between the two groups of rats although the AUC of DA-8164 was significantly smaller in PCM rats, and this may be due to the minor metabolic pathway of DA-8164 in rats. However, after oral administration of DA-8159, the AUC of DA-8159 was significantly greater in PCM rats (194 vs 122 μg min mL^{-1}). This was not due to enhanced absorption of DA-8159 from the gastrointestinal tract in the rats but may be due to a decreased intestinal first-pass effect of DA-8159 in the rats.

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Introduction

Drugs are generally metabolized to more water-soluble derivatives by enzymes located in the endoplasmic reticulum and cytoplasm of the liver. The rate of drug metabolism may be influenced by various physiological, genetic and environmental factors. Nevertheless, nutritional status was not usually investigated as a factor that may affect the pharmacokinetics and hence the pharmacodynamics of drugs. Of particular importance for the majority of the world's population is malnutrition, specifically protein–calorie malnutrition (PCM). The changes in drug metabolism and pharmacokinetics in malnutrition have been reviewed (Buchanan 1978; Krishnaswamy 1978). Changes in the pharmacokinetics and pharmacodynamics of theophylline (Jung 1985a), paracetamol (Jung 1985b), furosemide, bumetanide, adriamycin, azosemide, phenytoin, chlorzoxazone, itraconazole, 2-(allylthio)pyrazine (a new chemopreventive agent), clarithromycin, DA-7867 (a new oxazolidinone) (Bae et al 2004b and references therein) and torasemide (Bae et al 2004a) in PCM rats have been reported.

DA-8159 (Figure 1) has been synthesized (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, Korea) as an inhibitor of cyclic guanosine monophosphate (cGMP)-specific phophodiesterase type V (PDE V), the predominant isozyme metabolizing cGMP in the corpus cavernosum (Goldenberg 1998). DA-8159 was incubated with microsomes containing Baculovirus-expressed rat hepatic microsomal cytochrome P450s (CYPs) and was metabolized to three metabolites – M1, M2 and DA-8164 (Figure 1) (Choi et al 2002). M1 was the predominant metabolic pathway 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 μL min^{-1} (mg protein)$^{-1}$, respectively. Glucuronide and sulfate conjugations were not involved in DA-8159 metabolism.

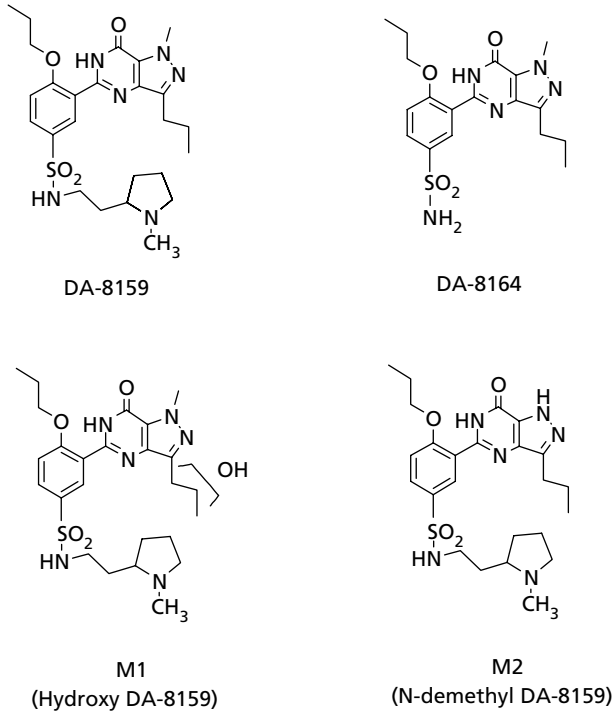

Figure 1 Chemical structures of DA-8159 and its metabolites in rats.

Work in our laboratories has shown that DA-8164 was formed mainly via CYP3A1/2 in male Sprague–Dawley rats pretreated with various enzyme inducers or inhibitors (our unpublished data). It has been reported (Cho et al 1999) that in male Sprague–Dawley rats with PCM, CYP3A1/2 level decreased 40–50% as compared with control. Hence, it would be expected that the pharmacokinetics of DA-8164 could be changed in PCM rats. DA-8159 is now being evaluated in a phase III clinical trial for the treatment of male erectile dysfunction.

The purpose of this study was to compare the pharmacokinetics of DA-8159 and DA-8164 after intravenous and oral administration of DA-8159 at a dose of $30\,mg\,kg^{-1}$ to control and PCM rats. It has been reported (Lado-Abeal et al 1999) that blood levels of both total and free testosterone were subnormal in protein–energy malnutrition patients. It has been also reported (Jannini et al 1999; Shabsigh 2003) that erectile dysfunction is associated with a reduced testosterone blood level. Hence, DA-8159 was chosen in this study.

