JPP Journal of Pharmacy and Pharmacology

JPP 2007, 59: 955–963

Received February 6, 2007
Accepted March 28, 2007
DOI 10.1211/jpp.59.7.0007
ISSN 0022-3573

Pharmacokinetics of DA-7218, a new oxazolidinone, and its active metabolite, DA-7157, after intravenous and oral administration of DA-7218 and DA-7157 to rats

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Abstract

DA-7218 (a prodrug of DA-7157), a new oxazolidinone, was hydrolysed via phosphatase to form its active metabolite, DA-7157, in rats. The pharmacokinetic parameters of DA-7218 and DA-7157 were evaluated after intravenous (5, 10 and 20 mg kg^{-1}) and oral (20, 50 and 100 mg kg^{-1}) administration of DA-7218 to rats. DA-7218 and DA-7157 exhibited dose-proportional pharmacokinetics after both intravenous and oral administration of DA-7218 to rats. The stability of DA-7218 and DA-7157, blood partition of DA-7157, and the plasma protein binding of DA-7157 were also evaluated. DA-7218 was unstable in rat blood, plasma, bile and liver homogenates, but DA-7157 was stable, suggesting that DA-7218 is hydrolysed via phosphatase. DA-7157 rapidly reached equilibrium between plasma and blood cells, and the mean equilibrium plasma-to-blood cells ratio was 3.18, indicating that binding of DA-7157 to blood cells was not considerable. The protein binding of DA-7157 in fresh rat plasma was 93.4%.

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Funding: This study was supported in part by a grant from the Korea Ministry of Health and Welfare (01-PJ1-PG4-01PT01-0005), 2001–2005.

Introduction

Oxazolidinone antibiotics, which inhibit bacterial protein synthesis (Diekema & Jone 2001; Fung et al 2001), have been synthesized to overcome the problem of emerging resistance in Gram-positive bacteria. The emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (*Enterococcus faecium* and *Enterococcus faecalis*), vancomycin/glycopeptide-intermediate *S. aureus*, penicillin-resistant *Streptococcus pneumoniae* and multidrug-resistant coagulase-negative staphylococci has been reported (Fung et al 2001). Recently, a new oxazolidinone, DA-7218 ((*R*)-[3-(4-(2-(2-methyltetrazol-5-yl)pyridin-5-yl)-3-fluorophenyl)-2-oxo-5-oxazolidinyl]methyl disodiumphosphate; Figure 1A) was synthesized (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, South Korea). DA-7218 (a basic compound having a molecular weight of 494.28 Da) is a prodrug and produces its active metabolite DA-7157 ((*R*)-3-(4-(2-(2-methyltetrazol-5-yl)pyridin-5-yl)-3-fluorophenyl)-5-hydroxymethyl oxazolidin-2-on; Figure 1B) by hydrolysis via the enzyme phosphatase. The solubility of DA-7157 in distilled water and various buffer solutions having a pH of 1, 3, 5, 7 and 9 was 0.00434, 0.0289, 0.00344, 0.00317, 0.00259 and 0.00346 mg mL^{-1}, respectively, and that of DA-7218 was greater than 150 mg mL^{-1}, except in pH 1 solution (0.015 mg mL^{-1}). The log partition coefficients of DA-7157 (compared with *n*-octanol) were greater than 1.3 in all the solutions.

DA-7157 showed excellent potency (approx. 4 times more active than linezolid) against medically significant clinical isolates of Gram-positive pathogens in South Korea (Lee et al 2004). For example, DA-7157 demonstrated excellent in-vitro activity against MRSA and coagulase-negative staphylococci (nearly all isolates were inhibited at a concentration of 0.5 μg mL^{-1} or less), vancomycin-resistant enterococci (inhibited at a concentration of 1 μg mL^{-1} or less), *S. pneumoniae* including penicillin-resistant strains and *Streptococcus pyogenes* (MIC90 of 0.25 μg mL^{-1} and all isolates were inhibited at a concentration of 0.25 μg mL^{-1} or less), *Streptococcus agalactiae* (Gram-negative; MIC90 of 0.5 μg mL^{-1}) and anaerobes including *Peptostreptococcus* spp., *Clostridium* spp. and other Gram-positive rods (MIC90 ranging from 0.25 to 0.5 μg mL^{-1}). Although DA-7157 lacked in-vitro activity against

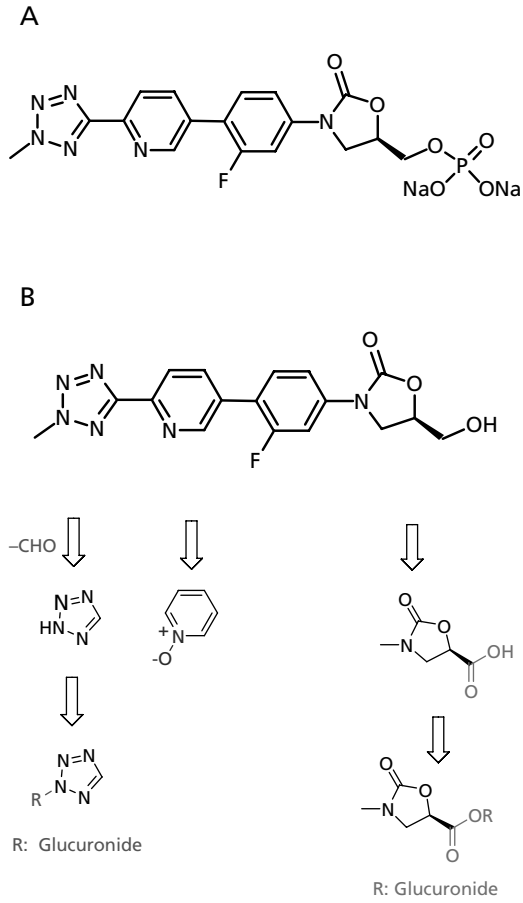

Figure 1 Chemical structure of DA-7218 (A) and DA-7157 (B) and possible metabolic pathways of DA-7157.

