BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **28**: 373–383 (2007)
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/bdd.564

Effects of Water Deprivation on the Pharmacokinetics of Metformin in Rats

Young H. Choi[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: It was reported that metformin was mainly metabolized via hepatic CYP2C11, 2D1 and 3A1/2 in rats, and in a rat model of dehydration, the expressions of hepatic CYP2C11 and 3A1/2 were not changed. Hence, it could be expected that the Cl_{nr} of metformin is comparable between two groups of rats if the contribution of CYP2D1 in the rat model of dehydration is not considerable. It was also reported that the timed-interval renal clearance of metformin was dependent on the urine flow rate in rats. In the rat model of dehydration, the 24 h urine output was significantly smaller than in the controls. Hence, the urinary excretion of metformin was expected to be smaller than the controls. The above expectations were proven as follows. After intravenous administration of metformin (100 mg/kg) to the rat model of dehydration, the Cl_{nr} were comparable between the two groups of rats. After both intravenous and oral administration of metformin (both 100 mg/kg) to the rat model of dehydration, the 24 h urinary excretion of the drug was significantly smaller than in the controls. After oral administration of metformin to the rat model of dehydration, the *AUC* was significantly greater (99.2% increase) than the controls. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: metformin; pharmacokinetics; dehydration; hepatic CYP2C11 and 3A1/2; rats

Introduction

Metformin, a biguanide antihyperglycemic agent, is widely used in the management of type 2 diabetes mellitus; it lowers the blood glucose concentration without causing hypoglycemia [1]. After intravenous (at doses of 0.25–1.0 g) and oral (at doses of 0.5–1.5 g) administration of metformin to four healthy volunteers, the terminal half-lives of the drug were 1.52–4.50 h, 78.9–99.9% of the dose was excreted in the urine via active renal tubular secretion, absorption of the drug was not complete (20–30% of the oral dose was recovered from the feces) possibly due to an active, saturable absorption process, and the extent of the absolute oral bioavailability (*F*) values of the drug were 33–55% [1]. Binding of metformin to human plasma proteins does not occur [2,3]. The metabolism of metformin was suggested in humans [1] based on incomplete recovery of the drug in the urine after intravenous administration of the drug [2], in accordance with a further study in which 20% of the dose was not accounted for [3]. Recently, it was reported [4] that metformin was mainly metabolized via the hepatic microsomal cytochrome P450 (CYP) 2C11, 2D1 and 3A1/2 (not via the CYP1A2, 2B1/2 and 2E1) in male Sprague–Dawley rats.

*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 25 August 2006
Revised 13 March 2007
Accepted 15 March 2007

Dehydration can occur by excessive sweating, polyuria, severe diarrhea and hyperthermia [5]. It was reported [6] that in male Sprague–Dawley rats with 72 h water deprivation (rat model of dehydration), the expressions of hepatic CYP1A2, 2B1/2, 2C11 and 3A1/2 were not changed, however, the expression and mRNA level of hepatic CYP2E1 increased compared with the controls. Water deprivation also causes significant hormonal, physiological and biochemical changes in the body [7]. Hence, it could be expected that the pharmacokinetics of metformin could be altered in the rat model of dehydration. Since the first report on the effects of water deprivation on aspirin disposition kinetics in rats [5], pharmacokinetic changes of drugs in the rat model of dehydration were reported [7].

It was reported that a hyperglycemic hyperosmolar syndrome, characterized by severe hyperglycemia, hyperosmolarity and dehydration in the absence of significant ketosis, is a major acute complication of decompensated diabetes mellitus [8]. Physical findings of the hyperosmolar hyperglycemic state include those associated with profound dehydration and various neurological symptoms such as coma [9]. Although pharmacokinetic changes of drugs in the rat model of dehydration were reported [7], changes with respect to hepatic CYP isozyme changes in rat model of dehydration [6] were scarce except oltipraz [7], DA-8159 (Zydena®), a new erectogenic [10] and chlorozoxazone [11]. Moreover, the hepatic CYP isozymes responsible for the metabolism of metformin in rats was reported recently [4]. Hence, metformin was chosen in this study using dehydrated rats as an animal model.

