JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 635–642

Received October 1, 2003
Accepted January 30, 2004
DOI 10.1211/0022357023277
ISSN 0022-3573

Effects of protein–calorie malnutrition on the pharmacokinetics of DA-7867, a new oxazolidinone, in rats

Soo Kyung Bae, Shin Jung Lee, Jong Won Kwon, Won Bae Kim and Myung Gull Lee

Abstract

The pharmacokinetic parameters of DA-7867, a new oxazolidinone, were compared after intravenous and oral administration at a dose of 10 mg kg^{-1} to control rats and rats with protein–calorie malnutrition (rats with PCM). After intravenous administration of 10 mg kg^{-1} DA-7867 to rats, metabolism of the drug was not considerable and after 14 days approximately 85.0% of the dose was recovered as unchanged drug from urine and faeces. After intravenous administration to rats with PCM, the area under the plasma concentration–time curve from time zero to time infinity (AUC) was significantly smaller (10 800 vs 6990 μg min mL^{-1}) compared with control rats. This may have been due to significantly faster total body clearance (CL, 0.930 vs 1.44 mL min^{-1} kg^{-1}). The faster CL in PCM rats could have been due to significantly faster non-renal clearance (0.842 vs 1.39 mL min^{-1} kg^{-1} due to significantly greater gastrointestinal (including biliary) excretion; the amount of unchanged DA-7867 recovered from the entire gastrointestinal tract at 24 h was significantly greater (1.19 vs 4.28% of intravenous dose)) because the renal clearance was significantly slower in PCM rats (0.0874 vs 0.0553 mL min^{-1} kg^{-1}). After oral administration to PCM rats, the AUC was significantly smaller compared with control rats (7900 vs 4310 μg min mL^{-1}). This could have been due to a decrease in absorption from the gastrointestinal tract.

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, Korea

Soo Kyung Bae, Shin Jung Lee, Myung Gull Lee

Research Laboratory, Dong-A Pharmaceutical Company Ltd, 47 Sanggal-Ri, Kiheung-Up, Yongin, Kyunggi-Do 449-900, Korea

Jong Won Kwon, Won Bae Kim

Correspondence: M. G. Lee, College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, Korea.
E-mail: leemg@snu.ac.kr

Funding: This study was supported in part by a grant from the Korea Ministry of Health & Welfare (01-PJ1-PG4-01PT01-0005), 2001–2004.

Introduction

Protein–calorie malnutrition (PCM) is considered to be a global problem, especially for children, infants, and institutional elderly who are more susceptible to PCM (Denke & Wilson 1998). The rate of drug metabolism may be influenced by various physiological, genetic, and environmental factors. Nevertheless, nutritional status is not usually investigated as a factor which may affect the pharmacokinetics of drugs, and hence, the pharmacodynamics of drugs. The pharmacokinetic and/or pharmacodynamic changes of the following drugs in rats with PCM have been reported from our laboratories; furosemide (Kim et al 1993), bumetanide (Kim & Lee 1993), itraconazole (Lee et al 2003), phenytoin (Kim et al 2001a), chlorzoxazone (Kim et al 2002), 2-(allylthio)pyrazine, a new chemoprotective agent (Kim et al 2003), clarithromycin (Ahn et al 2003), azosemide (Kim et al 2001b), and adriamycin (Kim et al 2000). The changes in drug metabolism and pharmacokinetics in malnutrition have been reviewed (Buchanan 1978; Krishnaswamy 1978).

The oxazolidinones, which inhibit bacterial protein synthesis, have been synthesized to overcome the problem of emerging resistance in Gram-positive bacteria (Daniel & Ronald 2001; Fung et al 2001). A new oxazolidinone, DA-7867 ((*S*)-[N-3-(4-(2-(1-methyl-5-tetrazolyl)-pyridine-5-yl)-3-fluorophenyl)-2-oxo-5-oxazolidinyl]methyl acetamide, a basic compound having a MW of 411.39 Da) has been synthesized (Research Laboratory of Dong-A Pharmaceutical Company, Yongin, Korea). DA-7867 showed antibacterial activity 4- to 8-fold better than linezolid against Gram-positive and Gram-negative pathogens, including multidrug resistant bacteria (Im et al 2002). We have reported (Bae et al 2004a) our investigations on the pharmacokinetics of DA-7867. Firstly, we found that the total area under the plasma concentration–time curve from

time zero to time infinity (AUC) of DA-7867 was dose-proportional after intravenous (at doses of 1–20 mg kg^{-1}) and oral (at doses of 1–20 mg kg^{-1}) administration. The mean extent of absolute oral bioavailability (F) was approximately 71.6%. Secondly, we found that the first-pass effects of DA-7867 in the lung, heart, and liver were negligible, and intestinal first-pass effect of DA-7867 was approximately 21.8% at an oral dose of 10 mg kg^{-1}. Metabolism of DA-7867 was not considerable while renal and gastrointestinal (including biliary) excretion was considerable; approximately 85.0% of the intravenous dose was recovered from urine (17.0% of intravenous dose), faeces (64.0% of intravenous dose), and the rinsing water from the metabolic cage (3.16% of intravenous dose), when collected for up to 14 days after intravenous administration at a dose of 10 mg kg^{-1} to 10 rats (our unpublished data).