Gram-negative pathogens, it had in-vitro activity against *Moraxella catarrhalis* (MIC90 of 1 μg mL^{-1}), *Haemophilus influenzae* (MIC90 of 4 μg mL^{-1}) and anaerobic Gram-negative rods including *Bacteroides* spp. (MIC90 ranging from 2 to 4 μg mL^{-1}). The activity of DA-7218 was also assessed in the murine systemic and localized infection models (Choi et al 2004). For example, intravenous and oral DA-7218 had high efficacy (2–5 fold better than linezolid) in murine systemic infection models with intraperitoneally Gram-positive infection, demonstrating activity against *S. aureus*, coagulase-negative staphylococci, *S. pneumoniae, E. faecalis* and *E. faecium* including multidrug-resistant strains. DA-7218 was also active in respiratory tract infections and demonstrated oral efficacy in neutropenic mouse thigh infection and mouse pouch infection due to MRSA.

The aims of this study were to report: (i) the pharmacokinetics of DA-7218 and DA-7157 after intravenous (5, 10 and 20 mg kg^{-1}) and oral (20, 50 and 100 mg kg^{-1}) administration of DA-7218 to rats; (ii) the pharmacokinetics of DA-7157 after intravenous and oral administration of DA-7157 at a dose of 10 mg kg^{-1} to rats; (iii) the biliary excretion of DA-7157 after intravenous administration of DA-7157 at a dose of 10 mg kg^{-1} to rats; (iv) the stability of DA-7218 and DA-7157; (v) the blood partition of DA-7157 between plasma and blood cells of rat blood; and (vi) the factors influencing the protein binding of DA-7157 to 4% human serum albumin (HSA) using the equilibrium dialysis technique.

Materials and Methods

Chemicals

DA-7218, DA-7157, sildenafil (an internal standard for high-performance liquid chromatographic (HPLC) analysis of DA-7218 and DA-7157), and hydroxypropylmethylcellulose (HPMC) (HPMC type 2910; Shin-Etsu Chemical Company, Tokyo, Japan) were supplied by the Research Laboratory of Dong-A Pharmaceutical Company (Yongin, South Korea). Dimethylacetamide, α_1-acid glycoprotein (AAG), cefaclor, enoxacin, salicylic acid and sulfisoxazole were purchased from Sigma-Aldrich Corporation (St Louis, MO, USA). HSA (20%) and polyethylene glycol 400 were obtained from Dong-Shin Pharmaceutical Company (Seoul, South Korea) and Duksan Chemical Company (Seoul, South Korea), respectively. Various buffer solutions having pH ranging from 2 to 12 were purchased from Shinyo Pure Chemicals (Osaka, Japan). Other chemicals were of reagent grade or HPLC grade.

Animals

Male Sprague-Dawley rats, 9 weeks of age, 270–300 g, were purchased from Charles River Company Korea (Orient, Seoul, South Korea) and maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea) at a temperature of 20–23°C with a 12-h light–dark cycle (lights on 0700–1900 hours) and a relative humidity of 50 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) and supplied with filtered pathogen-free air, and food (Samyang Company, Pyeongtaek, South Korea) and water ad libitum. The protocol of this animal study was approved by the Animal Care and Use Committee of College of Pharmacy of Seoul National University.

Intravenous administration of DA-7218 to rats

The jugular vein (for drug administration) and the carotid artery (for blood sampling) of each rat were cannulated with polyethylene tubes (Clay Adams, Parsippany, NJ, USA) while each rat was under light ether anaesthesia (Kim et al 1993). Both cannulae were exteriorized to the dorsal side of the neck, where each cannula was terminated with a long silastic tube (Dow Corning, Midland, MI, USA). Both silastic tubes were inserted into a wire sheath to allow free movement of the rat (hence, they were not restrained during the study). Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from the anaesthesia for 4–5 h before the study was started. DA-7218 (dissolved in distilled water) at doses of 5 (n = 7), 10 (n = 7) and 20 (n = 9) mg kg^{-1} was infused over 1 min via the jugular vein (total infusion volume of 2 mL kg^{-1}). A blood sample (approx. 0.12-mL) was

collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480, 600 and 720 min after the start of the intravenous infusion of DA-7218. Approximately 0.3 mL of heparinized 0.9% NaCl injectable solution (20 units mL^{-1}) was used to flush the cannula immediately after each blood sampling to prevent blood clotting. Blood samples were centrifuged immediately and a 50-μL aliquot of each plasma sample was transferred into an Eppendorf tube that contained 200-μL of acetonitrile (to minimize the hydrolysis of DA-7218 in rat plasma). At the end of 24 h, each metabolic cage was rinsed with 20 mL of distilled water, and the rinsed material was combined with the 24-h urine sample. After the exact volume of the combined urine sample was measured, two 50-μL aliquots of the samples were collected. At the same time (24 h), each rat was exsanguinated and killed by cervical dislocation. Then, the entire gastrointestinal tract (including contents and faeces) of each rat was removed, transferred into a beaker that contained 100 mL of methanol (to facilitate the extraction of DA-7218 and DA-7157), and cut into small pieces using scissors. After manual shaking and stirring with a glass rod for 1 min, two 50-μL samples of the supernatant were collected from each beaker. The urine and gastrointestinal tract samples were also transferred into an Eppendorf tube that contained a 200-μL of acetonitrile. DA-7218 and DA-7157 concentrations were measured as soon as the samples were collected to minimize hydrolysis of DA-7218 to DA-7157.

Oral administration of DA-7218 to rats

DA-7218 (the same solution that was used in the intravenous study) at doses of 20 (n = 10), 50 (n = 8) and 100 (n = 9) mg kg^{-1} was administered orally (total oral volume of 3 mL kg^{-1}) using a feeding tube. Blood samples were collected via the carotid artery at 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720, 960 and 1440 min after oral administration of DA-7218. Other procedures were similar to those in the intravenous study.

Intravenous and oral administration of DA-7157 to rats

DA-7157 (dissolved in dimethylacetamide/polyethylene glycol 400/distilled water in a ratio of 3:5:2 v/v/v) at a dose of 10 mg kg^{-1} was administered intravenously (total injection volume of 2 mL kg^{-1}) over 1 min to rats (n = 8). The same dose of DA-7157 (suspended in 1% HPMC) was administered orally (total oral volume of 3 mL kg^{-1}) using a feeding tube (n = 10). For intravenous studies, 0.12-mL aliquots of blood samples were collected at 0, 1, 5, 15, 30, 60, 90, 120, 240, 360, 480 and 600 min. For oral studies, blood samples were collected at 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720, 960 and 1440 min. The same dose of DA-7157 was also administered via the tail vein of six rats after bile duct cannulation (without cannulation of the carotid artery and the jugular vein and without blood sampling), and the 24-h bile sample was collected.