This paper reports the significantly greater total area under the plasma concentration–time curve from time zero to time infinity (*AUC*) and the significantly slower time-averaged renal clearance (Cl_r) of metformin after both intravenous and oral administration of the drug in the rat model of dehydration than in the controls, and the comparable Cl_{nr} of metformin after intravenous administration of the drug to control rats and the rat model of dehydration.

Materials and Methods

Chemicals

Metformin hydrochloride and ipriflavone (an internal standard of high-performance liquid chromatographic (HPLC) analysis of metformin) were supplied from Dalim Medical (Seoul, Republic of Korea) and Research Laboratory of Dong-A Pharmaceutical Company (Yongin, Republic of Korea), respectively. Reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), ethylenediamine tetraacetic acid (EDTA) and tri(hydroxymethyl)-aminomethane (Tris®)-buffer were purchased from Sigma–Aldrich Corporation (St Louis, MO). Other chemicals were of reagent grade or HPLC grade.

Animals

Male Sprague–Dawley rats of 6–10 weeks of age (weighing 210–420 g) were purchased from Taconic Farms Inc. (Samtako Bio Korea, O-San, 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 University, Seoul, Republic of Korea). The rats were randomly divided into two groups, control rats and rat model of dehydration. For control rats, food (Sam Yang Company, Pyeongtaek, Republic of Korea) and water were supplied *ad libitum* for 72 h; for the rat model of dehydration, water was deprived for 72 h with free access to food. Animal Care and Use Committee of Collage of Pharmacy of Seoul National University approved this animal study protocol.

Preliminary study

The following preliminary study was performed in control rats and in the rat model of dehydration ($n = 5$; each) to measure the liver and kidney functions. On day 4 after water deprivation, the 24 h urine was collected for the measurement of creatinine level. After the hematocrit value was measured (Readacroit Centrifuge; Clay Adams, Parsippany, NJ), plasma was collected for the

DOI: 10.1002/bdd

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 metformin using the equilibrium dialysis technique. 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. The body weight and food intake were measured.

Measurement of V_{max}, K_m, and Cl_{int} for the disappearance of metformin in hepatic microsomal fractions of control rats and rat model of dehydration

The procedures were similar to the previously reported methods [7]. The livers of control rats and rat model of dehydration ($n = 5$; each) were homogenized (Ultra-Turrax T25; Janke & 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 homogenates were centrifuged at 10000*g* for 30 min and the supernatant fraction was further centrifuged at 100000*g* for 90 min. The protein content was measured using the reported method [12]. 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 metformin were determined after incubating the above microsomal fractions (equivalent to 0.5 mg protein), a 5 µl aliquot of 0.9% NaCl-injectable solution that contained 1, 2.5, 5, 7.5, 10, 50, 100 and 200 µM of metformin as metformin base, and a 50 µl aliquot of 0.1 M phosphate buffer of pH 7.4 that contained 1 mM of NADPH in a final volume of 0.5 ml by adding 0.1 M phosphate buffer of pH 7.4, in a water-bath shaker kept at 37°C and at a rate of 500 oscillations per min (opm). All of the above microsomal incubation conditions were linear. The reaction was terminated by the addition of 1 ml of acetonitrile after 15 min incubation. The kinetic constants (K_m and V_{max}) for the disappearance of metformin were calculated using the nonlinear regression method [13]. The intrinsic clearance (Cl_{int}) for the disappearance of metformin was calculated by dividing the respective V_{max} by the respective K_m.

Intravenous administration of metformin to rats

The procedures for the pretreatment of rats including the cannulation of the jugular vein (for drug administration) and the carotid artery (for blood sampling) were similar to previously reported methods [14]. Both cannulas were exteriorized to the dorsal side of the neck where each cannula was terminated with a long silastic tube (Dow Corning, Midland, MI). Both silastic tubes were inserted into a wire sheath to allow free movement of the rat. Each rat was 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.