PCM as well as many selective mineral and vitamin deficiencies deteriorate the immune and inflammatory metabolic response and increase the frequency of infections (Benito López 1993). Hence, DA-7867 could be used to treat microbial infections in such patients. In this study we have investigated the pharmacokinetic changes of DA-7867 after intravenous administration at a dose of 10 mg kg^{-1} to rats with PCM. Tissue distribution of DA-7867 was compared after intravenous administration at a dose of 10 mg kg^{-1} to control rats and rats with PCM.

Materials and Methods

Chemicals

DA-7867, DA-7858 ((*S*)-[N-3-(4-(2-(5-methyl-1,2,4-oxadiazol-3-yl)-pyridine-5-yl)-3-fluorophenyl)-2 -oxo-5-oxazolidinyl]methyl acetamide, an internal standard for HPLC analysis), and hypromellose (hydroxypropyl methylcellulose, HPMC type 2910, Shin-Etsu Chemical Company, Tokyo, Japan) were from Research Laboratory of Dong-A Pharmaceutical Company. N,N-dimethylacetamide (DMA) and polyethylene glycol 400 (PEG 400) were purchased from Sigma Chemical Company (St Louis, MO) and Duksan Chemical Company (Seoul, Korea), respectively. Other chemicals were of reagent grade or HPLC grade, and were used without further purification.

Animals

Male Sprague–Dawley rats (155–175 g) were purchased from Charles River Company Korea (Biogenomics, Seoul, Korea). The rats were assigned randomly to one of two diets containing either 23% (control rats) or 5% (rats with PCM) casein. Both diets were isocaloric and the compositions of the diets listed (Cho et al 1999). All rats were provided with food and water, which were freely available. The animals were maintained on each diet for a four-week period (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Korea).

The Animal Care and Use Committee of the College of Pharmacy, Seoul National University, approved the animal study protocol.

Preliminary study

The following preliminary study was performed after a four-week period on each diet (n = 5, each). The 24-h urine was collected for measuring the creatinine level. Serum was collected for measuring the protein binding using an equilibrium dialysis technique (Bae et al 2004b) and total proteins, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and creatinine levels (analysed by Green Cross Reference Lab., Seoul, Korea). The whole kidney and liver of each rat were excised, rinsed with 0.9% NaCl-injectable solution, blotted dry with tissue paper, and weighed. To assess the influence of the low protein diet, food intake and body weight were recorded at least once a week.

Pretreatment of rats

In the early morning, after a four-week period on each diet, the rats were put under light ether anaesthesia. The jugular vein and the carotid artery of each rat were then cannulated with polyethylene tubing (Clay Adams, Parsippany, NJ) (Kim et al 1993). Both cannulas were exteriorized to the dorsal side of the neck where each cannula terminated with long Silastic tubing (Dow Corning, Midland, MI). Both Silastic tubings were inserted into a wire coil to allow free movement of the rat. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, Korea) and allowed 4–5 h to recover from the anaesthesia before the study began. The animals were not restrained during the study. Heparinized 0.9% NaCl-injectable solution (20 U mL^{-1}), 0.3 mL, was used to flush each cannula to prevent blood clotting.

Intravenous study

DA-7867 (the DA-7867 powder was dissolved in DMA: PEG 400:distilled water, 3:5:2, v/v/v) at a dose of 10 mg kg^{-1} was infused (total injection volume was approximately 0.6 mL) over 1 min via the jugular vein of control rats (n = 8) and rats with PCM (n = 13). Blood (0.12-mL sample) was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of infusion), 5, 30, 60, 90, 120, 240, 360, 480, 720, 960, and 1440 min after intravenous administration of DA-7867. Approximately 0.3 mL heparinized 0.9% NaCl-injectable solution was used to flush each cannula immediately after each blood sampling. Blood samples were centrifuged immediately and 50 μL of each plasma sample was stored in a −70 °C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until HPLC analysis of DA-7867 (Bae et al 2003). At the end of 24 h, each metabolic cage was rinsed twice with 10 mL distilled water and the rinsing water was combined with the urine sample. After measuring the exact volume of the combined urine sample, two 50-μL portions of the combined urine sample were stored at −70 °C until HPLC analysis of DA-7867 (Bae et al 2003). At the same time (24 h), the entire gastrointestinal tract (including its contents and faeces) was removed, transferred into a beaker containing 100 mL methanol (to facilitate extraction of DA-7867), and cut

into small pieces using scissors. After manual shaking and stirring with a glass rod, two 50-μL samples of the supernatant were collected from each beaker and stored at $-70\,^{\circ}C$ until HPLC analysis of DA-7867 (Bae et al 2003).

Oral study

DA-7867 (the DA-7867 powder was suspended in 1% hypromellose) at a dose of $10\,mg\,kg^{-1}$ was administered orally (total oral volume was approximately 2 mL) to control rats (n = 8) and rats with PCM (n = 11) using a feeding tubing. Blood samples were collected at 0, 15, 30, 60, 120, 180, 240, 360, 480, 720, 960, and 1440 min. Other procedures were similar to those in the intravenous study.