Stability of DA-7218 and DA-7157

The procedures for stability of DA-7218 and DA-7157 were similar to previously reported methods (Yu et al 2003). DA-7218 stock solution in distilled water and DA-7157 stock solution in dimethylsulfoxide was added (10 μL mL^{-1}) to Eppendorf tubes containing rat blood, plasma, urine, bile, 9000-*g* supernatant fractions of liver homogenates and gastric juices (pH of 1.44 and 2.86, respectively), isotonic Sørensen phosphate buffer of pH 7.4 and various buffer solutions having pH ranging from 2 to 12. Rat liver was homogenized with 4 vols of 0.9% NaCl injectable solution using a tissue homogenizer (Ultra-Turrax, T25; Janke & Kunkel, IKA-Labortechnik, Staufeni, Germany) at 4°C in an ice-bath. Each sample was incubated in a water-bath shaker kept at a temperature of 37 ± 2°C and at a rate of 50 oscillations min^{-1} for 24 or 48 h (4 h for rat gastric juices). The plasma samples for both DA-7218 and DA-7157 were stored in a −70°C freezer for 48 h. In the case of DA-7218, a stability test after sample preparation (deproteinization with acetonitrile) in rat plasma at room temperature was performed. The concentrations of DA-7218 and DA-7157 in the above samples were analysed as soon as the sample was collected.

In-vitro distribution kinetics of DA-7157 between plasma and blood cells of rat blood

The procedures for the in-vitro distribution kinetics of DA-7157 between plasma and blood cells of rat blood were similar to previously reported methods (Lee et al 1981). A total of 1 mL of heparinized rat blood (blood was freshly withdrawn via the carotid artery from seven unanaesthetized rats and pooled together) was pipetted into each glass test tube (22 tubes for each concentration). The initial DA-7157 concentrations in rat blood were 0.2, 2 and 20 μg mL^{-1}. After 30 s gentle manual mixing, each tube was incubated in a water-bath shaker kept at 37°C and a rate of 50 oscillations min^{-1}. At 0, 1, 3, 5, 7, 10, 15, 30, 60, 90 and 120 min, blood samples were centrifuged and plasma samples collected. Whole blood concentrations of DA-7157 were also measured at each time by adding two volumes of distilled water to facilitate the haemolysis and increase the reproducibility of HPLC analysis of DA-7157 (Lee et al 1981).

Factors influencing the binding of DA-7157 to 4% HSA using the equilibrium dialysis technique

The procedures for the measurement of protein binding of DA-7157 to 4% HSA using the equilibrium dialysis technique are similar to previously reported methods (Shim et al 2000). Briefly, 1 mL of 4% HSA (20% HSA was diluted with isotonic Sørensen phosphate buffer pH 7.4) was dialysed against 1 mL of isotonic Sørensen phosphate buffer of pH 7.4 that contained 3% (w/v) dextran ("the buffer") in a 1-mL dialysis cell (Spectrum Medical Industries, Los Angeles, CA, USA) using a Spectra/Por 4 membrane (molecular weight cutoff of 12 000–14 000 Da; Spectrum Medical Industries). The dialysis cell was incubated in a water-bath shaker kept at 37°C and at a rate of 50 oscillations min^{-1}. After 24 h incubation, two 50-μL samples were collected from each compartment and stored in a −70°C freezer (Model DF8517; Ilshin Laboratory Company, Seoul, South Korea) until HPLC analysis of DA-7157. The effects of DA-7157 concentrations, HSA concentrations, various buffers, buffer pH, amounts of heparin and AAG and other drugs

(sulfisoxazole, salicylic acid, cefaclor and enoxacin) were also evaluated. Protein binding of DA-7157 in mice, rats, rabbits, dogs and human fresh plasma (n=5–7) was also measured at a DA-7157 concentration of 5 μg mL^{-1}.

HPLC analysis of DA-7218 and DA-7157

The concentrations of DA-7218 and DA-7157 in the above samples were analysed by a HPLC method developed in our laboratories. A 50-μL aliquot of biological sample was deproteinized with 200 μL of acetonitrile (only for studies on DA-7157), and a 50-μL aliquot of citrate buffer (pH 5.0) containing 2.5 μg mL^{-1} of sildenafil (internal standard) was added. After vortex-mixing and centrifugation, the supernatant was transferred into a clean tube and evaporated (Dry thermobath; Eyela, Tokyo, Japan) under a gentle stream of nitrogen gas at 40°C. The residue was reconstituted in 100 μL of the mobile phase and a 50-μL aliquot was injected directly onto the RP-18 reversed-phase HPLC column (15 cm in length, 4.6 mm i.d., particle size 3.5 μm; Hichrome, Berkshire, England). The mobile phase (20 mM KH_2PO_4/acetonitrile in a ratio of 80:20 v/v), was run at a flow rate of 1.5 mL min^{-1} and the column effluent was monitored using a UV detector (UV/VIS-151; Gilson, Middleton, WI, USA) set at 307 nm. The retention times of DA-7218, sildenafil and DA-7157 were approximately 5, 8 and 15.2 min, respectively. The detection limits of DA-7218 and DA-7157 in rat plasma were 0.02 and 0.02 μg mL^{-1}, respectively, and the corresponding values in rat urine were 0.05 and 0.05 μg mL^{-1}. The coefficients of variation (within- and between-day) were below 8.84%. The HPLC chromatograms of DA-7218 and DA-7157 in rat plasma samples are shown in Figure 2.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to infinity (AUC) was calculated using the trapezoidal rule extrapolation method. This method uses the logarithmic trapezoidal rule (Chiou 1978) to calculate the area during the declining plasma level phase, and the linear trapezoidal rule for the rising plasma level phase. The area from the last point to time infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods (Gibaldi & Perrier 1982) were used to calculate the following pharmacokinetic parameters using non-compartmental analysis (WinNonlin; Pharsight Corporation, Mountain View, CA, USA): the time-averaged total body (CL), renal (CL_R) and non-renal (CL_{NR}) clearance, terminal half-life (t1/2), first moment of the AUC (AUMC), mean residence time (MRT), apparent volume of distribution at steady state (V_{ss}) and extent of absolute oral bioavailability (F) (Kim et al 1993). The F values of DA-7218 were measured by dividing the AUC values after various oral doses by the AUC after intravenous administration of DA-7218 at a dose of 20 mg kg^{-1}. The peak plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were read directly from the experimental data. The mean values of V_{ss} (Chiou 1979a), t1/2 (Eatman et al 1977) and CL, CL_R and CL_{NR} (Chiou 1980) were calculated using the harmonic mean method.