On day 4 after water deprivation, metformin (metformin hydrochloride was dissolved in 0.9% NaCl-injectable solution) at a dose of 100 mg/kg as metformin base was infused (total infusion volume of approximately 0.6 ml) over 1 min via the jugular vein of control rats ($n = 8$) and rat model of dehydration ($n = 12$). An approximately 0.12 ml aliquot of blood sample was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of infusion), 5, 15, 30, 60, 90, 120, 180, 240, 360 and 480 min after the start of the intravenous administration of metformin. An approximately 0.3 ml aliquot of the heparinized 0.9% NaCl-injectable solution (20 units/ml) was used to flush each 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 stored in a –70°C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until HPLC analysis of metformin [15]. At the end of 24 h, each metabolic cage was rinsed with 20 ml of distilled water and the rinsings were combined with the urine sample. After measuring the exact volume of the 24 h urine output and the combined urine samples, two 50 µl aliquots of the combined urine sample were stored in a –70°C freezer until HPLC analysis of metformin

DOI: 10.1002/bdd

[15]. At the same time (24 h), each rat was exsanguinated and killed by cervical dislocation. Then the entire gastrointestinal tract (including its contents and feces) of each rat was removed, transferred into a beaker that contained 100 ml of methanol (to facilitate the extraction of metformin), and cut into small pieces using scissors. After manual shaking and stirring with a glass rod for 1 min, two 50 μl aliquots of the supernatant were collected from each beaker and stored in a –70°C freezer until HPLC analysis of metformin [15].

Oral administration of metformin to rats

Metformin (the same solution that was used in the intravenous study) at a dose of 100 mg/kg was administered orally (total oral volume of approximately 1.5 ml) to the control rats ($n = 8$) and rat model of dehydration ($n = 11$) using a feeding tube. Blood samples were collected at 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, 600, 720, 960, 1200 and 1440 min after oral administration of metformin. Other procedures were similar to those in the intravenous study.

Measurement of plasma protein binding of metformin using the equilibrium dialysis technique

Plasma protein binding of metformin was measured using the equilibrium dialysis technique [16]. One milliliter of plasma 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) using a Spectra/Por 4 membrane (mol. wt. cutoff of 12000–14000 Dalton; Spectrum Medical Industries). After 24 h incubation, two 50 μl aliquots were collected from each compartment and stored in a –70°C freezer until HPLC analysis of metformin [15]. The binding of metformin to 4% human serum albumin was independent of metformin concentrations ranging from 1 and 200 μg/ml; the mean value was 10.1% [16]. Hence, the concentration of metformin at a 10 μg/ml was arbitrarily chosen in this plasma protein binding study.

HPLC analysis of metformin

The concentrations of metformin in the above samples were determined by a slight modification of the reported HPLC method [15]; ipriflavone instead of hydrocodeine was used as an internal standard. A 50 μl aliquot of biological sample was deproteinized with a 100 μl aliquot of acetonitrile, and a 50 μl aliquot of methanol that contained 10 μg/ml of ipriflavone (an internal standard) was added. After vortex-mixing and centrifugation at 16000*g* for 10 min, a 50 μl aliquot of the supernatant was injected directly onto a reversed-phase (C_{18}) HPLC column. The mobile phase (pH = 6), 10 mM KH_2PO_4: acetonitrile (40: 60; v/v), was run at a flow-rate of 1.5 ml/min, and the column effluent was monitored using an ultraviolet detector set at 235 nm. The retention times of metformin and the internal standard were approximately 4 and 6.5 min, respectively. The quantitation limits of metformin in the rat plasma and urine were 0.05 and 1 μg/ml, respectively. The inter- and intra-day coefficients of variation were below 9.91% and 7.52% for rat plasma and urine samples, respectively, in the concentration ranges 0.05–5000 μg/ml and 1–1000 μg/ml for rat plasma and urine samples, respectively.

Pharmacokinetic analysis

The *AUC* was calculated using the trapezoidal rule–extrapolation method; this method uses the logarithmic trapezoidal rule recommended by Chiou [17] 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 time infinity was estimated by dividing the last measured concentration in plasma by the terminal-phase rate constant.

Standard methods [18] were used to calculate the following pharmacokinetic parameters using the noncompartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, CA); the time-averaged total body (*Cl*), renal (Cl_r) and nonrenal (Cl_{nr}) clearances, terminal half-life, first moment of *AUC* (*AUMC*), mean residence time (*MRT*), apparent volume of distribution at steady state (V_{ss}) and *F* [14]. The peak plasma concentration

DOI: 10.1002/bdd

(C_{max}) and time to reach a 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. 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 mean values of V_{ss} [19], terminal half-life [20] and each clearance [21] were calculated using the harmonic mean method.

Statistical analysis

A value of $p<0.05$ was considered to be statistically significant using the *t*-test between the two means for the unpaired data. All data are expressed as mean $\pm$ standard deviation except median (ranges) for T_{max}.