Tissue distribution after intravenous administration

DA-7867 (the same solution as used in the intravenous study) at a dose of $10\,mg\,kg^{-1}$ was administered intravenously to control rats (n = 5 at each time) and rats with PCM (n = 5 at each time). At 0.5 and 12 h after 1-min intravenous infusion, as much blood as possible was collected via the abdominal artery and each rat was killed by cervical dislocation. Blood samples were centrifuged immediately and plasma was collected. Approximately 1 g of each liver, kidney, lung, heart, spleen, muscle, mesentery, fat, brain, large intestine, stomach, and small intestine was excised. The samples were rinsed with cold 0.9% NaCl-injectable solution to eliminate any blood remaining in the tissues, blotted dry with paper tissue, and homogenized with 4 vol distilled water using a tissue homogenizer (Ultra-Turrax T25, Janke & Kunkel, IKA-Labortechnik, Staufeni, Germany). After centrifugation, two 50-μL samples of the 9000-*g* supernatant fraction were stored at $-70\,^{\circ}C$ until HPLC analysis of DA-7867 (Bae et al 2003). All the procedures were conducted at $4\,^{\circ}C$ in an ice-bath.

Protein binding study

The serum protein binding of DA-7867 to control rats and rats with PCM was determined using an equilibrium dialysis technique (Bae et al 2004b). Serum (1 mL) was dialysed against 1 mL isotonic Sørensen phosphate buffer (pH 7.4) containing 3% dextran to minimize volume shift (Boudinot & Jusko 1984), with 1 mL dialysis cell (Fisher Scientific, Fair Lawn, NJ) and a Spectra/Por 4 membrane (mol. wt. cut-off 12000–14000; Spectrum Medical Industries Inc., Los Angeles, CA). To reduce equilibrium time, DA-7867 $5\,\mu g\,mL^{-1}$ was added to the serum side (Øie & Guentert 1982), and the dialysis cell was incubated for 24 h in a water-bath shaker kept at $37\,^{\circ}C$ and at a rate of 50 oscillations min^{-1} (Bae et al 2004b).

HPLC analysis of DA-7867

The amount of DA-7867 in the above samples was analysed by the HPLC method developed from our laboratories (Bae et al 2003). A 50-μL biological sample was deproteinized (Chiou et al 1978) with 200-μL acetonitrile, and 50-μL methanol containing $10\,\mu g\,mL^{-1}$ DA-7858 (an internal standard) was added. After vortex-mixing, the supernatant was transferred into a clean tube and evaporated under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of the mobile phase and 50 μL was injected directly onto a reversed-phase (C_{18}) column. The mobile phase, 20 mM KH_2PO_4:acetonitrile (75:25, v/v), was run at a flow rate of $1.5\,mL\,min^{-1}$. The column effluent was monitored by a UV detector set at 300 nm. The retention times of DA-7867 and DA-7858 (an internal standard) were approximately 6.0 and 8.5 min, respectively. The detection limits of DA-7867 in human plasma, urine, and rat tissue homogenate were 0.02, 0.02, and $0.05\,\mu g\,mL^{-1}$, respectively. The coefficients of variation (within- and between-day) were generally low (below 8.02%).

Pharmacokinetic analysis

The AUC was calculated by the trapezoidal rule-extrapolation method; this method uses the logarithmic trapezoidal rule recommended by Chiou (1978) for the calculation of the area during the phase of a declining level in plasma and the linear trapezoidal rule for the phase of a rising level in plasma. The area from the last datum point to infinity was estimated by dividing the last measured concentration in plasma by the terminal rate constant. Standard methods (Gibaldi & Perrier 1982) were used to calculate the following pharmacokinetic parameters: the time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, area under the first moment of plasma concentration–time curve (AUMC), mean residence time (MRT), and apparent volume of distribution at steady state (Vd_{ss}) (Kim et al 1993).

The harmonic mean method was used for the calculation of mean values of Vd_{ss} (Chiou 1979), terminal half-life (Eatman et al 1977), and each clearance (Chiou 1980).

Glomerular filtration rate (GFR) was estimated by measuring the creatinine clearance (CL_{CR}), assuming kidney function was stable during the experimental period. The CL_{CR} was calculated by dividing the total amount of unchanged creatinine excreted in urine over 24-h by the $AUC_{0-24\,h}$ of creatinine in plasma.

Statistical analysis

A P value of less than 0.05 was considered statistically significant using the unpaired t-test. All data were expressed as mean ± s.d.

Results

Effects of PCM on body weight gain, food intake, and organ weight

Effects of dietary protein on body weight gain, food, protein, and calorie intakes, and liver and kidney weight in the preliminary studies are listed in Table 1. Protein deprivation for four weeks (5% casein diet, PCM) caused

Table 1 Body weight, food, protein, and calorie intakes, serum data, and liver and kidney weight in control rats and rats with PCM.

Parameters	Control	PCM
Initial body weight (g)	165 ± 8.71	167 ± 9.14
Final body weight (g)	400 ± 11.7	164 ± 8.22***
Food intake (g/day/rat)	17.9 ± 5.83	6.63 ± 0.642**
Protein intake (g/day/rat)	4.12 ± 1.34	0.318 ± 0.0321***
Calorie intake (kcal/day/rat)	72.4 ± 23.5	25.7 ± 2.60**
Serum		
Total proteins ($g\,dL^{-1}$)	5.36 ± 0.700	4.35 ± 0.208*
Albumin ($g\,dL^{-1}$)	3.26 ± 0.428	2.70 ± 0.428*
GOT ($IU\,L^{-1}$)	80.0 ± 67.7	72.3 ± 13.9
GPT ($IU\,L^{-1}$)	49.0 ± 43.3	58.5 ± 15.9
Protein binding (%)	75.3 ± 1.72	70.6 ± 8.66
CL_{CR} ($mL\,min^{-1}\,kg^{-1}$)	3.71 ± 1.21	2.92 ± 1.17
Liver weight (g)	17.5 ± 1.48	6.91 ± 1.62***
Liver weight (% body weight)	4.37 ± 0.327	4.20 ± 0.899
Kidney weight (g)	2.79 ± 1.51	1.29 ± 0.500***
Kidney weight (% body weight)	0.700 ± 0.0290	0.0788 ± 0.0533*

Values are mean ± s.d., n = 5, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with control.

