BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **27**: 361–370 (2006)
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/bdd.516

Pharmacokinetics of Omeprazole in Rats with Water Deprivation for 72 Hours

Dae Y. Lee[a], Hyun S. Shin[a], Inchul Lee[b] and Myung G. Lee[a,*]
[a] *College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Republic of Korea*
[b] *Department of Diagnostic Pathology, College of Medicine, University of Ulsan, Asan Foundation, Asan Medical Center, Seoul, Republic of Korea*

ABSTRACT: Dehydration can occur by excessive sweating, polyuria, severe diarrhea and hyperthermia. Previous studies reported that the expressions of CYP1A1/2 and 3A1(23)/2 were not changed in male Sprague–Dawley rats with 72 h water deprivation (dehydrated rats), and that the metabolism of omeprazole is mainly catalysed via CYP1A1/2, 2D1 and 3A23/2 in rats. Hence, it could be expected that the hepatic metabolism of omeprazole would not be changed considerably in dehydrated rats, if the contribution of CYP2D1 to the metabolism of omeprazole in dehydrated rats is not considerable. Therefore, the pharmacokinetics of omeprazole were compared after intravenous (20 mg/kg) and oral (40 mg/kg) administration in control rats and in dehydrated rats. After intravenous administration, the time-averaged nonrenal clearance (Cl_{nr}) values of omeprazole were comparable between the two groups of rats. This could be supported by comparable *in vitro* intrinsic clearance (Cl_{int}) values for the disappearance of omeprazole in rat hepatic microsomes and the comparable free (unbound to plasma proteins) fractions of omeprazole in plasma in the two groups of rats. After oral administration, the *AUC* values of omeprazole were also comparable in the two groups of rats. The above data suggest that the dehydration state did not affect considerably the pharmacokinetics of omeprazole in rats. Copyright © 2006 John Wiley & Sons, Ltd.

Key words: omeprazole; pharmacokinetics; dehydration; CYP1A2 and 3A1(23)/2; rats

Introduction

Omeprazole, 5-methoxy-2-[{(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl}sulphoxide]-1H-benzimidazole, is a substituted benzimidazole proton pump inhibitor in gastric parietal cells. The drug has greater antisecretory activity than histamine H_2-receptor antagonists and has been used widely in the treatment of peptic ulcer, efflux esophagitis and Zollinger–Ellison syndrome [1,2]. Recently, it was reported that omeprazole is mainly metabolized via the hepatic microsomal cytochrome P450 (CYP) 1A1/2, 2D1 and 3A1(23)/2 in male Sprague–Dawley rats [Lee DY, Shin HS, Bae SK, Lee MG. Effects of enzyme inducers and inhibitors on the pharmacokinetics of intravenous omeprazole in rats. *Biopharm Drug Dispos* 2006, in press]. For example, in rats pretreated with 3-methylcholanthrene and dexamethasone (main inducers of CYP1A1/2 and 3A23/2 in rats, respectively), the time-averaged nonrenal clearance (Cl_{nr}) of intravenous omeprazole (the Cl_{nr} of omeprazole could represent the metabolic clearance of omeprazole in rats) was significantly faster (43.8% and 33.2%

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

Received 19 January 2006
Revised 15 May 2006
Accepted 31 May 2006

increase, respectively) than in the respective controls. On the other hand, in rats pretreated with quinine and troleandomycin (main inhibitors of CYP2D1 and 3A23/2 in rats, respectively) the Cl_{nr} of intravenous omeprazole was significantly slower (12.9% and 20.9% decrease, respectively) than in the respective controls. However, the Cl_{nr} of intravenous omeprazole was not changed significantly in rats pretreated with orphenadrine, isoniazid and sulfaphenazole (main inducers of CYP2B1/2 and 2E1, and a main inhibitor of 2C11, respectively, in rats) compared with the respective controls.

Dehydration could occur by excessive sweating, polyuria, severe diarrhea and hyperthermia [3]. Water deprivation may cause significant hormonal, physiological and biochemical changes in the body [4 and references therein]. For example, the kidney and/or liver functions seemed to be impaired in water deprivation based on blood and urine chemistry data and/or microscopic examination of the kidney and liver. Therefore, it could be expected that the pharmacokinetics and hence the pharmacodynamics of drugs could be altered with water deprivation. Since the first report on the effects of water deprivation on aspirin disposition kinetics in rats [3], water deprivation has been reported to alter the disposition kinetics of various drugs [4 and references therein].