Results

Preliminary study

Body weight, food intake, hematocrit, 24 h urine output, plasma chemistry data, plasma protein binding of metformin, Cl_{cr} and relative liver and kidney weights in the control rats and the rat model of dehydration are listed in Table 1. In control rats, the body weight increased with days; the mean body weights were 385 ± 35.0 g, 388 ± 36.0 g, 394 ± 33.8 g and 399 ± 36.1 g for before water deprivation and the first, second, and third days after water deprivation, respectively. However, in the rat model of dehydration, the body weight decreased with days; the corresponding values were 392 ± 27.0 g, 376 ± 28.8 g, 362 ± 25.6 g and 344 ± 28.4 g. In the control rats, the daily food intake was almost constant; the mean values were 24.0 ± 0.931 g, 26.0 ± 1.37 g, and 29.0 ± 3.20 g for the first, second, and third days after water deprivation, respectively. However, in the rat model of dehydration, food intake decreased within days; the corresponding values were 10.5 ± 1.12 g, 9.0 ± 0.913 g and 7.00 ± 0.456 g (43.8%, 34.6% and 24.1% of the controls, respectively). The above data indicate that the significant decrease in body weight gain in rat model of dehydration was due to lower food consumption in addition to the water deprivation.

Table 1. Mean ($\pm$standard deviation) body weight, food intake, hematocrit, 24 h urine output, plasma chemistry data, plasma protein binding of metformin, Cl_{cr} and relative liver and kidney weights in control rats and rat model of dehydration

Parameter	Control ($n = 5$)	Dehydration ($n = 5$)
Body weight (g)		
Initial	385 ± 35.0	392 ± 27.0
Final	399 ± 36.1	344 ± 28.4[a]
Food intake (g/day/rat)		
Initial	24.0 ± 0.931	10.5 ± 1.12[b]
Final	29.0 ± 3.20	7.00 ± 0.456[b]
Hematocrit (%)	44.8 ± 2.34	57.8 ± 3.13[b]
Urine output (ml/24 h)	21.2 ± 5.40	7.90 ± 0.742[b]
Plasma		
Total proteins (g/dl)	7.26 ± 1.19	7.20 ± 0.361
Albumin (g/dl)	3.74 ± 0.462	4.06 ± 0.445
Urea nitrogen (mg/dl)	12.8 ± 4.27	19.3 ± 2.51[a]
GOT (IU/l)	88.4 ± 15.8	101 ± 22.5
GPT (IU/l)	33.8 ± 5.54	33.4 ± 6.19
Protein binding of metformin (%)	6.36 ± 3.42	13.4 ± 2.44[c]
Cl_{cr} (ml/min/kg)	6.59 ± 0.646	5.75 ± 1.38
Liver weight (% of body weight)	2.75 ± 0.198	2.67 ± 0.382
Kidney weight (% of body weight)	0.655 ± 0.0310	0.687 ± 0.0401

IU, international unit.
[a] Significantly different ($p<0.05$) from the control.
[b] Significantly different ($p<0.001$) from the control.
[c] Significantly different ($p<0.01$) from the control.

In the rat model of dehydration, kidney function did not seem to be impaired considerably; the Cl_{cr} and relative kidney weight were comparable to the controls. Although the plasma level of urea nitrogen became significantly higher (50.8% increase) than the controls, the levels for both groups of rats were in the reported ranges in control rats, 5.0–29.0 mg/dl [22]. Not considerably impaired kidney function in rat model of dehydration was also supported by the kidney microscopy; there were no significant findings in the kidneys of both groups of rats. In the rat model of dehydration, the liver function also did not seem to be impaired considerably; the plasma levels of total proteins, albumin, GOT and GPT, and relative liver weight became comparable to the controls. This could also be supported by the

DOI: 10.1002/bdd

liver microscopy; no significant findings were also found in the livers of both groups of rats. In the rat model of dehydration, the hematocrit (29.0% increase), plasma protein binding of metformin (111% increase) and 24 h urine output (62.7% decrease) became significantly greater, greater and smaller, respectively, than the controls.