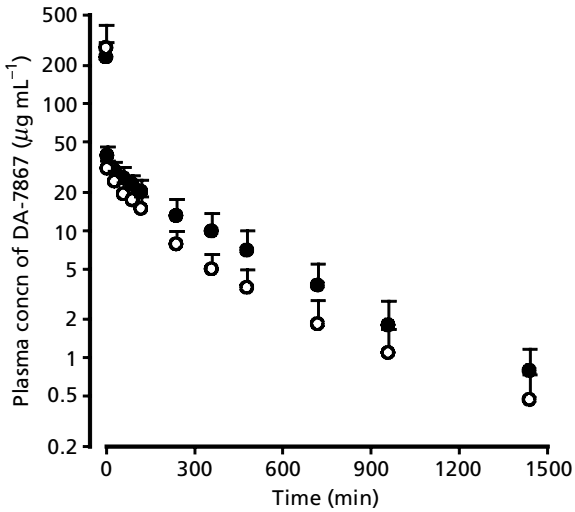

Figure 1 Mean arterial plasma concentration–time profiles of $10\,mg\,kg^{-1}$ DA-7867 after 1-min intravenous infusion to control rats (n = 8; ●) and rats with PCM (n = 13; ○). Vertical bars represent s.d.

a significant decrease in body weight gain and food consumption. For example, body weight gain decreased significantly in rats with PCM (from 167 g to 164 g) compared with control rats (from 165 to 400 g), and rats on the 5% protein diet (rats with PCM) consumed approximately 64.5% less food than the rats on the 23% protein diet (control rats), despite the supply of food being freely available. As a result, the protein and calorie intake decreased significantly by 92.3% and 64.5%, respectively, in rats with PCM. Since both the protein and calorie intakes decreased significantly in rats with PCM, it is important to realize that rats with PCM suffered from both protein and calorie deficiencies. Therefore, any changes in the pharmacokinetics of DA-7867 in rats with PCM should be attributed to PCM and not solely to protein deficiency. The absolute liver and kidney weight decreased significantly in rats with PCM and this could be due to significant decrease in body weight gain. However, the relative (% of body weight) weight of liver was not significantly different between control rats and rats with PCM. In rats with PCM, serum levels of total proteins (18.8% decrease) and albumin (17.2% decrease) were significantly lower than those in control rats, however, the levels of GOT, GPT, and protein binding, and CL_{CR} were comparable between the two groups of rats.

Pharmacokinetics of DA-7867 after intravenous administration to rats

The plasma concentration–time profiles of DA-7867 after intravenous administration to both groups of rats are shown in Figure 1. Table 2 shows the relevant pharmacokinetic parameters. After intravenous administration, plasma concentrations of DA-7867 declined in a polyexponential fashion for both groups of rats with lower levels in rats with PCM. This resulted in a significantly smaller AUC (35.3% decrease) in PCM rats compared with control rats. The smaller AUC in rats with PCM could be due to significantly faster CL (54.8% increase). The faster CL in rats with PCM was due to significantly faster CL_{NR} (65.1% increase), since the CL_R was significantly slower (39.0% decrease) compared with control rats. The total amount of unchanged DA-7867 excreted in 24-h urine ($Ae_{0-24\,h}$, expressed in

Table 2 Body weight and pharmacokinetic parameters of $10\,mg\,kg^{-1}$ DA-7867 after intravenous administration to control rats and rats with PCM.

Parameters	Control	PCM
Initial body weight (g)	188 ± 8.33	183 ± 12.9
Final body weight (g)	369 ± 18.8	176 ± 14.6***
AUC ($\mu g\,min\,mL^{-1}$)	10800 ± 3400	6990 ± 1800**
Terminal half-life (min)	334 ± 67.5	337 ± 29.7
MRT (min)	372 ± 44.9	315 ± 95.0
Vd_{ss} ($mL\,kg^{-1}$)	350 ± 94.8	442 ± 33.8
CL ($mL\,min^{-1}\,kg^{-1}$)	0.930 ± 0.348	1.44 ± 0.205*
CL_R ($mL\,min^{-1}\,kg^{-1}$)	0.0874 ± 0.0281	0.0553 ± 0.0100*
CL_{NR} ($mL\,min^{-1}\,kg^{-1}$)	0.842 ± 0.326	1.39 ± 0.198*
$Ae_{0-24\,h}$ (% of dose)	9.48 ± 1.66	4.02 ± 1.05***
$GI_{24\,h}$ (% of dose)	1.19 ± 0.459	4.28 ± 1.03***

Values are mean ± s.d., n = 8 for control rats and n = 13 for rats with PCM, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared with control.