Kim *et al*. reported [5] that in male Sprague–Dawley rats with 72 h water deprivation (dehydrated rats), the expressions of CYP1A2, 2B1/2, 2C11 and 3A23/2 were not changed, however, the expression and mRNA level of CYP2E1 were markedly increased compared with controls. Hence, it could be expected that the hepatic metabolism of omeprazole would not be changed considerably in dehydrated rats. Meko and Norton reported [6] that severe diarrhea (which may cause dehydration) may be the only presenting symptom of Zollinger–Ellison syndrome. Studies reported that every third person with Zollinger–Ellison syndrome had diarrhea [7], and that severe diarrhea (35%) was predominant in Zollinger–Ellison patients [8]. Therefore, omeprazole was chosen in this study using dehydrated rats as an animal model. Although pharmacokinetic changes of many drugs in dehydrated rats have been reported [4 and references therein], the pharmacokinetic changes of drugs in dehydrated rats with respect to CYP isozyme changes seemed not to have been reported, except for chlorzoxazone and theophylline (our unpublished data), DA-8159 [Kim JY, Kim YC, Kwon JW, Yoo M, Lee MG. Effects of water deprivation for 72 hours on the pharmacokinetics of DA-8159, a new erectogenic, in rats. *J Pharm Sci*, in press] and oltipraz [4]. The aim of this study was to report negligible changes in metabolism (comparable Cl_{nr} values of omeprazole between control and dehydrated rats) of omeprazole in dehydrated rats compared with controls with respect to CYP isozyme changes. In this paper, the pharmacokinetics of omeprazole after intravenous and oral administration at doses of 20 and 40 mg/kg, respectively, in control and dehydrated rats are reported with respect to CYP isozyme changes.

Materials and Methods

Chemicals

Omeprazole and torasemide (an internal standard for high-performance liquid chromatographic, HPLC, analysis of omeprazole) were donated from the Yungjin Pharmaceutical Company (Seoul, Republic of Korea) and Roche Pharmaceutical Company (Mannheim, Germany), respectively. Reduced form of nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), tris(hydroxymethyl) aminomethane (Tris®)-buffer and ethylenediamine tetraacetic acid (EDTA) were purchased from Sigma–Aldrich Corporation (St Louis, MO). Other chemicals were of reagent grade or HPLC grade.

Animals

Male Sprague–Dawley rats (weighing 230–295 g) were purchased from the Charles River Company Korea (Orient, Seoul, Republic of Korea). All rats were maintained in a light-controlled room (light: 0700–1900, dark: 1900–0700) kept at a temperature of $22 \pm 2\,^{\circ}C$ and a relative humidity of $55 \pm 5\%$ (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National

DOI: 10.1002/bdd

University, Seoul, Republic of Korea). The rats were randomly divided into two groups, control and dehydrated rats. For control rats, water and food (Sam Yang Company, Seoul, Republic of Korea) were supplied *ad libitum* for 72 h; for dehydrated rats, water was deprived for 72 h with free access to food. The Animal Care and Use Committee of the College of Pharmacy, Seoul National University, approved this animal study protocol.

Preliminary study

The following preliminary study was performed in control and dehydrated rats ($n = 5$; each). The body weight and food intake were measured daily for 4 days (before water deprivation and on days 1, 2 and 3 after water deprivation). On day 4, the 24 h urine was collected for the measurement of creatinine level. After the hematocrit was measured (Readacrit, Clay Adams, Parsippany, NJ), plasma was collected for the measurement of total proteins, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and creatinine levels (analysed by Green Cross Reference Laboratory, Seoul, Republic of Korea) and plasma protein binding of omeprazole. The whole kidney and liver of each rat were excised, rinsed with 0.9% NaCl-injectable solution, blotted dry with tissue paper and weighed. Small portions of each organ were fixed in a 10% neutral phosphate-buffered formalin and then processed for routine histological examination with hematoxylin–eosin staining.

Measurement of V_{max}, K_m, and Cl_{int} for the disappearance of omeprazole in hepatic microsomal fractions

The procedures were similar to the reported methods [9]. The livers of control and dehydrated rats ($n = 4$; each) were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) in an ice-cold buffer of 0.154 M KCl/50 mM Tris-HCl in 1 mM EDTA, pH 7.4. The homogenate was centrifuged at $10\,000 \times g$ for 30 min and the supernatant fraction was further centrifuged at $100\,000 \times g$ for 90 min. The protein content was measured using the reported method [10]. The V_{max} (the maximum velocity) and K_m (the Michaelis–Menten constant; the concentration at which the rate is one-half of V_{max}) for the disappearance of omeprazole were determined after incubating a 31.3–46.3 µl aliquot (equivalent to 0.5 mg protein) of the above microsomal fractions, a 5 µl aliquot of omeprazole (dissolved in 0.1 M carbonate buffer of pH 9.8 having substrate concentrations of 1, 2.5, 5, 10 and 20 µM), and a 50 µl aliquot of 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 (opm). All the above microsomal incubation conditions were linear. The reaction was terminated by the addition of a 1 ml aliquot of diethyl ether after 5 min incubation. Omeprazole was measured by the reported HPLC method [11]. The kinetic constants (K_m and V_{max}) for the disappearance of omeprazole were calculated using the nonlinear regression method [12]. The intrinsic clearance (Cl_{int}) for the disappearance of omeprazole was calculated by dividing the respective V_{max} by the respective K_m.

Intravenous and oral administration of omeprazole in rats

The procedures for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration for intravenous study only) were similar to the reported method [13]. Since it was reported that immobilization stress could change the pharmacokinetics of omeprazole in rats [14], the rats were not restrained during this study. Each rat was then housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, Republic of Korea) and allowed 4–5 h to recover from light ether anesthesia before the study began. Heparinized 0.9% NaCl-injectable solution (20 units/ml), approximately 0.3 ml, was used to flush each cannula to prevent blood clotting.