Materials and Methods

Chemicals

DA-8159, DA-8164 and sildenafil (an internal standard of high-performance liquid chromatographic, HPLC, assay) were supplied by Research Laboratory of Dong-A Pharmaceutical Company. Reduced form of nicotinamide adenine dinucleotide phosphate (NADPH, as a tetrasodium salt), ethylenediaminetetraacetic acid (EDTA), and Tris-buffer were products from Sigma-Aldrich Company (St Louis, MO). Other chemicals were of reagent grade or HPLC grade and were used without further purification.

Animals and diets

Male Sprague–Dawley rats, 150–200 g, were purchased from Charles River Company Korea (Orient, Seoul, Korea). Rats were assigned randomly to a diet containing either 23% (control rats) or 5% (PCM rats) casein. Both diets were isocaloric and the compositions of the diets were listed (Cho et al 1999). Rats were maintained under a 12-h light–dark cycle at a temperature of $22 \pm 2^{\circ}C$ and a relative humidity of $55 \pm 5\%$ for a 4-week period (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea). Food intake and body weight were recorded at least once a week to assess the influence of the low protein diet. The protocol of the animal study was approved by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University.

Preliminary study

In the early morning at the end of the 4-week period of each diet, the abdomen was opened and blood was collected via the abdominal artery from control and PCM rats ($n = 4$, each). Serum was stored at $-70^{\circ}C$ in a freezer (Revco ULT 1490 D-N-S; Western Mednics, Ashville, NC) for the measurement of total proteins, albumin, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), and protein binding of DA-8159 using an equilibrium dialysis technique. After sacrificing each rat by cervical dislocation, each whole liver, kidney and stomach was cut, rinsed or perfused with cold 0.9% NaCl injectable solution, blotted dry with tissue paper and weighed. Small portions of the liver and kidney were fixed in 10% neutral phosphate-buffered formalin and processed for routine histological examination with hematoxylin–eosin staining.

Disappearance of DA-8159 in 9000 *g* supernatant fraction of rat liver homogenates

The procedures were similar (Kim et al 2004) to the previously reported methods (Litterst et al 1975). Incubation was conducted with a 300-μL sample of the 9000 *g* supernatant fraction of liver homogenates of both groups of rats ($n = 5$, each), 10 μL of DA-8159 (5 μg) and 10 μL (1 mM) of NADPH in a final volume of 500 μL by adding a Tris-buffer in a water-bath shaker kept at $37^{\circ}C$ and at a rate of 500 oscillation per min for 30 min. The concentration of DA-8159 was determined by HPLC analysis.

Measurement of V_{max}, K_m, and CL_{int} for the formation of DA-8164 in hepatic microsomal fraction

The procedures were similar to previously reported methods (Kim et al 2004). Incubation was conducted with the hepatic microsomal fraction (equivalent to 1 mg protein)

of both groups of rats (n = 5, each), 10 μL of DA-8159 (to have substrate concentrations of 2, 5, 10, 50, and 200 μM) and 50 μL (1 mM) of NADPH in a final volume of 0.5 mL by adding 0.1 M phosphate buffer, pH 7.4, in a water-bath shaker kept at 37°C and at a rate of 500 oscillations per min. All of the above microsomal incubation conditions were linear. The intrinsic clearance (CL_{int}) for the formation of DA-8164 was calculated by dividing the maximum velocity for the formation of DA-8164 (V_{max}) by the Michaelis–Menten constant (K_m).

Intravenous infusion of DA-8159 in rats

The pretreatment procedures, including the cannulation of the carotid artery and the jugular vein of each rat, were similar to the reported methods (Kim et al 1993; Shim et al 2003). DA-8159 (the DA-8159 powder was dissolved in 0.05 M citric acid) at a dose of 30 mg kg^{-1} was infused (total infusion volume of 2 mL kg^{-1}) over 1 min via the jugular vein of control rats (n = 9) and PCM rats (n = 9). Approximately 0.12 mL of blood was collected via the carotid artery at time 0 (to serve as a control) and at 1 (at the end of the infusion), 5, 15, 30, 60, 120, 180, 240, 360, 480, 600 and 720 min, and 50 μL of each plasma sample was stored in a freezer at −70°C until HPLC analysis of DA-8159 and DA-8164. At 24 h, the metabolic cage was rinsed with 20 mL of distilled water and the rinsings were combined with urine samples. After measuring the exact volume of the combined 24-h urine, 2 × 50 μL of the combined 24-h urine sample were stored in a freezer at −70°C until HPLC analysis of DA-8159 and DA-8164. At the same time (24 h), each rat was sacrificed by cervical dislocation and the entire gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing 100 mL of methanol (to facilitate the extraction of DA-8159 and DA-8164) and then cut into small pieces using scissors. After shaking manually and stirring with a glass rod, 2 × 50 μL of the supernatant was collected from the beaker and stored in a freezer at −70°C until HPLC analysis of DA-8159 and DA-8164. Other procedures were similar to the reported methods (Kim et al 1993; Shim et al 2003).