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

The V_{max}, K_m and Cl_{int} for the disappearance of metformin in hepatic microsomal fractions of the control rats and the rat model of dehydration are listed in Table 2. The V_{max}, K_m and Cl_{int} in the rat model of dehydration were comparable to the controls, suggesting that the maximum velocity for the disappearance (mainly due to metabolism) of metformin, affinity of metformin to the enzyme(s) and metabolism of metformin were not affected considerably by dehydration.

Pharmacokinetics of metformin after intravenous administration of the drug to rats

The mean arterial plasma concentration–time profiles of metformin after intravenous administration of the drug at a dose of 100 mg/kg to the control rats and the rat model of dehydration are shown in Figure 1, and some relevant pharmacokinetic parameters are listed in Table 3. After intravenous administration of metformin to the rat model of dehydration, the changes in the pharmacokinetic parameters of the drug compared with the control rats were as follows; the

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

Parameter	Control ($n = 5$)	Dehydration ($n = 5$)
V_{max} (nmol/min/mg protein)	4.17 ± 2.24	5.60 ± 1.70
K_m (μM)	140 ± 69.0	210 ± 75.3
Cl_{int} (ml/min/mg protein)	0.0296 ± 0.00374	0.0270 ± 0.00191

Each value was not significantly different ($p < 0.05$) between two groups of rats.

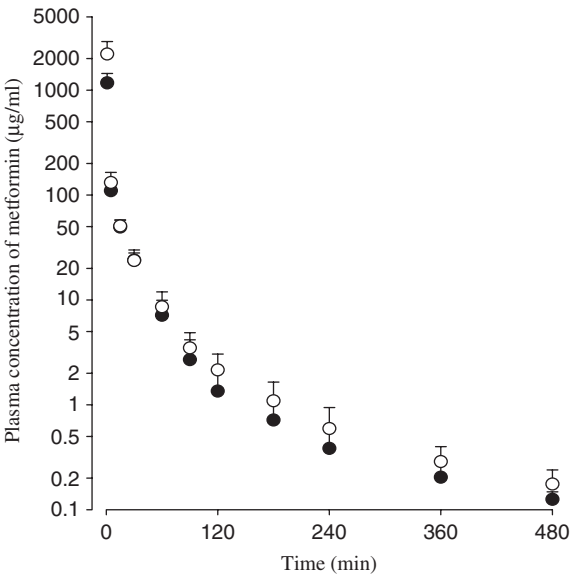

Figure 1. Mean arterial plasma concentration–time profiles of metformin after 1 min intravenous infusion of the drug at a dose of 100 mg/kg to the control rats (●; $n = 8$) and rat the model of dehydration (○; $n = 12$). Bars represent standard deviation

Table 3. Mean (±standard deviation) pharmacokinetic parameters of metformin after intravenous administration of the drug at a dose of 100 mg/kg to control rats and rat model of dehydration

Parameter	Control ($n = 8$)	Dehydration ($n = 12$)
Body weight (g)		
Initial	228 ± 10.7	234 ± 10.7
Final	249 ± 5.18	198 ± 9.88[a]
Food intake (g/day/rat)		
Initial	27.9 ± 0.741	9.58 ± 1.90[a]
Final	29.0 ± 0.880	5.97 ± 1.11[a]
Hematocrit (%)	41.5 ± 2.74	50.4 ± 2.14[a]
Urine output (ml/24 h)	27.1 ± 6.88	14.8 ± 1.66[a]
AUC (μg min/ml)	4620 ± 551	6460 ± 1370[b]
Terminal half-life (min)	154 ± 83.2	167 ± 33.0
MRT (min)	24.7 ± 9.96	22.5 ± 5.65
Cl (ml/min/kg)	21.7 ± 2.37	15.5 ± 3.43[a]
Cl_r (ml/min/kg)	13.6 ± 2.51	5.82 ± 1.78[a]
Cl_{nr} (ml/min/kg)	7.15 ± 2.88	9.24 ± 2.50
V_{ss} (ml/kg)	444 ± 289	323 ± 111[c]
$Ae_{0-24\,h}$ (% of dose)	64.4 ± 10.7	39.3 ± 7.82[a]
$GI_{24\,h}$ (% of dose)	1.53 ± 1.14	0.771 ± 1.09

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

DOI: 10.1002/bdd

AUC became significantly greater (39.8% increase), *Cl* (28.6% decrease) and Cl_r (57.2% decrease) became significantly slower and V_{ss} (27.3% decrease) and the percentage of intravenous dose of metformin excreted in 24 h urine as an unchanged drug ($Ae_{0-24\,h}$; 39.0% decrease) became significantly smaller than the controls. In the rat model of dehydration, the body weight gain and food intake became significantly smaller, the hematocrit became significantly greater (21.4% increase) and the 24 h urine output became significantly smaller (45.4% decrease) than the controls.