On day 4, omeprazole (dissolved in 0.1 M carbonate buffer, pH 9.8) at a dose of 20 mg/kg was infused (total infusion volume of 2 ml/kg) via the jugular vein over 1 min in control ($n = 9$) and dehydrated ($n = 8$) rats. A 0.22 ml aliquot of blood sample was collected via the carotid artery

DOI: 10.1002/bdd

at 0 (to serve as a control), 1 (at the end of the infusion), 3, 7, 15, 30, 45, 60, 70, 80 and 90 min after intravenous administration of omeprazole. Blood samples were centrifuged immediately and a 100 μl aliquot of plasma sample was stored in a −70°C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until the HPLC analysis of omeprazole [11]. At the end of the experiment (24 h), each metabolic cage was rinsed with 5 ml of distilled water and the rinsings were combined with 24 h urine. After measuring the exact volume of the combined urine, a 100 μl aliquot of urine sample was stored in a −70°C freezer until the HPLC analysis of omeprazole [11]. At the same time (24 h), each rat was killed by cervical dislocation, and then the entire gastrointestinal tract (including its contents and feces) was removed, transferred into a beaker containing 50 ml of methanol (to facilitate the extraction of omeprazole), and cut into small pieces using scissors. After manual shaking and stirring with a glass rod for 1 min, two 100 μl aliquots of the supernatant were collected from each beaker and stored in a −70°C freezer until the HPLC analysis of omeprazole [11].

Omeprazole (the same solution that was used in the intravenous study) at a dose of 40 mg/kg was administered orally (total oral volume of 5 ml/kg) using a feeding tube in control ($n = 9$) and dehydrated ($n = 7$) rats. Blood samples were collected at 0, 5, 15, 30, 60, 75, 90, 105, 120, 135, 150, 180 and 240 min after oral administration of omeprazole. Other procedures were similar to those in the intravenous study.

Measurement of plasma protein binding of omeprazole using an equilibrium dialysis technique

The plasma protein binding of omeprazole in the control and dehydrated rats ($n = 5$; each) was determined using an equilibrium dialysis technique [15]. One ml of plasma from the control and dehydrated rats was dialysed against 1 ml of isotonic Sørensen phosphate buffer, pH 7.4, containing 3% (w/v) dextran in a 1 ml dialysis cell (Spectrum Medical Industries, Los Angeles, CA) using a Spectra/Por 4 membrane (mol. wt cutoff of 12 000–14 000; Spectrum Medical Industries). In the preliminary study, the binding of omeprazole to 4% human serum albumin was constant, 91.7 ± 0.785%, at omeprazole concentrations ranging from 1 to 200 μg/ml. Therefore, an omeprazole concentration of 10 μg/ml was chosen arbitrarily in this plasma protein binding study.

HPLC analysis of omeprazole

Concentrations of omeprazole in the above samples were determined by a slight modification of the reported HPLC method [11]; torasemide instead of lansoprazole was used as an internal standard. In a 2.2 ml Eppendorf tube containing a 100 μl aliquot of a sample, a 50 μl aliquot of methanol that contained an internal standard (torasemide; 50 μg/ml) and a 50 μl aliquot of 0.2 M phosphate buffer (pH 7.0) were added. The mixture was then extracted with a 1 ml aliquot of diethylether. The organic layer was transferred into a clean Eppendorf tube and evaporated under a gentle stream of nitrogen gas at 50°C. The residue was reconstituted in a 125 μl aliquot of the mobile phase and a 50 μl aliquot was injected directly onto a reversed-phase (C_8) HPLC column. The mobile phase, phosphate buffer (0.2 M KH_2PO_4, pH 7.0): acetonitrile (77: 23; v/v) was run at a flow-rate of 1.3 ml/min and the column effluent was monitored by an ultraviolet detector set at 302 nm. The retention times of omeprazole and an internal standard were approximately 10.2 and 8.1 min, respectively. The detection limits of omeprazole in rat plasma and urine were 20 and 50 ng/ml, respectively. Coefficients of variation of omeprazole in plasma and urine were below 5.34% and 7.90%, respectively.

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 uses the logarithmic trapezoidal rule for the calculation of the area during the declining plasma-level phase [16] and the linear trapezoidal rule for the rising plasma-level phase. The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal phase rate constant.

DOI: 10.1002/bdd

Standard methods [17] were used to calculate the time-averaged total body (Cl), renal (Cl_r) and nonrenal (Cl_{nr}) clearances, terminal half-life, total area under the first moment of the plasma concentration–time curve from time zero to time infinity ($AUMC$), mean residence time (MRT), apparent volume of distribution at steady state (V_{ss}), and the extent of absolute oral bioavailability (F) [13]. The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were read directly from the experimental data.

The glomerular filtration rate (GFR) was estimated by measuring the creatinine clearance (Cl_{cr}) assuming that kidney function was stable during the experimental period (24 h). The Cl_{cr} was measured by dividing the total amount of unchanged creatinine excreted in 24 h urine by the $AUC_{0–24\,h}$ of creatinine in plasma.

The harmonic mean method was used to calculate the mean values of V_{ss} [18], terminal half-life [19], and each clearance [20].