Statistical analysis

Levels of statistical significance were assessed using Duncan's multiple range test in the SPSS posteriori analysis of variance

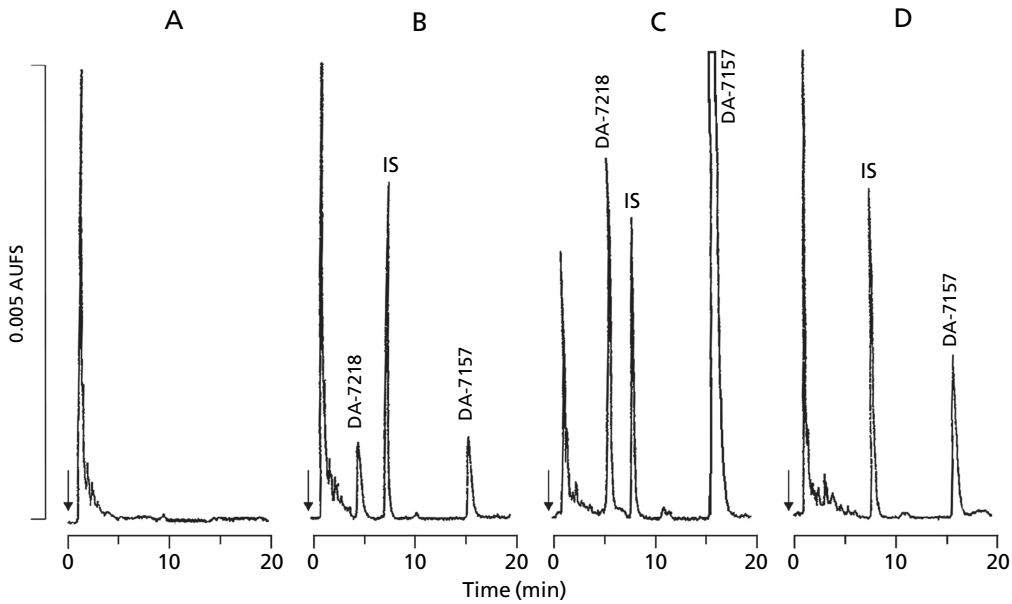

Figure 2 HPLC chromatograms of blank rat plasma (A), blank rat plasma with 0.5 μg mL^{-1} of both DA-7218 and DA-7157 and the internal standard (IS; 2.5 μg mL^{-1} of sildenafil) (B) and plasma collected from a male rat at 30 min (DA-7218: 1.95 μg mL^{-1}, DA-7157: 22.5 μg mL^{-1}) (C) and 6 h (DA-7157: 0.987 μg mL^{-1}) (D) after 1 min intravenous administration of DA-7218 at a dose of 20 mg kg^{-1}. DA-75218, 5 min; DA-7157, 15.2 min; and IS, 8 min. The arrow marks the point of injection. The sensitivity of the detector was set at 0.005 AUFS (absorption unit full scale).

among the three means for the unpaired data, or a *t*-test between the two means for unpaired data. A value of $P<0.05$ was considered significant. All results are expressed as mean ± s.d.

Results

Pharmacokinetics of DA-7218 and DA-7157 after intravenous administration of DA-7218 to rats

The mean arterial plasma concentration–time profiles of DA-7218 and DA-7157 after intravenous administration of various doses of DA-7218 to rats are shown in Figure 3A and B, respectively, and some relevant pharmacokinetic parameters are given in Table 1. Note that the AUC values of DA-7218 were proportional to the intravenous doses of DA-7218 studied; the dose-normalized (based on 5 mg kg^{-1} of DA-7218) AUC values were 313 ± 75.4, 333 ± 74.5 and 325 ± 63.8 μg min mL^{-1} for DA-7218 doses of 5, 10 and 20 mg kg^{-1}, respectively (Table 1). The slope between log AUC values of DA-7218 and log doses of DA-7218 was close to 1 (the value was 1.02). Moreover, other pharmacokinetic parameters of DA-7218 listed in Table 1 were not significantly different among the three DA-7218 intravenous