Pharmacokinetics of metformin after oral administration of the drug to rats

The mean arterial plasma concentration–time profiles of metformin after oral administration of the drug at a dose of 100 mg/kg to the control rats and the rat model of dehydration are shown in Figure 2, and some relevant pharmacokinetic parameters are listed in Table 4. After oral administration of metformin, absorption of the drug from the rat gastrointestinal tract was rapid; metformin was detected in plasma from the first or second blood sampling time (15 or 30 min) and rapidly reached T_{max} (30–90 min) for both groups of rats. After oral administration of metformin to the rat model of dehydration, the changes in the pharmacokinetic parameters of the drug compared with control rats were as follows; the *AUC* became significantly greater (99.2% increase), T_{max} became significantly longer (200% increase), Cl_r became significantly slower (53.4% decrease) and $Ae_{0-24\,h}$ became significantly smaller (11.3% decrease) than the controls. In the rat model of dehydration, the body weight gain and food intake became significantly smaller, the hematocrit became significantly greater (23.7% increase) and the 24 h urine output became significantly smaller (35.0% decrease) than the controls.

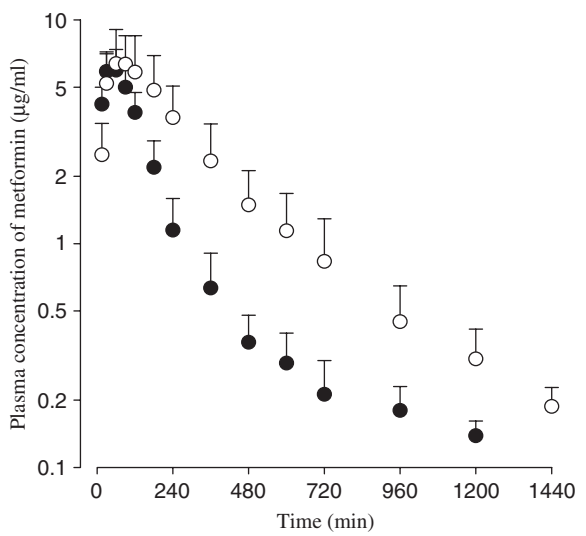

Figure 2. Mean arterial plasma concentration–time profiles of metformin after oral administration of the drug at a dose of 100 mg/kg to the control rats (●; $n = 8$) and the rat model of dehydration (○; $n = 11$). Bars represent standard deviation

Table 4. Mean (±standard deviation) pharmacokinetic parameters of metformin after oral administration of the drug at a dose of 100 mg/kg to control rats and rat model of dehydration

Parameter	Control ($n = 8$)	Dehydration ($n = 11$)
Body weight (g)		
Initial	253 ± 5.94	250 ± 8.79
Final	260 ± 5.35	208 ± 10.1[a]
Food intake (g/day/rat)		
Initial	22.8 ± 2.09	11.1 ± 1.54[a]
Final	23.5 ± 120	7.58 ± 1.49[a]
Hematocrit (%)	41.3 ± 2.73	51.1 ± 2.23[a]
Urine volume (ml/24-h)	24.0 ± 3.12	15.6 ± 1.31[a]
AUC (μg min/ml)	1210 ± 169	2410 ± 627[a]
Terminal half-life (min)	319 ± 127	310 ± 117
C_{max} (μg/ml)	6.38 ± 1.24	7.49 ± 2.39
T_{max} (min)	30 (30–60)	90 (30–120)[b]
Cl_r (ml/min/kg)	46.2 ± 6.33	21.5 ± 5.13[a]
$Ae_{0-24\,h}$ (% of dose)	55.9 ± 4.79	49.6 ± 7.32[b]
$GI_{24\,h}$ (% of dose)	5.68 ± 2.54	4.23 ± 3.43
F (%)	26.2	37.4

T_{max}, median (range).
[a]Significantly different ($p < 0.001$) from the control.
[b]Significantly different ($p < 0.05$) from the control.