terms of percentage of intravenous dose) was significantly smaller (57.6% decrease) in rats with PCM. This resulted in a significantly slower CL_R of DA-7867 in rats with PCM. However, the total amount of unchanged DA-7867 recovered from the entire gastrointestinal tract at 24 h ($GI_{24\,h}$, expressed in terms of percentage of intravenous dose) was significantly greater (260% increase) in rats with PCM. As mentioned earlier, metabolism of DA-7867 in rats was not considerable while urinary and gastrointestinal (including biliary) excretion was considerable; 85.0% of an intravenous dose at 10 mg kg^{-1} was excreted via the urine and faeces for up to 14 days after intravenous administration. Hence, the significantly faster CL_{NR} of DA-7867 in rats with PCM could be due to significantly increased gastrointestinal (including biliary) excretion. DA-7867 5 μg mL^{-1} was stable for up to 3-h incubation in five human gastric juices; the recovery of DA-7867 after 3-h incubation was 96.2, 109, 98.3, 100, and 108% for five human gastric juices having pH values of 3.68, 3.31, 6.86, 2.41, and 4.43, respectively. DA-7867 5 μg mL^{-1} was stable for up to 24-h incubation in various buffer solutions over the pH range 1–11; the recovery of the DA-7867 after 24-h incubation was 104, 100, 99.4, 102, 96.0, 104, 100, 105, 103, 98.1, and 105% for pH values of 1–11, respectively.

In rats with PCM, the Vd_{ss} of DA-7867 was comparable between the two groups of rats. This could have been expected since the free (unbound in plasma proteins) fraction of DA-7867 was comparable between the two groups of rats; the values were 24.7 and 29.4% for control rats and rats with PCM, respectively (Table 1). Similar results have been reported for the Vd_{ss} values of azosemide (Kim et al 2001b), bumetanide (Kim & Lee 1993), furosemide (Kim et al 1993), clarithromycin (Ahn et al 2003), and itraconazole (Lee et al 2003). However, the apparent volume of distribution of phenytoin (Kim et al 2001a), chlorzoxazone (Kim et al 2002), and 2-(allylthio)pyrazine (Kim et al 2003) in rats with PCM was significantly larger (47.3, 52.7 and 128%, respectively) compared with control rats. This was mainly due to the increase in the free (unbound in plasma proteins) fraction in plasma (43.3, 97.4, and 24% increase, respectively). The terminal half-life and MRT were not significantly different between two groups of rats. Note that the body weight gain was also decreased significantly in rats with PCM (from 183 to 176 g) compared with control rats (from 188 to 369 g).

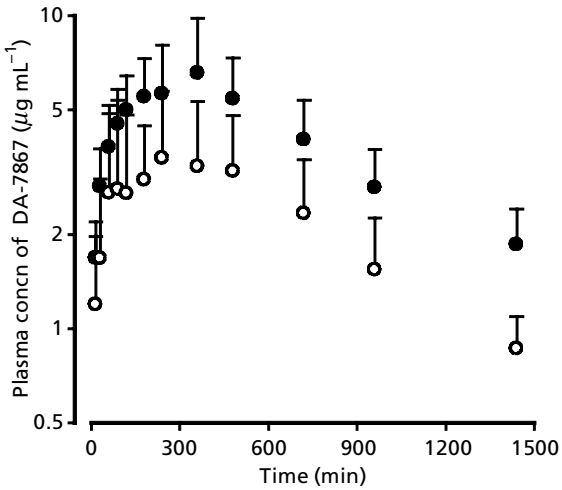

Figure 2 Mean arterial plasma concentration–time profiles of 10 mg kg^{-1} DA-7867 after oral administration to control rats (n = 8; ●) and rats with PCM (n = 11; ○). Vertical bars represent s.d.

Table 3 Body weight and pharmacokinetic parameters of 10 mg kg^{-1} DA-7867 after oral administration to control rats and rats with PCM.

Parameters	Control	PCM
Initial body weight (g)	170 ± 8.44	169 ± 11.7
Final body weight (g)	371 ± 21.7	175 ± 10.5***
AUC (μg min mL^{-1})	7900 ± 2360	4310 ± 1810**
Terminal half-life (min)	713 ± 175	541 ± 98.0
CL_R (mL min^{-1} kg^{-1})	0.0259 ± 0.0165	0.0460 ± 0.0202*
$Ae_{0-24\,h}$ (% of dose)	2.59 ± 1.24	2.33 ± 1.48
$GI_{24\,h}$ (% of dose)	12.0 ± 5.88	20.9 ± 10.2*
C_{max} (μg mL^{-1})	7.29 ± 2.75	5.50 ± 2.74
T_{max} (min)	308 ± 113	290 ± 150
F (%)	73.2	61.7

Values are mean ± s.d., n = 8 for control rats and n = 11 for rats with PCM, *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with control.

Pharmacokinetics of DA-7867 after oral administration to rats

The plasma concentration–time profiles of DA-7867 after oral administration to both groups of rats are shown in Figure 2. Table 3 shows the relevant pharmacokinetic parameters. After oral administration, plasma concentrations of DA-7867 reached (T_{max}) a peak (C_{max}) at 308 and 290 min for control rats and rats with PCM, respectively. After reaching respective C_{max}, plasma concentrations of DA-7867 declined in a monoexponential fashion for both groups of rats with mean terminal half-lives of 713 and 541 min for control rats and rats with PCM, respectively. In rats with PCM, plasma concentrations of DA-7867 and C_{max} were lower (24.6% decrease) compared with control rats. This resulted in a significantly smaller AUC (45.4% decrease) in rats with PCM. The $Ae_{0-24\,h}$ values were comparable between the two groups of rats, but AUC was significantly smaller in PCM rats. Hence, the CL_R was significantly faster in rats with PCM (77.6% increase). In rats with PCM, the $GI_{24\,h}$ was significantly greater (74.2% increase) than that in control rats. Hence, the significantly smaller AUC in rats with PCM could have been due to a significant decrease in absorption in the rats, as will be discussed later. The

Table 4 Amount of DA-7867 recovered from each g tissue ($\mu g\,mL^{-1}$ for plasma or $\mu g\,g^{-1}$ for other tissues) 30 min and 12 h after intravenous administration at a dose of 10 mg kg^{-1} to control rats and rats with PCM. The numbers in parentheses represent tissue-to-plasma (T/P) ratios.