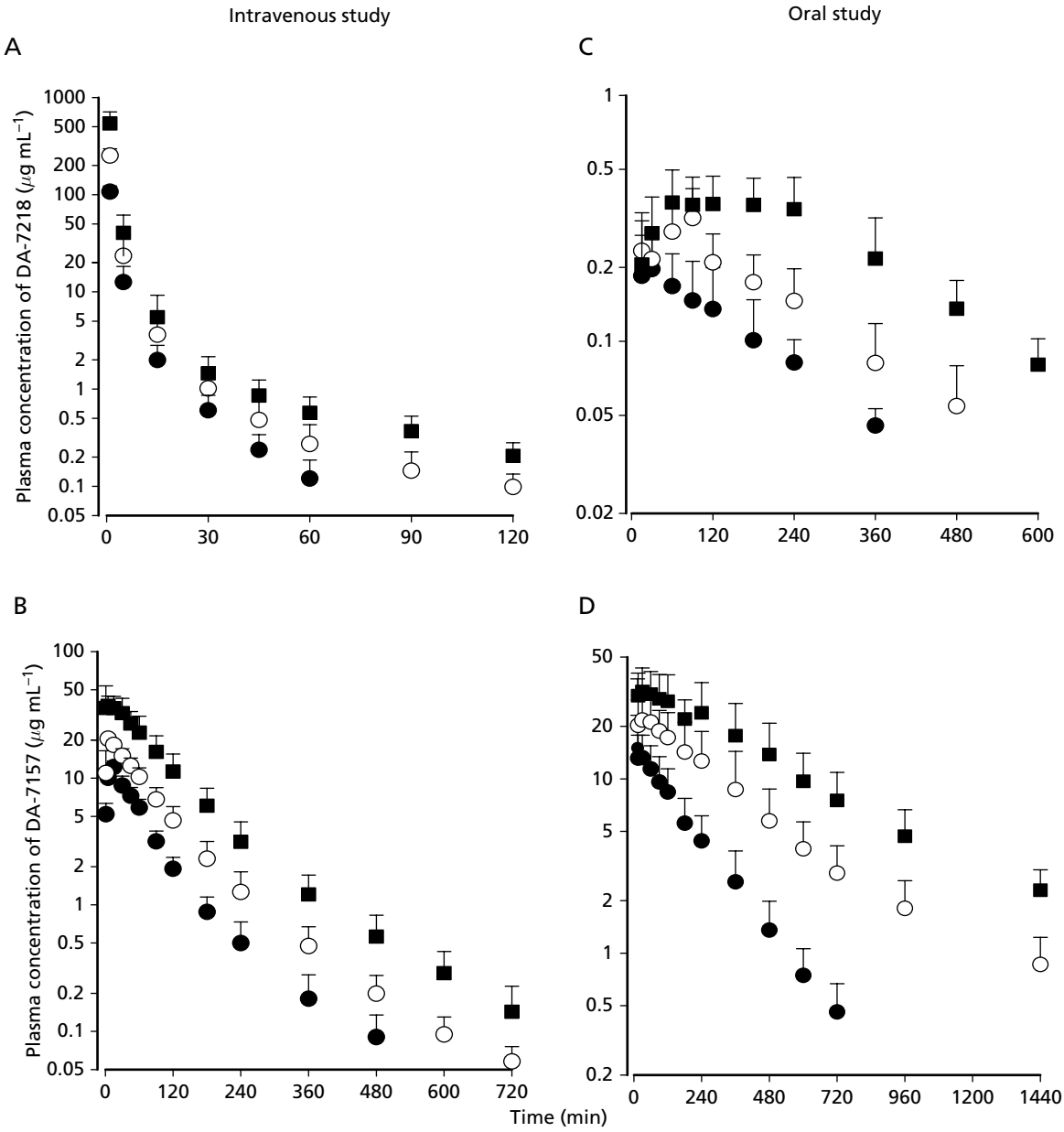

Figure 3 Mean arterial plasma concentration–time profiles of DA-7218 and DA-7157 after 1 min intravenous infusion of DA-7218 at doses of 5 (●; n = 7), 10 (○; n = 7) and 20 (■, n = 9) mg kg^{-1}, and oral administration of DA-7218 at doses of 20 (●; n = 10), 50 (○; n = 8) and 100 (■, n = 9) mg kg^{-1} to rats. Vertical bars represent the s.d.

Table 1 Pharmacokinetic parameters of DA-7218 and DA-7157 after intravenous and oral administration of various doses of DA-7218 to rats

Parameter	Intravenous study			Oral study		
	5 mg kg^{-1} (n = 7)	10 mg kg^{-1} (n = 7)	20 mg kg^{-1} (n = 9)	20 mg kg^{-1} (n = 10)	50 mg kg^{-1} (n = 8)	100 mg kg^{-1} (n = 9)
DA-7218						
AUC[a] (μg min mL^{-1})	313 ± 75.4	44.7 ± 16.9	86.3 ± 27.9	44.7 ± 16.9	86.3 ± 27.9	163 ± 40.0
t1/2 (min)	(13.8 ± 3.64)[b]	133 ± 30.8	159 ± 32.1	133 ± 30.8	159 ± 32.1	162 ± 29.6
MRT (min)	5.34 ± 1.05	1.05 ± 1.27	1.23 ± 0.924			
V_{ss} (mL kg^{-1})	84.7 ± 14.2	0.214 ± 0.0833	0.320 ± 0.102			
CL (mL min^{-1} kg^{-1})	16.0 ± 3.40	45.0 ± 24.5	80.6 ± 26.5			
$Ae_{0-24\,h}$ (% of DA-7218 dose)	BD[c]	BD	BD	0.373 ± 0.281	0.248 ± 0.0840	0.239 ± 0.0961
$GI_{24\,h}$ (% of DA-7218 dose)	BD	BD	BD	0.455 ± 0.260	1.18 ± 0.989	0.781 ± 1.04
C_{max}[a] (μg mL^{-1})				0.214 ± 0.0833[d]	0.320 ± 0.102	0.440 ± 0.0995
T_{max} (min)				45.0 ± 24.5	80.6 ± 26.5	130 ± 73.5[e]
F (%)				3.44	2.66	2.51
DA-7157						
AUC[a] (μg min mL^{-1})	905 ± 105	1780 ± 334	4070 ± 1140	2890 ± 857	8580 ± 3230	17500 ± 6710
t1/2 (min)	106 ± 12.0	112 ± 19.2	115 ± 29.8	(158 ± 24.5)[b]	276 ± 113	366 ± 85.7
C_{max}[a] (μg mL^{-1})	12.5 ± 1.16	20.8 ± 3.41	45.4 ± 8.95	14.0 ± 4.43[d]	22.9 ± 8.13	34.6 ± 10.1
T_{max} (min)	12.1 ± 4.88	7.85 ± 4.88	10.6 ± 12.6	25.5 ± 14.2	43.1 ± 24.6	65.0 ± 72.3
$Ae_{0-24\,h}$ (% of DA-7218 dose)	BD	BD	BD			
$GI_{24\,h}$ (% of DA-7218 dose)	BD	BD	BD			