Oral administration of DA-8159 in rats

DA-8159 (the same solution as used in the intravenous study) at a dose of 30 mg kg^{-1} was administered orally (total oral volume of 3 mL kg^{-1}) to control rats (n = 9) and PCM rats (n = 9) using a feeding tube. Blood sampling times were 0, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600 and 720 min. The other procedures were similar to those of the intravenous study.

Serum protein binding studies

The serum protein binding of DA-8159 to control and PCM rats (n = 5 each) was determined using an equilibrium dialysis technique (Shim et al 2000). One millilitre of serum was dialysed against 1 mL of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% dextran, with a 1-mL dialysis cell (Fisher Scientific, Fair Lawn, NJ) and Spectra/Por 4 membrane (MW cutoff 12 000–14 000; Spectrum Medical Industries Inc., Los Angeles, CA). Serum protein binding of DA-8159 was determined at a DA-8159 concentration of 0.2 μg mL^{-1} with or without the presence of 0.1 μg mL^{-1} 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 (Shim et al 2000).

HPLC analysis of DA-8159 and DA-8164

The concentrations of DA-8159 and DA-8164 in the above biological samples were assayed using a slight modification of the reported HPLC method using the extraction procedure (Shim et al 2002). To a 50-μL biological sample, 0.1 mL of 0.1 M Na_2CO_3 containing 3 μg mL^{-1} of sildenafil (an internal standard) and 1 mL of ethyl ether were added. After vortex centrifugation at 13 400 *g* for 2 min, the ether layer was collected and dried under a gentle stream of nitrogen gas. Mobile phase (0.1 mL) was added to reconstitute the residue and 0.05 mL 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^{-1} and the column effluent was monitored by a 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^{-1}. The coefficients of variation (inter- and intra-day) were generally low (below 7.95%).

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (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 plasma-level phase (Chiou 1978) and the linear trapezoidal rule for the rising plasma-level phase. The total area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal rate constant.

Standard methods (Gibaldi & Perrier 1982) were used to calculate the following pharmacokinetic parameters: the total area under the first moment of plasma concentration–time curve from time zero to time infinity (AUMC), terminal half-life, mean residence time (MRT), apparent volume of distribution at steady state (Vd_{SS}), time-averaged total body (CL), renal (CL_R) and nonrenal (CL_{NR}) clearances and extent of absolute oral bioavailability (F) (Kim et al 1993). The maximum plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were read directly from the experimental data.

The mean values of each clearances (Chiou 1980), Vd_{SS} (Chiou 1979) and terminal half-life (Eatman et al 1977) were calculated by the harmonic mean method.

Statistical analysis

A P value of less than 0.05 was considered to be statistically significant using an unpaired t-test. All data were expressed as mean ± standard deviation (s.d.).

Results

Preliminary study

Effects of dietary protein on the body weight gain, intakes of food, protein and calorie, certain serum values and organ weights from the preliminary studies are listed in Table 1. Protein deprivation for 4 weeks (5% protein diet) caused a significant decrease in body weight gain and food consumption. Rats on the 5% protein diet (PCM rats) consumed approximately 45.8% less food than those on the 23% protein diet (control rats) despite the supply of food being freely available. As a result, the protein and calorie intakes decreased significantly by 88.2 and 45.6%, respectively, in PCM rats. Since both the protein and calorie intakes decreased significantly in PCM rats, it is important to realize that PCM rats suffered from both protein and calorie deficiencies. Therefore, any changes in pharmacokinetics of DA-8159 and DA-8164 in PCM rats should be attributed to PCM and not solely to protein deficiency. In addition, significantly lower serum levels of total proteins (22.7% decrease) and albumin (21.7% decrease) were observed in PCM rats. The absolute liver (50.7% decrease), kidney (44.3% decrease) and stomach (37.3% decrease) weights were significantly lighter in PCM rats than those in control rats due to significant decrease in body weight gain in PCM rats. However, liver weight based on whole body weight was not significantly different between the two groups of rats.