Tissue	Thirty minutes		Twelve hours	
	Control	PCM	Control	PCM
Plasma	36.0 ± 1.39	25.4 ± 2.93	3.39 ± 0.532	1.74 ± 0.700
Liver	22.3 ± 1.94 (0.622 ± 0.0570)	17.5 ± 0.953 (0.696 ± 0.0888)	2.57 ± 1.28 (0.739 ± 0.282)	1.93 ± 0.625 (1.15 ± 0.172)*
Kidney	20.3 ± 2.29 (0.565 ± 0.0650)	17.9 ± 3.23 (0.718 ± 0.202)	1.95 ± 1.09 (0.559 ± 0.231)	1.49 ± 0.299 (0.932 ± 0.234)*
Lung	9.43 ± 1.35 (0.262 ± 0.0330)	9.83 ± 1.38 (0.395 ± 0.0970)*	0.862 ± 0.526 (0.245 ± 0.117)	0.740 ± 0.216 (0.452 ± 0.124)*
Spleen	10.1 ± 0.973 (0.281 ± 0.0279)	8.16 ± 0.452 (0.325 ± 0.0479)	0.977 ± 0.500 (0.280 ± 0.0984)	0.810 ± 0.230 (0.484 ± 0.0976)*
Heart	13.2 ± 2.06 (0.367 ± 0.0646)	9.85 ± 0.497 (0.391 ± 0.0374)	1.21 ± 0.706 (0.343 ± 0.148)	1.31 ± 0.881 (0.865 ± 0.776)
Muscle	6.66 ± 0.508 (0.185 ± 0.0137)	4.86 ± 0.466 (0.193 ± 0.0291)	0.491 ± 0.243 (0.141 ± 0.0535)	0.355 ± 0.0310 (0.239 ± 0.120)
Mesentery	3.68 ± 0.570 (0.103 ± 0.0172)	4.35 ± 0.868 (0.171 ± 0.0218)**	0.305 ± 0.193 (0.0860 ± 0.0373)	0.274 ± 0.110 (0.158 ± 0.0263)**
Fat	1.57 ± 0.215 (0.0435 ± 0.00449)	2.63 ± 0.642 (0.103 ± 0.0232)**	BD	BD
Brain	0.679 ± 0.104 (0.0189 ± 0.00315)	0.517 ± 0.153 (0.0207 ± 0.00722)	BD	BD
Large intestine	7.56 ± 0.871 (0.210 ± 0.0214)	4.48 ± 0.785 (0.177 ± 0.0321)	2.38 ± 0.630 (0.720 ± 0.243)	1.82 ± 0.277 (1.19 ± 0.503)
Stomach	8.98 ± 3.22 (0.249 ± 0.0870)	5.10 ± 0.280 (0.205 ± 0.0344)	0.572 ± 0.289 (0.163 ± 0.0523)	0.664 ± 0.411 (0.370 ± 0.0919)**
Small intestine	12.0 ± 0.780 (0.334 ± 0.0300)	14.7 ± 3.26 (0.583 ± 0.158)*	2.91 ± 1.35 (0.851 ± 0.364)	1.69 ± 0.409 (1.12 ± 0.539)

Values are mean ± s.d., n = 5, *$P < 0.05$ and **$P < 0.01$ compared with control. BD, below detection.

F values were 73.2 and 61.7% for control rats and rats with PCM, respectively. Body weight gain again decreased significantly in rats with PCM (from 169 to 175 g) compared with control rats (from 170 to 371 g).

Tissue distribution after intravenous administration to rats

Table 4 lists the amount of DA-7867 in each tissue ($\mu g\,mL^{-1}$ for plasma and $\mu g\,g^{-1}$ for other tissues) and tissue-to-plasma (T/P) ratios 30 min and 12 h after intravenous administration ($10\,mg\,kg^{-1}$) to control rats and rats with PCM. In both groups of rats, the T/P ratios of DA-7867 were less-than-unity in all rat tissues studied for both times except in large intestine and small intestine at 12 h. This suggested that the rat tissues studied had a poor affinity to DA-7867. In rats with PCM, the T/P ratios in lung, mesentery, fat, and small intestine at 30 min and liver, kidney, lung, spleen, mesentery, and stomach at 12 h were significantly greater compared with control rats.