Values are mean ± s.d. [a]Dose-normalized (based on 5 mg kg^{-1} of intravenous DA-7218 and 20 mg kg^{-1} of oral DA-7218) AUC and C_{max} values were compared. [b]Numbers in parenthesis represent the pharmacokinetic parameters of DA-7218 at an intravenous DA-7218 dose of 5 mg kg^{-1} and of DA-7157 at an oral DA-7218 dose of 20 mg kg^{-1}. Because the detection of plasma concentrations of DA-7218 at an intravenous DA-7218 dose of 5 mg kg^{-1} and DA-7157 at an oral DA-7218 dose of 20 mg kg^{-1} was shorter than those at other doses, these data were not included in the statistical analysis. [c]BD, below the detection limit. [d]The 20 mg kg^{-1} dose was significantly different ($P < 0.05$) compared with 50 and 100 mg kg^{-1}. [e]The 100 mg kg^{-1} dose was significantly different ($P < 0.05$) compared with 20 and 50 mg kg^{-1}.

doses studied. The above results indicate that DA-7218 exhibits dose-proportional pharmacokinetics for the three intravenous doses of DA-7218 in rats. DA-7218 was below the detection limit in the 24-h urine ($Ae_{0-24\,h}$) and gastrointestinal tract (including contents and faeces) at 24 h ($GI_{24\,h}$) for all three intravenous doses of DA-7218 (Table 1). After intravenous administration of DA-7218, the formation of DA-7157 was fast; DA-7157 was detected in plasma at the first blood sampling time (1 min) and rapidly reached T_{max} (7.85–12.1 min) for all three doses studied (Figure 3B). Note that the AUC values of DA-7157 were also proportional to the DA-7218 intravenous doses; the dose-normalized (based on 5 mg kg^{-1} of DA-7218) AUC values of DA-7157 were 905 ± 105, 890 ± 167 and 1020 ± 285 μg min mL^{-1} for DA-7218 doses of 5, 10 and 20 mg kg^{-1}, respectively (Table 1). The slope between log AUC values of DA-7157 and log doses of DA-7218 was also close to 1 (the value was 1.08). Moreover, other pharmacokinetic parameters of DA-7157 listed in Table 1 were also independent of the three intravenous doses of DA-7218. These results also indicate that DA-7157 exhibits dose-proportional pharmacokinetics for the three intravenous doses of DA-7218 in rats. DA-7157 was also below the detection limit in the $Ae_{0-24\,h}$ and $GI_{24\,h}$ (Table 1).

Pharmacokinetics of DA-7218 and DA-7157 after oral administration of DA-7218 to rats

The mean arterial plasma concentration–time profiles of DA-7218 and DA-7157 after oral administration of various doses of DA-7218 to rats are shown in Figure 3C and D, respectively, and some relevant pharmacokinetic parameters are given in Table 1. After oral administration of DA-7218, absorption of the drug from the rat gastrointestinal tract was rapid; DA-7218 was detected in plasma at the first blood sampling time (15 min) and rapidly reached T_{max} (43.6–130 min) for all three doses studied (Figure 3C). Note that the AUC values of DA-7218 were also proportional to the oral doses of DA-7218; the dose-normalized (based on 5 mg kg^{-1} of DA-7218) AUC values of DA-7218 were 11.2 ± 4.23, 8.63 ± 2.79 and 8.15 ± 2.00 μg min mL^{-1} for DA-7218 doses of 20, 50 and 100 mg kg^{-1}, respectively (Table 1). The slope between log AUC values of DA-7218 and log doses of DA-7218 was close to 1.0 (the value was 1.12). Hence, the F values of DA-7218 were also independent of the oral doses of DA-7218; the values were 3.44%, 2.66% and 2.51% for DA-7218 doses of 20, 50 and 100 mg kg^{-1}, respectively (Table 1). The other pharmacokinetics parameters of DA-7218 given in Table 1 were not significantly different among the three oral doses of DA-7218, except C_{max} and T_{max}. The above results suggest

that DA-7218 exhibits dose-proportional pharmacokinetics for the three oral doses of DA-7218 in rats. The values of the $Ae_{0-24\,h}$ and $GI_{24\,h}$ of DA-7218 after oral administration of DA-7218 were almost negligible (Table 1). After oral administration of DA-7218, the formation of DA-7157 was also rapid; DA-7157 was detected in plasma at the first blood sampling time (15 min) and rapidly reached T_{max} (25.5–65.0 min) for all the three doses studied (Figure 3D). Note that the AUC values of DA-7157 were also proportional to the DA-7218 oral doses studied; the dose-normalized (based on 20 mg kg^{-1} of DA-7218) AUC values of DA-7157 were 2890 ± 857, 3430 ± 1290 and 3500 ± 1340 μg min mL^{-1} for DA-7218 doses of 20, 50 and 100 mg kg^{-1}, respectively (Table 1). The other pharmacokinetic parameters of DA-7157 given in Table 1 were not significantly different among the three oral doses of DA-7218, except for C_{max}. The above results suggest that DA-7157 exhibits dose-proportional pharmacokinetics for the three oral doses of DA-7218 in rats.

Pharmacokinetics of DA-7157 after intravenous and oral administration of DA-7157 to rats

The mean arterial plasma concentration–time profiles of DA-7157 after intravenous and oral administration of DA-7157 at a dose of 10 mg kg^{-1} to rats are shown in Figure 4A and B, respectively, and some relevant pharmacokinetic parameters are given in Table 2. After intravenous administration of DA-7157, the plasma concentrations of the drug declined in a poly-exponential fashion (Figure 4A) with a $t^1/_2$ of 114 min (Table 2). After intravenous administration, only 0.249% of the intravenous dose of DA-7157 was excreted in the 24-h urine as unchanged DA-7157 (Table 2).

After oral administration of DA-7157 at a dose of 10 mg kg^{-1} to rats, the absorption of DA-7157 from the rat gastrointestinal tract was fast; DA-7157 was detected in plasma at the first blood sampling time (15 min) (Figure 4B). The F value of DA-7157 was 38.3% (Table 2).

Biliary excretion of DA-7157 after intravenous administration of DA-7157 to rats

The 24-h biliary excretion of DA-7157 at a dose of 10 mg kg^{-1} was not considerable; 9.23% of the intravenous dose of DA-7157 was recovered from six rats after bile duct cannulation. The negligible $Ae_{0-24\,h}$ (0.249% of the intravenous dose) (Table 1) and low biliary excretion (9.23% of the intravenous dose) of DA-7157 suggest that DA-7157 is mainly metabolized in rats.