Table 1 Body weight, intakes of food, protein, and calorie, serum data, and liver, kidney, and stomach weights in control and PCM rats

Parameter	Control (n=4)	PCM (n=4)
Initial body weight (g)	180 ± 9.13	195 ± 4.08
Final body weight (g)	369 ± 28.2	189 ± 7.42***
Food intake (g daily/rat)	21.2 ± 0.800	11.5 ± 0.347**
Protein intake (g daily/rat)	4.88 ± 0.184	0.577 ± 0.0174***
Calorie intake (kcal daily/rat)	85.7 ± 3.23	46.6 ± 1.40**
Serum		
Total protein ($g\,dL^{-1}$)	5.630 ± 0.359	4.35 ± 0.208***
Albumin ($g\,dL^{-1}$)	3.450 ± 0.129	2.70 ± 0.0816***
GOT ($IU\,L^{-1}$)	86.5 ± 70.3	75.3 ± 13.9
GPT ($IU\,L^{-1}$)	53.3 ± 48.8	53.3 ± 48.8
Protein binding (%)		
without DA-8164	63.9 ± 7.15	51.8 ± 10.4
with DA-8164	66.4 ± 7.25	55.70 ± 6.82***
Liver weight (g)	13.8 ± 1.63	6.81 ± 0.69***
Liver weight (% of body weight)	3.700 ± 0.256	3.630 ± 0.281
Kidney weight (g)	2.640 ± 0.296	1.4700 ± 0.0710***
Kidney weight (% of body weight)	0.7100 ± 0.0456	0.7870 ± 0.0485*
Stomach weight (g)	1.930 ± 0.218	1.210 ± 0.117***
Stomach weight (% of body weight)	0.5200 ± 0.0493	0.6450 ± 0.0620**

Values are mean ± s.d. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with control. IU, international unit.

Serum levels of GOT and GPT were not significantly different between the two groups of rats. Based on tissue microscopy, there were no significant findings in the liver of control rats and kidneys of both control rats and PCM rats. However, mild hepatocellular degeneration was observed in the liver of PCM rats. No significant histological changes in the kidney of PCM rats have been reported in other rat studies (Kim et al 1993). Our finding is also consistent with the report (Krishnaswamy 1978) stating that kidneys are relatively resistant to starvation.

Disappearance of DA-8159 in the 9000 *g* supernatant fraction of rat liver homogenates

The percentages of DA-8159 disappeared per g liver after a 30-min incubation of 5 μg of DA-8159 with the 9000 *g* supernatant fraction were comparable between the two groups of rats; the mean values were 65.6 and 66.1%, respectively, for control and PCM rats.

Measurement of V_{max}, K_m and CL_{int} for the formation of DA-8164 in hepatic microsomal fraction

In PCM rats, the V_{max} for the formation of DA-8164 was significantly slower (0.0426 ± 0.00873 vs 0.0758 ± 0.0169 nmol min^{-1} (mg protein)$^{-1}$; $P < 0.01$) than in control rats, but the K_m was similar to that in control rats (21.9 ± 4.73 vs 24.3 ± 3.51 μM). This suggested that the maximum velocity for the formation of DA-8164 was significantly slower in PCM rats, but affinity to the enzyme(s) was not changed in PCM rats. As a result, the CL_{int} for the formation of DA-8164 was significantly slower (2.04 ± 0.646 vs 3.15 ± 0.693 μL min^{-1} (mg protein)$^{-1}$; $P < 0.05$) in PCM rats suggesting that formation of DA-8164 decreased significantly.

Pharmacokinetics of DA-8159 and DA-8164 in rats after intravenous infusion of DA-8159

After intravenous infusion of DA-8159, the mean arterial plasma concentrations of DA-8159 declined similarly in a polyexponential fashion for both groups of rats (Figure 2A). In PCM rats, the terminal half-life (26.4% increase) and MRT (65.7% increase) of DA-8159 were significantly longer, and Vd_{SS} (63.4% increase) of DA-8159 were significantly larger than those in control rats (Table 2). Other pharmacokinetic parameters of DA-8159 listed in Table 2 were not significantly different between the two groups of rats. Body weight gain decreased significantly in PCM rats compared with that in control rats (Table 2).

After intravenous administration of DA-8159, formation of DA-8164 was fast; DA-8164 was detected in plasma from the second blood sampling time, 5 min, for both groups of rats (Figure 2B). In PCM rats, the plasma concentrations of DA-8164 were lower (Figure 2B) and the C_{max} of DA-8164 was significantly lower (61.9% decrease) than in control rats (Table 2). This resulted in a significantly smaller AUC for DA-8164 (46.3% decrease) in the rats (Table 2). In PCM rats, the terminal half-life of DA-8164