Statistical analysis

A value of $p<0.05$ was considered to be statistically significant using an unpaired t-test. All data were expressed as mean $\pm$ standard deviation.

Results

Preliminary study

Body weight, hematocrit, 24 h urine output, plasma chemistry data, protein binding of omeprazole, Cl_{cr}, and liver and kidney weights in control and dehydrated rats are listed in Table 1. In control rats, the body weight increased with time; the mean body weights were 266 ± 23.4 g, 281 ± 11.4 g, 285 ± 9.56 g and 300 ± 12.5 g before water deprivation and on days 1, 2 and 3 after water deprivation, respectively. However, in dehydrated rats the body weight decreased significantly; the corresponding values were 273 ± 11.7 g, 255 ± 8.06 g, 244 ± 7.78 g and 237 ± 6.51 g. In control rats, the daily food intake was almost constant; the mean values were 23.9 ± 1.92 g, 25.8 ± 2.20 g and 21.9 ± 1.73 g on days 1, 2 and 3 after water deprivation, respectively. However, in dehydrated rats, the food intake decreased with time; the corresponding values were 36.3% (15.2 ± 2.39 g), 71.0% (7.50 ± 1.67 g) and 85.4% (3.19 ± 0.636 g) compared with the controls (23.9 ± 1.92 g, 25.8 ± 2.20 g and 21.9 ± 1.73 g, respectively). The above data indicate that significant body weight loss in dehydrated rats was due to lower

Table 1. Mean ($\pm$ standard deviation) body weight, hematocrit, urine output, plasma chemistry data, plasma protein binding of omeprazole, creatinine clearance, and liver and kidney weights in control and dehydrated rats

Parameter	Control ($n = 5$)	Dehydration ($n = 5$)
Body weight (g)		
Initial[a]	266 ± 23.4	273 ± 11.7
Final[b]	300 ± 12.5	237 ± 6.51[c]
Hematocrit (%)	52.6 ± 2.37	67.3 ± 2.95[d]
Urine output (ml/24 h)	25.2 ± 18.9	1.64 ± 1.50[c]
Plasma		
Total proteins (g/dl)	5.86 ± 0.207	6.62 ± 0.415[e]
Albumin (g/dl)	3.58 ± 0.130	4.02 ± 0.239[e]
Urea nitrogen (mg/dl)	12.3 ± 1.04	17.7 ± 1.80[d]
GOT (IU/l)	59.6 ± 6.31	62.0 ± 14.0
GPT (IU/l)	17.6 ± 3.42	18.0 ± 9.95
Protein binding of omeprazole (%)	77.8 ± 3.61	81.9 ± 4.81
Cl_{cr} (ml/min/kg)	2.59 ± 0.584	2.61 ± 0.0878
Liver weight (% of body weight)	3.10 ± 1.38	2.72 ± 0.293[c]
Kidney weight (% of body weight)	0.718 ± 0.0497	0.744 ± 0.0446

[a] Measured just before starting the 72 h water deprivation.
[b] Measured just before starting the experiment.
[c] Significantly different ($p<0.05$) from control group.
[d] Significantly different ($p<0.001$) from control group.
[e] Significantly different ($p<0.01$) from control group.

DOI: 10.1002/bdd

food consumption in addition to the water deprivation. The dehydration state causes body weight loss due to a reduction in food intake to prevent elevations in extracellular fluid osmolarity and sodium concentration [21]. Similar results were also reported from other rat studies [5].

In dehydrated rats, kidney function seemed not to be impaired considerably; the Cl_{cr} and kidney weights were comparable to the controls. Although, the plasma level of urea nitrogen was significantly higher (43.9% increase) than in the controls, the level was in the reported range (5.0–29.0 mg/dl) in control rats [22]. There were no significant findings in the kidneys from both groups of rats based on kidney microscopy. In dehydrated rats, the liver function also seemed not to be impaired considerably; the plasma levels of GOT and GPT were comparable to the controls and no significant findings were found in either group of rats by microscopic examination of the liver, although the liver weight was significantly lighter (12.3% decrease) than in the controls.

In dehydrated rats, the hematocrit was significantly greater (27.9% increase), the plasma levels of total proteins (13.0% increase) and albumin (12.3% increase) were significantly higher, and the 24 h urine output was significantly smaller (93.5% decrease) than in the controls. Similar results were also reported from other rat studies [4 and references therein]. Note that the plasma levels of total proteins (4.70–8.15 g/dl) and albumin (2.70–5.10 g/dl) were in the range of the control rats [22]. The plasma protein binding values of omeprazole in control and dehydrated rats were 77.8% and 81.9%, respectively; they were not significantly different.

Measurement of V_{max}, K_m, and Cl_{int} for the disappearance of omeprazole in rat liver microsomes

The V_{max}, K_m and Cl_{int} for the disappearance of omeprazole in hepatic microsomal fractions of both groups of rats are listed in Table 2. The V_{max}, K_m and Cl_{int} for the disappearance of omeprazole were comparable between the two groups of rats, suggesting that the maximum velocity for the disappearance (mainly due to metabolism) of omeprazole, affinity of omeprazole to the enzyme(s) and formation of metabolite(s) of omeprazole, respectively, were not affected considerably by dehydration.