Stability of DA-7218 and DA-7157

DA-7218 and DA-7157 (both at 0.1 μg mL^{-1}) were stable up to 48 h in various buffer solutions having pH ranging from 2 to 11; more than 94.9% of the added amounts were recovered. However, they were unstable in pH 12 solution; 80.7 and 82.1% of the added amounts of DA-7157 and DA-7218, respectively, were recovered after 2 h incubation, and the corresponding values after 48 h incubation were 4.59 and 8.85%. DA-7218 and DA-7157 (both at 0.1 μg mL^{-1}) were also stable up to 4 h in rat gastric juices (pH of 1.44 and 2.86, respectively), up to 24 h in rat urine (both at 1 μg mL^{-1}), up to 48 h in Sørensen phosphate buffer pH 7.4 (both at 0.1 μg mL^{-1}), and up to 48 h in storage in a −70°C freezer in rat plasma (both at 5 μg mL^{-1}); more than 98.8% was recovered.

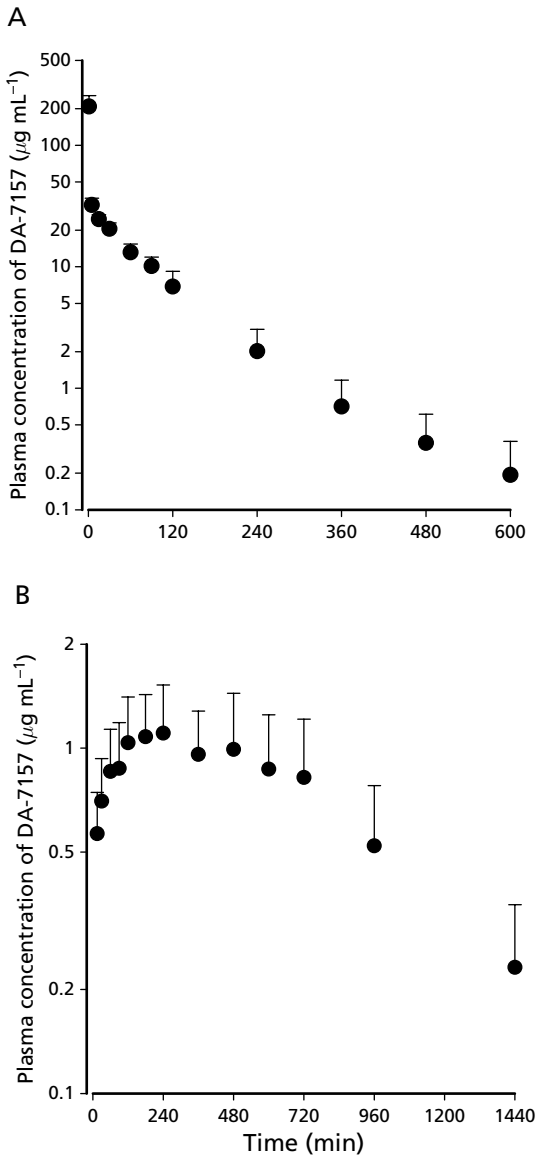

Figure 4 Mean arterial plasma concentration–time profiles of DA-7157 after 1 min intravenous infusion (n = 8) (A) and oral administration (n = 10) (B) of DA-7157 at a dose of 10 mg kg^{-1} to rats. Vertical bars represent the s.d.

DA-7157 was stable up to 24 h in rat plasma (at 1 μg mL^{-1}), blood (at both 1 and 10 μg mL^{-1}), bile (at 1 μg mL^{-1}) and liver homogenates (at both 0.1 and 10 μg mL^{-1}); more than 96.6% was recovered. However, DA-7218 was unstable up to 24 h in rat plasma (at 5 μg mL^{-1}; 95.9% and 2.57% were recovered after 2 and 24 h incubation, respectively), blood (at 5 μg mL^{-1}; 52.4% was recovered after 1 h incubation and below the detection limit from 4 h incubation), bile (at 1 μg mL^{-1}; 58.3%

Table 2 Pharmacokinetic parameters of DA-7157 after intravenous and oral administration of DA-7157 at a dose of 10 mg kg^{-1} to rats

Parameter	Intravenous (n = 8)	Oral (n = 10)
AUC (μg min mL^{-1})	2950 ± 518	1130 ± 402
t1/2 (min)	114 ± 27.0	372 ± 180
MRT (min)	90.9 ± 24.0	
CL (mL min^{-1} kg^{-1})	3.40 ± 0.527	
CL_R (mL min^{-1} kg^{-1})	0.00630 ± 0.00485	0.00330 ± 0.00141
CL_{NR} (mL min^{-1} kg^{-1})	3.39 ± 0.527	
C_{max} (μg mL^{-1})		1.32 ± 0.393
T_{max} (min)		324 ± 202
V_{ss} (mL kg^{-1})	301 ± 39.1	
$Ae_{0-24\,h}$ (% of DA-7157 dose)	0.249 ± 0.139	0.0413 ± 0.0173
$GI_{24\,h}$ (% of DA-7157 dose)	0.112 ± 0.0346	2.59 ± 2.57
F (%)		38.3

Values are mean ± s.d.

was recovered after 20 min incubation and below the detection limit from 4 h incubation) and liver homogenates (at 0.1 μg mL^{-1}; 1.40% was recovered after 20 min incubation and below the detection limit from 40 min incubation). Note that the formation of DA-7157 from DA-7218 in rat liver homogenates (75.4% of DA-7218 was formed into DA-7157) was less than the disappearance of DA-7218 (98.6%), suggesting that other compounds in addition to DA-7157 were formed from DA-7218 in the liver homogenates (Figure 1). DA-7218 was stable in rat plasma up to 24 h standing at room temperature after deproteinization with acetonitrile. The above results indicate that DA-7218 is hydrolysed via the enzyme phosphatase. For the exact measurement of the amounts of DA-7218 and DA-7157 remaining in the whole gastrointestinal tract (including contents and faeces) at 24 h ($GI_{24\,h}$), the stability test in rat gastric juices and various buffer solutions is required.