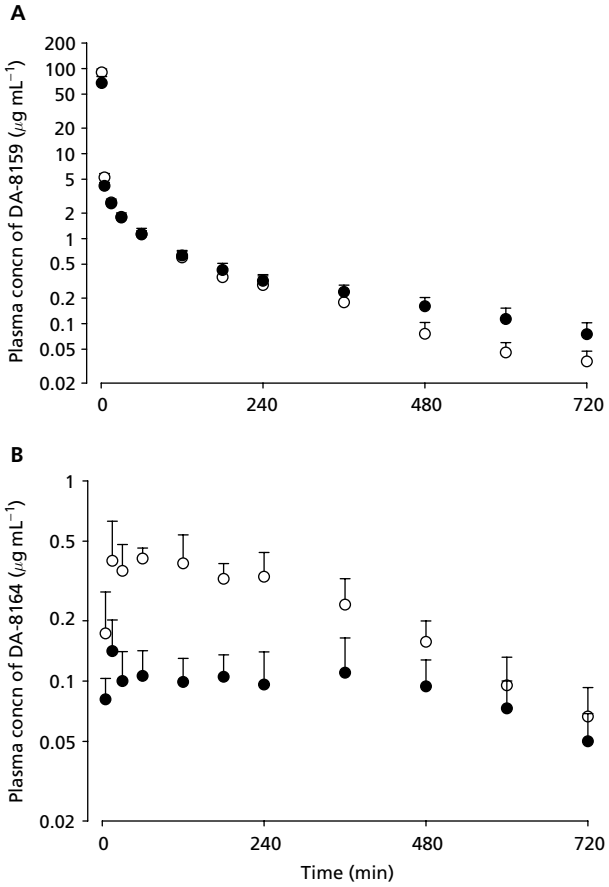

Figure 2 Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after 1-min intravenous infusion of DA-8159 at a dose of 30 mg kg^{-1} to control rats (○; n = 9) and PCM rats (●; n = 9). Bars represent s.d.

was significantly longer (42.1% increase) than that in control rats (Table 2). The total amounts of unchanged DA-8164 excreted in 24-h urine ($Ae_{0-24\,h}$, expressed in terms of intravenous dose of DA-8159) were comparable between the two groups of rats and the amounts recovered from the gastrointestinal tract at 24 h ($GI_{24\ h}$, expressed in terms of intravenous dose of DA-8159) were below the detection limit for both groups of rats (Table 2).

Pharmacokinetics of DA-8159 and DA-8164 in rats after oral administration of DA-8159

After oral administration of DA-8159, absorption of DA-8159 from rat gastrointestinal tract was rapid and almost complete. DA-8159 was detected in plasma from the first blood sampling time, 15 min, for both groups of rats (Figure 3A) and reached T_{max} of DA-8159 at 36.7 and 23.3 min for control and PCM rats, respectively; the values were significantly different (Table 3). Moreover, the $GI_{24\,h}$ values of DA-8159 were 1.42 and 1.84% for control and PCM rats, respectively (Table 3). In PCM rats, the plasma concentrations of DA-8159 were higher and the C_{max} of DA-8159 was significantly higher (76.5% increase) than

Table 2 Pharmacokinetic parameters of DA-8159 and DA-8164 in control and PCM rats after 1-min intravenous infusion of DA-8159 at a dose of 30 mg kg^{-1}

Parameter	Control (n = 9)	PCM (n = 9)
Initial body weight (g)	167.00 ± 3.54	171.00 ± 6.97
Final body weight (g)	357.0 ± 19.7	169.0 ± 21.7***
DA-8159		
AUC (μg min mL^{-1})	446.0 ± 49.9	444.0 ± 44.8
Terminal half-life (min)	159.0 ± 40.3	201.0 ± 35.7*
MRT (min)	102.0 ± 22.7	169.0 ± 38.5***
Vd_{ss} (L kg^{-1})	6.730 ± 0.999	11.00 ± 2.02***
CL (mL min^{-1} kg^{-1})	67.3 ± 7.8	67.60 ± 5.63
CL_R (mL min^{-1} kg^{-1})	3.08 ± 1.93	3.59 ± 2.35
CL_{NR} (mL min^{-1} kg^{-1})	63.40 ± 8.01	61.5 ± 10.1
$Ae_{0-24\,h}$ (% of dose of DA-8159)	5.71 ± 2.63	6.60 ± 3.28
$GI_{24\,h}$ (% of dose of DA-8159)	0.684 ± 0.515	0.997 ± 0.553
DA-8164		
AUC (μg min mL^{-1})	162.0 ± 45.7	87.0 ± 24.3***
Terminal half-life (min)	183.0 ± 46.5	260 ± 81.1**
C_{max} (μg mL^{-1})	0.443 ± 0.178	0.1690 ± 0.0506***
T_{max} (min)	80.0 ± 56.1	203 ± 132*
$Ae_{0-24\,h}$ (% of dose of DA-8159)	0.105 ± 0.076	0.0821 ± 0.0766
$GI_{24\,h}$ (% of dose of DA-8159)	BD	BD

Values are mean ± s.d. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with control. BD, below detection limit.

those in control rats (Table 3). This resulted in a significantly greater AUC for DA-8159 (59.0% increase; Table 3). Other pharmacokinetic parameters of DA-8159 listed in Table 3 were not significantly different between two groups of rats. Body weight gain also decreased significantly in PCM rats compared with that in control rats (Table 3).