Discussion

After intravenous administration of DA-7867, the CL values (0.930 and $1.44\,mL\,min^{-1}\,kg^{-1}$ based on plasma data, Table 2) were extremely slower than the reported cardiac output of $295\,mL\,min^{-1}\,kg^{-1}$ based on blood data in rats (Davies & Morris 1993). This suggested that first-pass effect of DA-7867 in the lung and heart could be almost negligible, if any, in both groups of rats. It has been reported (Bae et al 2004a) from our laboratories that the hepatic first-pass effect of DA-7867 was almost negligible in rats. The estimated CL_R values of DA-7867 based on free (unbound in plasma proteins) fraction (Table 1) were 0.354 and $0.188\,mL\,min^{-1}\,kg^{-1}$ for control rats and rats with PCM, respectively, based on CL_R (Table 2) and serum protein binding values (Table 1). The values were considerably slower than the reported glomerular filtration rate of $5.24\,mL\,min^{-1}\,kg^{-1}$ in rats (Davies & Morris 1993). This indicated that DA-7867 was mainly reabsorbed in rat renal tubules. Considering the CL_R values of DA-7867 (Table 2), reported kidney blood flow rate of $36.8\,mL\,min^{-1}\,kg^{-1}$ (Davies & Morris 1993), and haematocrit of approximately 45% (Mitruka & Rawnsley 1981) in rats, the estimated renal extraction ratios (CL_R of DA-7867/kidney plasma flow rate, only for urinary excretion of unchanged DA-7867) were 0.432 and 0.273% for control rats and rats with PCM, respectively. The above data indicated that DA-7867 was a poor renal extraction ratio drug.

After oral administration of $10\,mg\,kg^{-1}$ DA-7867 to rats with PCM, the AUC was significantly smaller (45.4% decrease) compared with control rats (Table 3). This could have been due to decreased absorption of DA-7867 from the entire gastrointestinal tract in the rats. After oral administration, the $GI_{24\,h}$ values were 12.0 and 20.9% for control rats and rats with PCM, respectively (Table 3). It was possible that this unchanged DA-7867, 12.0 and 20.9%, might be partly attributed to the gastrointestinal (including biliary) excretion of the absorbed drug. Based on the linear pharmacokinetics, the mean true fraction of dose unabsorbed (F_{unabs}) in this study could be estimated by the following equations (Lee & Chiou 1983);

$$\text{for control rats: } 0.12 = F_{unabs} + (0.732 \times 0.0119) \quad (1)$$

$$\text{for rats with PCM: } 0.209 = F_{unabs} + (0.617 \times 0.0428) \quad (2)$$

where 0.732 (0.617) and 0.0119 (0.0428) are F (Table 3) and $GI_{24\,h}$ after intravenous administration to control rats (rats with PCM), respectively (Table 2). The calculated F_{unabs} values were 11.1 and 18.3% for control rats and rats with PCM, respectively. Hence, approximately 89 and 82% of the oral dose was absorbed from the entire gastrointestinal tract for control rats and rats with PCM, respectively.

Conclusion

After intravenous administration of DA-7867 to rats with PCM, the AUC was significantly smaller compared with control rats and this was due to significantly faster CL in the rats (Table 2). The faster CL in rats with PCM was due to significantly faster CL_{NR} since CL_R was significantly slower in the rats (Table 2). The faster CL_{NR} in rats with PCM could have been due to a significant increase in gastrointestinal (including biliary) excretion in the rats. After oral administration of DA-7867 to rats with PCM, the AUC was significantly smaller compared with control rats (Table 3) and this could have been due to the decrease in gastrointestinal absorption in the rats.

References

Ahn, C. Y., Kim, E. J., Kwon, J. W., Chung, H. J., Kim, S. G., Shim, C.-K., Lee, M. G. (2003) Effects of cysteine on the pharmacokinetics of intravenous clarithromycin in rats with protein–calorie malnutrition. *Life Sci.* **73**: 1783–1794

Bae, S. K., Kim, E. J., Kwon, J. W., Kim, W. B., Lee, M. G. (2003) High-performance liquid chromatographic analysis of DA-7867, a new oxazolidinone, in plasma and urine and rat tissue homogenates. *J. Chromatogr. B.* **794**: 397–403

Bae, S. K., Chung, W.-S., Kim, E. J., Rhee, J. K., Kwon, J. W., Kim, W. B., Lee, M. G. (2004a) Pharmacokinetics of DA-7867, a new oxazolidinone, after intravenous and oral administration to rats; intestinal first-pass effect. *Antimicrob. Agents Chemother.* **48**: 659–662

Bae, S. K., Chung, W.-S., Kim, E. J., Rhee, J. K., Kwon, J. W., Kim, W. B., Lee, M. G. (2004b) Pharmacokinetics, blood partition, and protein binding of DA-7867, a new oxazolidinone. *Biopharm. Drug Dispos.* In press

Benito López, P., Gálvez Moreno, M. A., Corpas Jiménez, M. S., Calanas Continente, A. (1993) Influence of nutritional status on the organism's response to infectious disease. *Rev. Clin. Esp.* **193**: 255–260

Boudinot, F. D., Jusko, W. J. (1984) Fluid shifts and other factors affecting plasma protein binding of prednisolone by equilibrium dialysis. *J. Pharm. Sci.* **71**: 774–780

Buchanan, N. (1978) Drug kinetics in protein energy malnutrition. *S. Afr. Med. J.* **53**: 327–330

Chiou, W. L. (1978) Critical evaluation of potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. *J. Pharmacokinet. Biopharm.* **6**: 539–546

Chiou, W. L. (1979) New calculation method for mean apparent drug volume of distribution and application to rational dosage regimens. *J. Pharm. Sci.* **68**: 1067–1069

Chiou, W. L. (1980) New calculation method of mean total body clearance of drugs and its application to dosage regimens. *J. Pharm. Sci.* **69**: 90–91