Table 2. Mean (± standard deviation) V_{max}, K_m and Cl_{int} for the disappearance of omeprazole in hepatic microsomes of control and dehydrated rats

Parameter	Control ($n = 4$)	Dehydration ($n = 4$)
V_{max} (nmol/min/mg protein)	2.42 ± 0.598	2.60 ± 0.314
K_m (μM)	16.4 ± 8.32	16.1 ± 1.50
Cl_{int} (ml/min/mg protein)	0.160 ± 0.0369	0.161 ± 0.00898

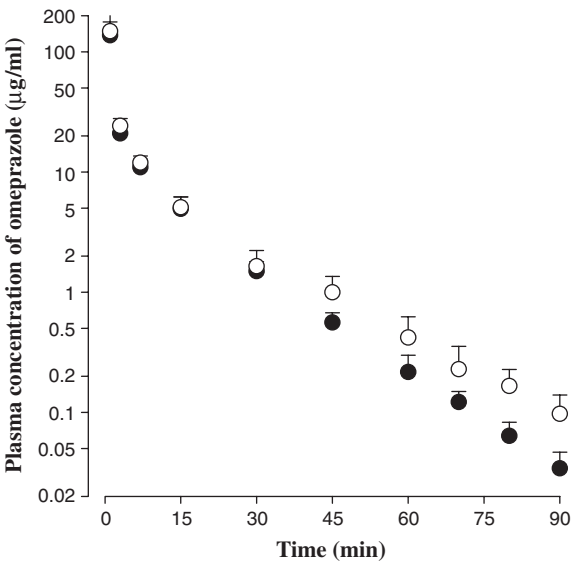

Figure 1. Mean arterial plasma concentration–time profiles of omeprazole after 1 min intravenous infusion at a dose of 20 mg/kg in control rats (●; $n = 9$) and dehydrated rats (○; $n = 8$). Vertical bars represent standard deviation

Pharmacokinetics of omeprazole after intravenous administration in rats

The mean arterial plasma concentration–time profiles of omeprazole after intravenous administration in both groups of rats are shown in Figure 1, and some relevant pharmacokinetic parameters are listed in Table 3. After intravenous administration in dehydrated rats, the Cl_r of omeprazole was significantly slower (56.0% decrease), and the percentage of the intravenous dose of omeprazole excreted in 24 h urine as

DOI: 10.1002/bdd

Table 3. Mean (± standard deviation) pharmacokinetic parameters of omeprazole after intravenous administration at a dose of 20 mg/kg in control and dehydrated rats

Parameter	Control ($n = 9$)	Dehydration ($n = 8$)
Body weight (g)		
Initial[a]	280 ± 9.35	271 ± 24.1
Final[b]	297 ± 13.5	236 ± 10.4[c]
Urine output (ml/24 h)	18.1 ± 4.88	0.625 ± 0.744[c]
AUC (μg min/ml)	380 ± 67.1	424 ± 131
Terminal half-life (min)	11.1 ± 2.32	12.6 ± 5.48
MRT (min)	7.94 ± 0.754	9.51 ± 1.19
Cl (ml/min/kg)	52.6 ± 9.30	47.2 ± 14.9
Cl_r (ml/min/kg)	0.168 ± 0.161	0.0740 ± 0.0301[d]
Cl_{nr} (ml/min/kg)	52.4 ± 9.27	46.7 ± 17.7
V_{ss} (ml/kg)	410 ± 102	412 ± 171
$Ae_{0-24\,h}$ (% of dose)	0.474 ± 0.240	0.166 ± 0.0909[d]
$GI_{24\,h}$ (% of dose)	BD[e]	BD

[a] Measured just before starting the 72 h water deprivation.
[b] Measured just before starting the experiment.
[c] Significantly different ($p < 0.001$) from control group.
[d] Significantly different ($p < 0.05$) from control group.
[e] Below the detection limit.

unchanged drug ($Ae_{0-24\,h}$; 65.0% decrease), 24 h urine output (96.5% decrease) and body weight gain (from 280 to 236 g compared with 271 to 297 g) were significantly smaller than in the controls. Other pharmacokinetic parameters of omeprazole listed in Table 3 were not significantly different between the two groups of rats. Omeprazole was below the detection limit in the gastrointestinal tract at 24 h ($GI_{24\,h}$) for both groups of rats.

Pharmacokinetics of omeprazole after oral administration in rats

The mean arterial plasma concentration–time profiles of omeprazole after oral administration in both groups of rats are shown in Figure 2, and some relevant pharmacokinetic parameters are listed in Table 4. After oral administration, omeprazole was absorbed rapidly from the rat gastrointestinal tract; omeprazole was detected in the plasma from the first blood sampling time (15 min) for both groups of rats, and rapidly reached T_{max} at 21.1 and 12.1 min for control and dehydrated rats, respectively. After oral administration of omeprazole in dehydrated rats, the terminal half-life of omeprazole was significantly longer (52.0% increase), the Cl_r of omeprazole was significantly slower (81.0% decrease), and