After oral administration of DA-8159, formation of DA-8164 was fast; DA-8164 was detected in plasma from first sampling time, 15 min, for both groups of rats (Figure 3B). The plasma concentration of DA-8164 kept increasing up to T_{max} (297 and 473 min for control and PCM rats, respectively); they were significantly different (Table 3). The longer T_{max} values (Table 3) suggested that DA-8164 was formed continuously from DA-8159 in both groups of rats. In PCM rats, the plasma concentrations of DA-8164 were lower (Figure 3B) and the C_{max} of DA-8164 was significantly lower (54.6% decrease) (Table 3) than in control rats. This resulted in a significantly smaller AUC for DA-8164 (54.9% decrease) (Table 3). Other pharmacokinetic parameters of DA-8164 listed in Table 3 were not significantly different between the two groups of rats.

Serum protein binding of DA-8159 in the absence or presence of DA-8164

After either intravenous (Figure 2) or oral (Figure 3) administration of DA-8159 to both groups of rats,

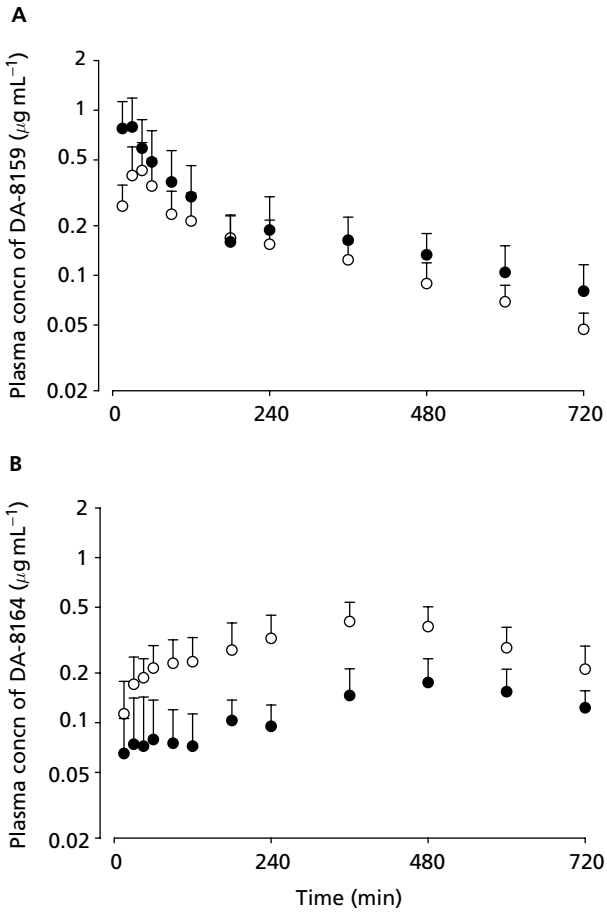

Figure 3 Mean arterial plasma concentration–time profiles of DA-8159 (A) and DA-8164 (B) after oral administration of DA-8159 at a dose of 30 mg kg^{-1} to control rats (○; n = 9) and PCM rats (●; n = 9). Bars represent s.d.

DA-8159 and DA-8164 plasma concentrations of 0.05–1.0 and 0.05–0.5 μg mL^{-1}, respectively, were maintained for a long period of time. Hence, in the protein binding study, DA-8159 and DA-8164 concentrations of 0.2 and 0.1 μg mL^{-1}, respectively, were arbitrarily chosen. Serum protein binding values of DA-8159 without addition of DA-8164 were comparable in control and PCM rats, although the value with addition of DA-8164 in PCM rats was significantly smaller (16.1% decrease; Table 1).

Discussion

DA-8164 is a main metabolite of DA-8159 in man and the pharmacological effect of DA-8164 in terms of PDE V inhibitory activity is half of that of DA-8159 (unpublished data). Hence, the pharmacokinetics of DA-8164 were evaluated in this study. It has been reported (Shim et al 2003) that the AUC values of DA-8159 were dose-proportional after intravenous doses of 5–30 mg kg^{-1} and oral doses of 20–30 mg kg^{-1}. Hence, 30 mg kg^{-1} of DA-8159 was arbitrarily chosen in this study.