Discussion

Induction of dehydration by water deprivation for 72 h in rats was evident based on the significantly greater hematocrit, and the significantly smaller 24 h urine output and body weight gain in the rat model of dehydration (Tables 1, 3 and 4). Similar results were also obtained from

DOI: 10.1002/bdd

other rat studies [7]. Food intake also decreased significantly in the rat model of dehydration (Tables 1, 3 and 4) and similar results were also reported from other rat studies [6,7,10,11,23,24]. The smaller body weight gain in the rat model of dehydration could be due to the lower food intake to prevent elevations in the extracellular fluid osmolarity and sodium concentration [6] in addition to water deprivation.

The contribution of gastrointestinal (including biliary) excretion of unchanged metformin to the Cl_{nr} of the drug was almost negligible; the percentages of the intravenous dose of metformin recovered from the gastrointestinal tract (including its contents and feces) at 24 h as an unchanged drug ($GI_{24\,h}$) were 1.53% and 0.771% for the control rats and the rat model of dehydration, respectively (Table 3). The smaller values of $GI_{24\,h}$, 1.53% and 0.771%, were not due to chemical and enzymatic degradation of metformin in the rat gastric fluids. It was reported [16] that metformin was stable up to 48 h incubation in various buffer solutions having pHs ranging from 1 to 12, and up to 24 h incubation with two rat gastric juices (pHs of 2.5 and 4.5, respectively). Moreover, it was also reported [16] that after intravenous administration of metformin at a dose of 100 mg/kg to six rats with bile duct cannulation, the biliary excretion of the unchanged drug was almost negligible; the percentage of the intravenous dose of metformin excreted in 24 h bile as unchanged drug was only 0.343%. Hence, the Cl_{nr} of metformin listed in Table 3 could represent the metabolic clearance of metformin. Therefore, the changes in the Cl_{nr} of metformin could represent changes in the metabolism of the drug in rats.

The *AUC* values of metformin were dose-proportional after both intravenous and oral administration of the drug at doses of 50, 100 and 200 mg/kg to rats [16]. Hence, the intravenous and oral metformin doses of 100 mg/kg were arbitrarily chosen in the present study.

After intravenous administration of metformin to the rat model of dehydration, the *AUC* of the drug was significantly greater than in the controls (Table 3). This could be due to the significantly slower *Cl* than in the controls (Table 3). The slower *Cl* could be due mainly to significantly slower Cl_r than the controls, since the Cl_{nr} values were comparable between the two groups of rats (Table 3). In the control rats, the contribution of Cl_r to *Cl* of metformin was considerable, 62.7% (Table 3), indicating that metformin is mainly excreted in the urine. It was reported that metformin was mainly metabolized via hepatic CYP2C11, 2D1 and 3A1/2 in rats [4], and the expressions of the hepatic CYP2C11 and 3A1/2 were not changed in the rat model of dehydration [6]. The comparable Cl_{nr} values of metformin between the two groups of rats (Table 3) suggest that the contribution of CYP2D1 to the metabolism of the drug did not seem to be considerable in the rat model of dehydration. Since metformin is a low hepatic extraction ratio drug (hepatic first-pass effect of 27.1% in rats [16]), the hepatic clearance of the drug depends more on the Cl_{int} and free (unbound to plasma proteins) fractions of metformin in plasma than on the hepatic blood flow rate [25]. The comparable Cl_{nr} values of metformin between the control rats and the rat model of dehydration (Table 3) could be supported by comparable *in vitro* Cl_{int} values for the disappearance of the drug (Table 2) and free fractions of the drug in plasma. Although the plasma protein binding value of metformin in the rat model of dehydration was significantly greater than the controls, the free fractions of metformin in plasma were 93.6% and 86.6% for the control rats and the rat model of dehydration, respectively; the difference was only 7.48% (Table 1). Therefore, the contribution of the decrease in free fractions of metformin in plasma in the rat model of dehydration to the comparable Cl_{nr} could not be considerable.