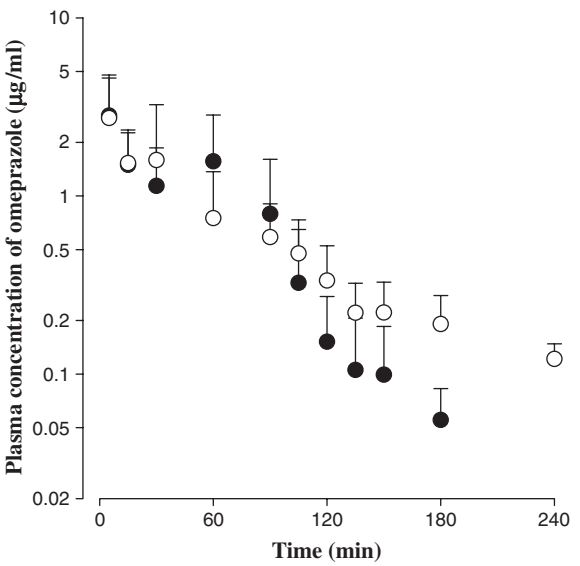

Figure 2. Mean arterial plasma concentration–time profiles of omeprazole after oral administration at a dose of 40 mg/kg in control rats (●; $n = 9$) and dehydrated rats (○; $n = 7$). Vertical bars represent standard deviation

$Ae_{0-24\,h}$ of omeprazole (76.1% decrease), 24 h urine output (98.5% decrease), and body weight gain (from 279 to 234 g compared with from 281 to 289 g) were significantly smaller than in the controls. Other pharmacokinetic parameters of

DOI: 10.1002/bdd

Table 4. Mean (± standard deviation) pharmacokinetic parameters of omeprazole after oral administration at a dose of 40 mg/kg in control and dehydrated rats

Parameter	Control ($n = 9$)	Dehydration ($n = 7$)
Body weight (g)		
Initial[a]	281 ± 10.9	279 ± 9.95
Final[b]	298 ± 8.25	234 ± 8.98[c]
Urine output (ml/24 h)	15.0 ± 4.24	0.222 ± 0.667[c]
AUC (μg min/ml)	136 ± 64.1	139 ± 45.3
C_{max} (μg/ml)	3.30 ± 1.65	3.51 ± 1.61
T_{max} (min)	21.1 ± 23.6	12.1 ± 12.2
Terminal half-life (min)	17.5 ± 13.4	26.6 ± 30.1[d]
Cl_r (ml/min/kg)	0.134 ± 0.348	0.0254 ± 0.0805[d]
$Ae_{0-24\,h}$ (% of dose)	0.103 ± 0.0719	0.0246 ± 0.0138[d]
$GI_{24\,h}$ (% of dose)	0.116 ± 0.0993	0.264 ± 0.329
F (%)	17.9	16.4

[a] Measured just before starting the 72 h water deprivation.
[b] Measured just before starting the experiment.
[c] Significantly different ($p < 0.001$) from control group.
[d] Significantly different ($p < 0.05$) from control group.

omeprazole listed in Table 4 were not significantly different between the two groups of rats. The *F* values were 17.9% and 16.4% for control and dehydrated rats, respectively.

Discussion

After intravenous administration of omeprazole at doses of 2.5, 5 and 10 mg/kg in rats, the $AUC_{0-2\,h}$ values were dose-proportional and the terminal half-life, V_{ss}, and *Cl* values were also dose-independent [23]. In the preliminary study, the $AUC_{0-2\,h}$ values of omeprazole after intravenous administration at a dose of 20 mg/kg in control rats were approximately 2-times that of $AUC_{0-2\,h}$ obtained after intravenous administration at a dose of 10 mg/kg in rats [23]. After oral administration of omeprazole at doses of 10, 20 and 40 mg/kg in rats, the pharmacokinetic parameters including $AUC_{0-3\,h}$, C_{max}, T_{max} and terminal half-life were dose-independent [23]. Hence, intravenous and oral doses of omeprazole of 20 and 40 mg/kg, respectively, were chosen arbitrarily in the present study.

The contribution of Cl_r to *Cl* of omeprazole after intravenous administration in rats was almost negligible; the values were less than 0.474% for both groups of rats (Table 3). This suggested that almost all the intravenously administered omeprazole was eliminated via the nonrenal route (Cl_{nr}). The contribution of biliary excretion of omeprazole to the Cl_{nr} of omeprazole was also negligible; it was reported that only 0.0436 ± 0.0159% of the dose was excreted as unchanged omeprazole in 24 h bile after intravenous administration of omeprazole at a dose of 20 mg/kg in ten control rats after bile duct cannulation [Lee *et al*., 2006]. This suggests that omeprazole is almost completely metabolized in rats. Hence, the Cl_{nr} of omeprazole listed in Table 3 could represent the metabolic clearance of omeprazole in rats. Therefore, the changes in the Cl_{nr} of omeprazole represent changes in the metabolism of omeprazole in rats.