Table 3 Pharmacokinetic parameters of DA-8159 and DA-8164 in control and PCM rats after oral administration of DA-8159 at a dose of 30 mg kg^{-1}

Parameter	Control (n = 9)	PCM (n = 9)
Initial body weight (g)	162.00 ± 7.12	174.0 ± 10.5
Final body weight (g)	327.0 ± 22.4	163.0 ± 23.1***
DA-8159		
AUC (μg min mL^{-1})	122.0 ± 32.3	194.0 ± 77.9*
Terminal half-life (min)	263 ± 146	305 ± 132
CL_R (mL min^{-1} kg^{-1})	5.19 ± 1.08	4.57 ± 3.04
C_{max} (μg mL^{-1})	0.486 ± 0.208	0.858 ± 0.385*
T_{max} (min)	36.7 ± 13.2	23.30 ± 7.91*
$Ae_{0-24\,h}$ (% of dose of DA-8159)	2.180 ± 0.681	3.51 ± 1.82
$GI_{24\,h}$ (% of dose of DA-8159)	1.42 ± 1.01	1.84 ± 1.31
F (%)	27.4	43.7
DA-8164		
AUC (μg min mL^{-1})	319 ± 113	144.0 ± 48.7***
Terminal half-life (min)	273 ± 131	353.0 ± 83.7
C_{max} (μg mL^{-1})	0.425 ± 0.121	0.1930 ± 0.0612***
T_{max} (min)	297 ± 141	473 ± 136*
$Ae_{0-24\,h}$ (% of dose of DA-8159)	0.0559 ± 0.0287	0.0894 ± 0.4220
$GI_{24\,h}$ (% of dose of DA-8159)	BD	BD

Values are mean ± s.d. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with control. BD, below detection limit.

Based on an in-vitro metabolism study with microsomes containing Baculovirus-expressed rat CYP isozymes, the CYP isozymes responsible for the formation of DA-8164 from DA-8159 were CYP1A1 and 2C12 (Choi et al 2002). However, our laboratories performed an in-vivo pharmacokinetic study of DA-8159 using various enzyme inducers or inhibitors to find what types of CYP isozymes are involved in the metabolism of DA-8159 to form DA-8164 in rats. The results showed that CYP3A1/2 is mainly involved in the metabolism of DA-8159 to form DA-8164 in rats (our unpublished data). This could be due to differences between in-vitro and in-vivo studies. In the in-vitro study, NADPH was added once, oxygen and nutrients were not supplied continuously and distribution (especially protein binding and tissue distribution) and elimination (mainly hepatic metabolism and renal excretion) were not considered, which was different from the in-vivo study.

After intravenous administration of DA-8159 to PCM rats, the terminal half-life of DA-8159 was significantly longer and this could be due to a significantly larger Vd_{SS} of DA-8159 in the rats since the CL values of DA-8159 were comparable between the two groups of rats (Table 2). The longer terminal half-life resulted in a significantly longer MRT of DA-8159 in the rats (Table 2). In PCM rats, the Vd_{SS} of DA-8159 was significantly larger than that in control rats, and this could be due to significant increase in the free (not bound to plasma proteins) fraction of DA-8159 in the rats; the free fraction was 33.6 and 44.3% for control

and PCM rats, respectively (Table 1). A larger volume of distribution in PCM rats has also been reported for 2-(allythio)pyrazine (128% increase; Kim et al 2003), phenytoin (47.3% increase; Kim et al 2001) and furosemide (31% increase; Kim et al 1993).

After intravenous administration of DA-8159 to PCM rats, the AUC for DA-8164 was significantly smaller than that in control rats (Table 2), and this could be due to decrease in expression of CYP3A1/2 (40–50% of control level) in the rats (Cho et al 1999). DA-8164 was mainly formed via CYP3A1/2 in rats as explained earlier. This could be supported by a significantly slower in-vitro CL_{int} for the formation of DA-8164 in hepatic microsomal fraction of PCM rats. After intravenous administration of DA-8159, the AUC values of DA-8159 were comparable between the two groups of rats although the AUC of DA-8164 was significantly smaller in PCM rats (Table 2). This could be due to the formation of DA-8164 not being a major metabolic pathway in rats (Choi et al 2002), hence contribution of formation of DA-8164 to the AUC of DA-8159 seemed to be minor.