Chiou, W. L., Nation, R. L., Peng, G. W., Huang, S. M. (1978) Improved micro-scale high-pressure liquid-chromatographic assay of gentamicin in plasma. *Clin. Chem.* **24**: 1846–1847

Cho, M. K., Kim, Y. G., Lee, M. G., Kim, S. G. (1999) Suppression of rat hepatic cytochrome P450s by protein–calorie malnutrition: complete or partial restoration by cysteine or methionine supplementation. *Arch. Biochem. Biophys.* **372**: 150–158

Daniel, J. D., Ronald, N. J. (2001) Oxazolidinone antibiotics. *Lancet* **358**: 1975–1982

Davies, B., Morris, T. (1993) Physiological parameters in laboratory animals and humans. *Pharm. Res.* **10**: 1093–1095

Denke, M., Wilson, J. D. (1998) Protein and energy malnutrition. In: Fauci, A. S., Braunwald, E., Isselbacher, K. J., Wilson, J. D., Martin, J. B., Kasper, D. L., Hauser, S. L., Longo, D. L. (eds) *Principles of internal medicine*, 14th edn. McGraw-Hill, New York, pp 452–454

Eatman, F. B., Colburn, W. A., Boxenbaum, H. G., Posmanter, H. N., Weinfeld, R. E., Ronfeld, R., Weissman, L., Moore, J. D., Gibaldi, M., Kaplan, S. A. (1977) Pharmacokinetics of diazepam following multiple dose oral administration to healthy human subjects. *J. Pharmacokinet. Biopharm.* **5**: 481–494

Fung, H. B., Kirschenbaum, H. L., Ojofeitimi, B. O. (2001) Linezoild: an oxazolidinone antimicrobial agent. *Clin. Ther.* **23**: 356–391

Gibaldi, M., Perrier, D. (1982) *Pharmacokinetics*, 2nd edn. Marcel-Dekker, New York

Im, W. B., Lee, T. H., Cho, J., Choi, S. H., Rhee, J. K., (2002) ASM's Annual Meetings On Infectious Diseases, 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, September, 27–30, San Diego, abstract p. 211. F1131. American Society for Microbiology, Washington, DC

Kim, S. H., Lee, M. G. (1993) Influence of protein and calorie malnutrition on the pharmacokinetics and pharmacodynamics of bumetanide in rats. *J. Pharm. Sci.* **82**: 838–843

Kim, S. H., Choi, Y. M., Lee, M. G. (1993) Pharmacokinetics and pharmacodynamics of furosemide in protein–calorie malnutrition. *J. Pharmacokinet. Biopharm.* **21**: 1–17

Kim, Y. G., Cho, M. K., Kwon, J. W., Kim, S. G., Lee, M. G. (2000) Effects of cysteine on the pharmacokinetics of intravenous adriamycin in rats with protein–calorie malnutrition. *Res. Commun. Mol. Pathol. Pharmacol.* **107**: 361–376

Kim, Y. G., Cho, M. K., Kwon, J. W., Kim, S. G., Chung, S. J., Shim, C.-K., Lee, M. G. (2001a) Effects of cysteine on the pharmacokinetics of intravenous phenytoin in rats with protein–calorie malnutrition. *Int. J. Pharm.* **229**: 45–55

Kim, Y. G., Cho, M. K., Kwon, J. W., Kim, S. G., Lee, M. G. (2001b) Effects of cysteine on the pharmacokinetics and pharmacodynamics of intravenous and oral azosemide in rats with protein–calorie malnutrition. *Life Sci.* **68**: 2329–2345

Kim, Y. G., Cho, M. K., Kwon, J. W., Kim, S. G., Chung, S. J., Shim, C.-K., Lee, M. G. (2002) Effects of cysteine on the pharmacokinetics of intravenous chlorzoxazone in rats with protein–calorie malnutrition. *Biopharm. Drug Dispos.* **23**: 121–129

Kim, Y. G., Cho, M. K., Kwon, J. W., Kim, D.-H., Kim, S. G., Lee, M. G. (2003) Effects of cysteine on the pharmacokinetics of intravenous 2-(allylthio)pyrazine, a new chemoprotective agent, in rats with protein–calorie malnutrition. *Int. J. Pharm.* **255**: 1–11

Krishnaswamy, K. (1978) Drug metabolism and pharmacokinetics in malnutrition. *Clin. Pharmacokinet.* **3**: 216–240

Lee, M. G., Chiou, W. L. (1983) Evaluation of potential causes for the incomplete bioavailability of furosemide: Gastric first-pass metabolism. *J. Pharmacokinet. Biopharm.* **11**: 623–640

Lee, A. K., Ahn, C. Y., Kim, E. J., Kwon, J. W., Kim, S. G., Chung, S. J., Shim, C.-K., Lee, M. G. (2003) Effects of cysteine on the pharmacokinetics of itraconazole in rats with protein–calorie malnutrition. *Biopharm. Drug Dispos.* **24**: 64–70

Mitruka, B. M., Rawnsley, H. M. (1981) *Clinical biochemical and hematological reference values in normal experimental animals and normal humans*, 2nd edn. Masson Publishing USA, New York

Øie, S., Guentert, T. W. (1982) Comparison of equilibrium time in dialysis experiments using spiked plasma or spiked buffer. *J. Pharm. Sci.* **71**: 127–128