After intravenous administration, the Cl_{nr} values of omeprazole were comparable between the control and dehydrated rats (Table 3). This result could be expected because omeprazole is mainly metabolized via CYP1A1/2, 2D1 and 3A23/2 in rats [Lee *et al*., 2006], and CYP1A1/2 and 3A23/2 were not changed in dehydrated rats [5]. Omeprazole is mainly metabolized in the liver of humans [24] and rats [23]. Since omeprazole is an intermediate hepatic extraction ratio drug (hepatic first-pass effect of approximately 60%) in rats [23], the hepatic clearance of omeprazole in rats depends on hepatic blood flow rate, free (unbound to plasma proteins) fractions of omeprazole in plasma and intrinsic clearance (Cl_{int}) [25]. The comparable Cl_{nr} values of omeprazole in the control and dehydrated rats

DOI: 10.1002/bdd

(Table 3) could be supported by comparable *in vitro* Cl_{int} values for the disappearance of omeprazole (Table 2) and comparable free fractions of omeprazole in plasma between the two groups of rats (Table 1). However, the contribution of the hepatic blood flow rate to the comparable Cl_{nr} values of omeprazole (Table 3) seemed not to be considerable, since hepatic blood flow rate was slower in dehydrated rats [26]. Although, the above data suggest that the contribution of CYP2D1 to the *AUC* of omeprazole in dehydrated rats seemed not to be considerable, studies on the changes of CYP2D1 in dehydrated rats are required.

After intravenous administration of omeprazole in dehydrated rats, the Cl_r of omeprazole was significantly slower than in the controls (Table 3). However, the *Cl* of omeprazole was not significantly different between the two groups of rats. This could be due to the almost negligible contribution of Cl_r to *Cl* of omeprazole in rats; the values being 0.319% and 0.157% for control and dehydrated rats, respectively (Table 3). Hence, the similar *Cl* values of omeprazole between the two groups of rats were mainly the result of comparable Cl_{nr} values between the two groups of rats (Table 3). Although the Cl_r values were almost negligible, the significantly slower Cl_r in dehydrated rats could be mainly the result of a significantly smaller $Ae_{0-24\,h}$, since *AUC* values of omeprazole were comparable between the two groups of rats (Table 3). The smaller $Ae_{0-24\,h}$ in dehydrated rats could be due to urine flow rate-dependent Cl_r of omeprazole; it was shown by our laboratories that the $Ae_{0-24\,h}$ of omeprazole decreased with decreasing urine flow rate in control rats. The 24 h urine output was significantly smaller in dehydrated rats (Tables 1, 3 and 4).

After intravenous administration, the Cl_r values of omeprazole were estimated as free (unbound to plasma proteins) fractions of omeprazole in plasma based on Cl_r values (Table 3) and plasma protein binding values (Table 1); the values thus estimated were 0.757 and 0.409 ml/min/kg for control and dehydrated rats, respectively. The values of 0.757 and 0.409 ml/min/kg were considerably slower than the GFR values of 2.59 and 2.61 ml/min/kg for control and dehydrated rats (Table 1). The above data suggest that omeprazole is mainly reabsorbed in the renal tubules of both groups of rats. The renal extraction ratio of omeprazole (Cl_r of omeprazole/renal blood flow rate; only for urinary excretion of unchanged drug) were estimated based on Cl_r values of omeprazole (Table 3), hematocrit ($45.2 \pm 5.43\%$ and $56.5 \pm 1.57\%$ for control and dehydrated rats, respectively [27]), and reported renal blood flow rate, 36.8 ml/min/kg for control rats [28] and 12.9 ml/min/kg for dehydrated rats [29]. The renal blood flow rate decreased by 65% in rats dehydrated for 8 days compared with controls [29]. The renal extraction ratios thus estimated were 0.833% and 1.32% for control and dehydrated rats, respectively. The above data suggested that omeprazole is a low renal extraction ratio drug for both groups of rats.

After oral administration, the *AUC* values of omeprazole were also comparable between the two groups of rats (Table 4). Hence, the *F* values were also comparable between the two groups of rats (Table 4). The above data suggest that the absorption and/or first-pass effect of omeprazole seemed not to be changed considerably in dehydrated rats. Similar *F* values of 12.6% and first-pass effects of 88.9% were estimated in other rat studies [23].

In conclusion, after intravenous administration, the Cl_{nr} values of omeprazole were comparable between control and dehydrated rats (Table 3), since omeprazole is metabolized mainly via CYP1A1/2, 2D1 and 3A23/2 in rats [Lee *et al.*, 2006], and the expressions of 1A1/2 and 3A23/2 were not changed in dehydrated rats [5]. After oral administration, the *AUC* values of omeprazole were also comparable between the two groups of rats. If the present rat data could be extrapolated to humans, modification of the oral dosage regimen of omeprazole would seem not to be required in patients in a dehydrated state. More studies are required in humans to prove the above hypothesis, since the pharmacokinetic changes of omeprazole in patients with dehydration with respective CYP isozyme changes have not been reported.

Acknowledgements

This work was supported in part by the Korea Research Foundation Grant funded by the

DOI: 10.1002/bdd

Korean Government (MOEHRD) (KRF-2005-015-E00081).