In-vitro distribution kinetics of DA-7157 between plasma and blood cells of rat blood

DA-7157 rapidly reached equilibrium (within 30 s manual mixing) between plasma and blood cells of rat blood; the whole blood and plasma concentrations of DA-7157 were constant up to 2 h incubation at initial DA-7157 blood concentrations of 0.2, 2 and 20 μg mL^{-1}, respectively (data not shown). The equilibrium plasma-to-blood cell partition ratios of DA-7157 were independent of initial DA-7157 blood concentrations studied; the mean value was 3.18 (range 2.87–3.69), suggesting that the binding of DA-7157 to blood cells was not considerable.

Factors influencing the binding of DA-7157 to 4% HSA using the equilibrium dialysis technique

Equilibrium of DA-7157 between 4% HSA and "the buffer" compartments was found to be established after 8 h incubation, and the binding values to 4% HSA were not influenced up to 24 h incubation. The mean binding value of DA-7157 to 4% HSA was 63.4% at DA-7157 concentrations ranging from 0.1 to 50 μg mL^{-1}, however the value was 57.4% at 100 μg mL^{-1}; this could be due to limited binding sites in HSA. Therefore, in the following experiments, the 24-h incubation and a DA-7157 concentration of 5 μg mL^{-1} were used.

The binding of DA-7157 seemed to be dependent on HSA concentrations (binding values of 9.30, 17.8, 34.6, 55.3, 64.9 and 67.1% for 0.5, 1, 2, 3, 4 and 5% HSA concentrations, respectively), buffer pH (68.9, 75.6, 75.9 and 79.7% for buffer solutions having a pH of 5.8, 6.4, 7.0 and 8.0, respectively, at 4% HSA) and AAG concentrations (65.5, 73.5 and 76.4% for 0.08, 0.16 and 0.32% AAG, respectively). However, the binding of DA-7157 to 4% HSA seemed to be independent of other drugs, such as salicylic acid (150 and 300 μg mL^{-1}), sulfisoxazole (100 and 300 μg mL^{-1}), cefaclor (2 and 20 μg mL^{-1}), enoxacin (1 and 10 μg mL^{-1}) and heparin concentrations (at 10 and 40 units mL^{-1}).

The protein binding values of DA-7157 in mice, rats, rabbits, dogs and human fresh plasma (n = 5–7) were 65.5 ± 2.84, 93.4 ± 1.65, 81.3 ± 1.92, 66.8 ± 2.72 and 74.4 ± 3.59%, respectively.

Discussion

In pharmacokinetic studies, accurately measured plasma drug concentrations are usually assumed to be equal to their in-vivo plasma concentrations. Such an assumption may be valid for drugs that have very rapid or instantaneous rates of equilibration between plasma and blood cells (Chiou 1979b; Lee et al 1981). If this equilibration process is slow or irregular, then the length of time elapsed between collection and centrifugation of blood sample may have a profound effect on the measured drug concentration (the so-called blood storage effect); the sooner the centrifugation, the higher the plasma concentration measured will be (Chiou 1979b; Lee et al 1981). This factor might have contributed in part to the reported inconsistencies in the time to achieve the peak plasma level after intravenous administration, to the calculated time-dependent renal clearances, and to the unsmooth plasma level–time profiles reported in the literature (Chiou 1979b). Moreover, it was reported that the bound fractions of adriamycin (Lee & Chiou 1989a) and propranolol (Lee & Chiou 1989b) to red blood cells act as barriers for their elimination. Hence, the experiments on the distribution kinetics of DA-7157 between plasma and blood cells of rat blood were performed.

It was reported that the binding of drugs to plasma proteins was dependent on the buffer pH (unionized forms of drugs have higher affinity to proteins than ionized forms of drugs), concentrations of proteins (due to limited binding sites in proteins), heparin (heparin influences the protein binding of imipramine), AAG (especially for basic drugs), other antibiotics (cefaclor and enoxacin) and other drugs frequently used in medical practice (sulfisoxazole and salicylic acid) (Shim et al 2000). These factors were evaluated in the present study.

The mean t1/2 values of both DA-7218 and DA-7157 after oral administration of DA-7218 (Table 2) were considerably longer than those after intravenous administration

of DA-7218 (Table 1). This could not be due to a flip-flop model of absorption of DA-7218. The $t_{1/2}$ values of the absorption phase of DA-7218 using the residual method were 35.2 and 57.8 min for DA-7218 oral doses of 50 and 100 mg kg^{-1}, respectively; the values were considerably shorter than the $t_{1/2}$ values of oral DA-7218 (Table 2). This could be due to different blood sampling time schedules between the two routes of administration. For comparison, the $t_{1/2}$ values of both DA-7218 and DA-7157 after oral administration of DA-7218 were estimated up to 120 min (for DA-7218) and 480 min (for DA-7157) for plasma data (the same times as in the intravenous study; Figure 3C and D for DA-7218 and DA-7157, respectively); the values for both DA-7218 and DA-7157 were close to those after the intravenous administration of DA-7218 (Table 1); the value for DA-7218 at a dose of 20 mg kg^{-1} was 67.1 min, and the value for DA-7157 at a DA-7218 dose of 20 mg kg^{-1} was 147 min.

After intravenous administration of DA-7157, the CL_R of DA-7157 was estimated as free (unbound to plasma proteins) fractions of DA-7157 in plasma based on the CL_R and plasma protein binding of DA-7157 in rats; the estimated value was 0.0955 mL min^{-1} kg^{-1}. The value of 0.0955 mL min^{-1} kg^{-1} was considerably slower than the reported glomerular filtration rate of 5.24 mL min^{-1} kg^{-1} in rats (Davies & Morris 1993), suggesting that DA-7157 is reabsorbed in rat renal tubules.

Conclusion

DA-7218 and DA-7157 exhibit dose-proportional pharmacokinetics after both intravenous (5–20 mg kg^{-1}) and oral (20–100 mg kg^{-1}) administration of DA-7218 to rats. DA-7218 was unstable in rat blood, plasma, bile and liver homogenates but DA-7157 was stable, suggesting that DA-7218 is hydrolysed via the enzyme phosphatase. DA-7157 rapidly reached equilibrium between plasma and blood cells, and the mean equilibrium plasma-to-blood cells ratio was 3.18, indicating that binding of DA-7157 to blood cells was not considerable. The protein binding of DA-7157 in fresh rat plasma was 93.4%.

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