After intravenous administration of DA-8159, the CL values of DA-8159 based on plasma data, 67.3 and 67.6 $mL\,min^{-1}\,kg^{-1}$ (Table 2), were considerably slower than the reported cardiac output based on blood data, 296 $mL\,min^{-1}\,kg^{-1}$ (Davies & Morris 1993), and a haematocrit of approximately 45% (Mitruka & Rawnsley 1981) in rats. This suggested that the first-pass effect of DA-8159 in the lung and heart is almost negligible, if indeed it has any effect at all, in rats. The CL_{NR} values of DA-8159 based on plasma data, 63.4 and 61.5 $mL\,min^{-1}\,kg^{-1}$ (Table 2), were faster than the reported hepatic blood flow rate, 78.4 $mL\,min^{-1}\,kg^{-1}$ (Davies & Morris 1993) and hematocrit of approximately 45% (Mitruka & Rawnsley 1981) in rats. This suggested existence of extrahepatic metabolism of DA-8159 in rats.

After intravenous administration of DA-8159, the estimated CL_R values of DA-8159 as free (not bound to serum proteins) fractions were 9.17 and 8.10 $mL\,min^{-1}\,kg^{-1}$ for control and PCM rats, respectively, based on the CL_R (Table 2) and protein binding values of DA-8159 in the presence of DA-8164 (Table 1). The values, 9.17 and 8.10 $mL\,min^{-1}\,kg^{-1}$, were considerably faster than the reported glomerular filtration rate of 5.24 $mL\,min^{-1}\,kg^{-1}$ in rats (Davies & Morris 1993) indicating that DA-8159 is secreted in rat renal tubules. However, the CL_R of sildenafil, chemically similar to DA-8159, as unbound fraction was 0.277 $mL\,min^{-1}\,kg^{-1}$ based on CL_R of total drug in plasma (0.0341 $mL\,min^{-1}\,kg^{-1}$) and protein binding (87.7%) after intravenous administration of 10 $mg\,kg^{-1}$ to rats (our unpublished data). This suggested that sildenafil is reabsorbed in rat renal tubules.

After intravenous administration of DA-8159, the AUC values of DA-8159 were comparable between the two groups of rats (Table 2). However, after oral administration of DA-8159 to PCM rats, the AUC for DA-8159 was significantly greater than in control rats (Table 3). This was not due to increase in absorption of DA-8159 in PCM rats. After oral administration of DA-8159 to PCM rats, the F was 43.7% and the $GI_{24\,h}$ was 1.84% of the oral dose (Table 3). It is possible that this unchanged DA-8159, 1.84%, might be partly attributed to the gastrointestinal (including biliary) excretion of the absorbed drug. Based on the linear pharmacokinetics (Shim et al 2003), the mean true fraction of oral dose unabsorbed (F_{unabs}) in this study could be estimated by the following equations (Lee & Chiou 1983):

$$0.0142 = F_{unabs} + (0.274 \times 0.00684) \text{ for control rats} \quad (1)$$

$$0.0184 = F_{unabs} + (0.437 \times 0.00997) \text{ for PCM rats} \quad (2)$$

where 0.274 (0.437) and 0.00684 (0.00997) are the F (Table 3) and $GI_{24\,h}$ (Table 2) values, respectively, in control (PCM rats). The calculated F_{unabs} values were 1.23 and 1.40% for control rats and PCM rats, respectively. Hence, approximately 98% of the oral dose of DA-8159 was absorbed for both groups of rats. The significantly greater AUC for DA-8159 after oral administration of DA-8159 to PCM rats (Table 3) may be due to a decrease in the intestinal first-pass effect of DA-8159. It has been reported that the intestinal first-pass effect of DA-8159 at a dose of 30 $mg\,kg^{-1}$ was approximately 59% of the oral dose in rats based on AUC difference between intraportal and intraduodenal administration (Shim et al 2003). The hepatic first-pass effect of DA-8159 at a dose of 30 $mg\,kg^{-1}$ was 23% (equivalent to 9.6% of the oral dose since approximately 40% of the oral dose was absorbed into the portal vein) based on the difference in AUC after intravenous and intraportal administration (Shim et al 2003). The decrease in intestinal first-pass effect in PCM rats could also be supported by the considerably smaller $AUC_{DA\text{-}8164}/AUC_{DA\text{-}8159}$ ratio after oral administration to the rats; the ratios were 261 and 74.2% for control and PCM rats, respectively (Table 3). A decrease in intestinal first-pass effect of DA-8159 in PCM rats could explain the considerably greater F value of DA-8159 after oral administration to the rats (Table 3).

In conclusion, after both intravenous and oral administration of DA-8159 to PCM rats, the AUC values for DA-8164 were significantly smaller than in control rats (Tables 2 and 3). This could be due to decrease in CYP3A1/2 in the rats (Cho et al 1999) because DA-8164 is mainly formed via CYP3A1/2 in rats. After oral administration of DA-8159 to PCM rats, the AUC for DA-8159 was significantly greater and this may be due to a decrease in the intestinal first-pass effect.

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