References

1. Berglindh T, Sachs G. Emerging strategies in ulcer therapy: pumps and receptors. *Scand J Gastroenterol* 1985; **108**(Suppl): 7–14.
2. Im W, Blakeman DP, Davis JP. Irreversible inactivation of rat gastric (H^+-K^+)-ATPase *in vivo* by omeprazole. *Biochem Biophys Res Commun* 1985; **126**: 78.
3. Bakar SK, Niazi S. Effect of water deprivation on aspirin disposition kinetics. *J Pharm Sci* 1983; **72**: 1030−1034.
4. Bae SK, Lee SJ, Kim JW, Kim YH, Kim SG, Lee MG. Pharmacokinetics of oltipraz after intravenous and oral administration in rats with dehydration for 72 hours. *Biopharm Drug Dispos* 2005; **26**: 77–83.
5. Kim SG, Kim EJ, Kim YG, Lee MG. Expression of cytochrome P-450 s and glutathione S-transferases in the rat liver during water deprivation: effects of glucose supplementation. *J Appl Toxicol* 2001; **21**: 123−129.
6. Meko JB, Norton JA. Management of patients with Zollinger–Ellison syndrome. *Annu Rev Med* 1995; **46**: 395–411.
7. Arendt R. Zollinger–Ellison syndrome. *Z Gesamte Inn Med* 1975; **30**: 749–752.
8. Kaplan EL, Horvath K, Udekwu A, *et al*. Gastrinomas: A 42-year experience. *World J Surg* 1990; **14**: 365–376.
9. Kim YC, Kwon JW, Kim WB, Lee I, Lee MG. Pharmacokinetic changes of intravenous DA-8159, a new erectogenic, in rats with diabetes mellitus induced by streptozotocin. *J Pharm Sci* 2004; **93**: 2374–2387.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* 1976; **72**: 248–254.
11. Kang WK, Kim DS, Kwon KI. Advanced method for determination of omeprazole in plasma by HPLC. *Arch Pharm Res* 1999; **22**: 86–88.
12. Duggleby RG. Analysis of enzyme progress curves by nonlinear regression. *Methods Enzymol* 1995; **249**: 61–90.
13. Kim SH, Choi YM, Lee MG. Pharmacokinetics and pharmacodynamics of furosemide in protein–calorie malnutrition. *J Pharmacokinet Biopharm* 1993; **21**: 1−17.
14. Watanabe K, Matsuka N, Okazaki M, Hashimoto Y, Araki H, Gomita Y. The effect of immobilization stress on the pharmacokinetics of omeprazole in rats. *Acta Med Okayama* 2002; **56**: 19–23.
15. Shim HJ, Lee EJ, Kim SH, *et al*. Factors influencing the protein binding of a new phosphodiesterase V inhibitor, DA-8159, using an equilibrium dialysis technique. *Biopharm Drug Dispos* 2000; **21**: 285–291.
16. Chiou WL. Critical evaluation of the 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* 1978; **6**: 539–546.
17. Gibaldi M, Perrier D. *Pharmacokinetics*, 2nd edn. Marcel-Dekker: New York, 1982.
18. Chiou WL. New calculation method for mean apparent drug volume of distribution and application to rational dosage regimen. *J Pharm Sci* 1979; **68**: 1067–1069.
19. Eatman FB, Colburn WA, Boxenbaum HG, *et al*. Pharmacokinetics of diazepam following multiple dose oral administration to healthy human subjects. *J Pharmacokinet Biopharm* 1977; **5**: 481–494.
20. Chiou WL. New calculation method of mean total body clearance of drugs and its application to rational dosage regimens. *J Pharm Sci* 1980; **69**: 90−91.
21. Kutscher CL. Hematocrit, plasma osmolality, and plasma protein concentrations as estimators of plasma volume in hooded rats during food and water deprivation. *Physiol Behav* 1971; **7**: 283–285.
22. Mitruka BM, Rawnsley HM. *Clinical, Biochemical and Hematological Reference Values in Normal Experimental Animals and Normal Humans*, 2nd edn. Masson: New York, 1981.
23. Watanabe K, Furuno K, Eto K, Oishi R, Gomita Y. First-pass metabolism of omeprazole in rats. *J Pharm Sci* 1994; **83**: 1131–1134.
24. Karam WG, Goldstein JA, Lasker JM, Ghanayem BI. Human CYP2C19 is a major omeprazole 5-hydroxylase, as demonstrated with recombinant cytochrome P450 enzymes. *Drug Metab Dispos* 1996; **24**: 1081–1087.
25. Wilkinson GR, Shand DG. A physiological approach to hepatic drug clearance. *Clin Pharmacol Ther* 1975; **18**: 377–390.
26. Bencsath P, Takacs L. Effect of dehydration on cardiac output and organ blood flow in anaesthetized and unanaesthetized rats. *Acta Med Acad Sci Hung* 1966; **22**: 275–282.
27. Kim YC, Kim YG, Kim EJ, Cho MK, Kim SG, Lee MG. Pharmacokinetics of intravenous chlorzoxazone in rats with dehydration and rehydration: effects of food intakes. *Biopharm Drug Dispos* 2003; **24**: 53–61.
28. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993; **10**: 1093–1095.
29. Hope A, Tyssebotn I. The effect of water deprivation on local renal blood flow and filtration in the laboratory rat. *Circ Shock* 1983; **11**: 175–186.

DOI: 10.1002/bdd