After intravenous administration of metformin, the Cl_r values of the drug were estimated as free fractions in plasma based on the Cl_r (Table 3) and plasma protein binding values (Table 1) of the drug. The values thus estimated were 14.5 and 6.72 ml/min/kg for the control rats and the rat model of dehydration, respectively. The 14.5 and 6.72 ml/min/kg were considerably faster than and comparable to the GFR (as estimated by Cl_{cr}) for the control rats and the rat model of dehydration (Table 1), respectively. This indicates that metformin is excreted in urine mainly via secretion and glomerular filtration for the control rats and the rat model of dehydration,

DOI: 10.1002/bdd

respectively. The above data suggest that the in rat model of dehydration, the secretion of metformin decreased more considerably than the controls. Active renal tubular secretion of metformin was also reported in humans [1]. The renal extraction ratios of metformin (Cl_r of metformin/renal plasma flow rate; only for urinary excretion of unchanged drug) were estimated based on the Cl_r of metformin (Table 3), hematocrit (Table 3) and reported renal blood flow rate of 36.8 and 12.9 ml/min/kg for the control rats [26] and the rat model of dehydration [27]. The renal blood flow rate decreased by 65% in rats with dehydration for 8 days compared with the controls [27]. The renal extraction ratios thus estimated were 63.2% and 91.0% for control rats and rat model of dehydration, respectively. The above data suggest that the renal extraction of metformin is considerable for both groups of rats.

After both intravenous and oral administration of metformin to the rat model of dehydration, the $Ae_{0-24\,h}$ was significantly smaller than the controls (Tables 3 and 4). Some factors might be proposed to explain the above phenomena. First, the decrease in the expression of the renal organic cation transporter 2 (rOCT2) in the rat model of dehydration might be a factor. It was reported that metformin was excreted in urine via rOCT2 [28]. However, this factor is unknown, since changes in the expression of rOCT2 did not seem to be reported in the rat model of dehydration. Second, the impaired kidney function in the rat model of dehydration could be another factor. However, this could be ruled out because kidney function was not impaired in the rat model of dehydration as mentioned earlier. Third, the decrease in free fractions of metformin in the plasma in the rat model of dehydration could also be another factor, since metformin was mainly excreted in the urine via the glomerular filtration as mentioned above. However, this was also remote, although the free fractions of metformin in plasma decreased in the rat model of dehydration, the difference was only 7.48% (Table 1). Hence, this 7.48% difference could not fully explain the 39.0% decrease in $Ae_{0-24\,h}$ of metformin after intravenous administration of the drug to the rat model of dehydration (Table 3). Finally, this could be due to urine flow rate-dependent timed-interval renal clearance of metformin in rats; there was a straight line between 1/timed-interval renal clearance of the drug and 1/urine flow rate [29] in both control rats and rats with diabetes mellitus induced by streptozotocin before and after insulin treatment (our unpublished data); the more the urine flow rate increased, the more metformin was excreted in 24 h urine. The 24 h urine output was significantly smaller in the rat model of dehydration (Tables 1, 3 and 4) and other rat studies [7]. The experiment on the urine flow rate-dependent timed-interval renal clearance of metformin could not be performed in the rat model of dehydration, since rehydration of 48 h water-deprived rats for the next 24 h with free access of food partially restored the *AUC* of chlorozoxazone to the controls [11]. The significant decrease in $Ae_{0-24\,h}$ of metformin after both intravenous and oral administration of the drug to the rat model of dehydration could contribute to the significantly slower Cl_r of the drug than the controls (Tables 3 and 4).

After intravenous administration of metformin to the rat model of dehydration, the V_{ss} of the drug was significantly smaller than the controls (Table 3). However, this could not be mainly due to a significant decrease in the free fractions of metformin in plasma in the rat model of dehydration; as mentioned earlier, the free fractions of the drug decreased only 7.48% in the rat model of dehydration. Although the exact reason is not clear, this could be related to the reduction in the extracellular and intracellular body fluids in rat model of dehydration [27] as shown in dehydrated goats [31,32]. The significantly smaller V_{ss} of antipyrine, sulfadiamidine and oxytetracycline hydrochloride were also reported in dehydrated goats [31,32].

After oral administration of metformin to the rat model of dehydration, the *AUC* of the drug was also significantly greater than in the controls (Table 4). However, this was not due to increased absorption of metformin from the gastrointestinal tract in the rat model of dehydration. After oral administration of metformin, the $GI_{24\,h}$ values of the drug were 5.68% and 4.23% of the oral dose for the control rats and the rat model of dehydration, respectively (Table 4). It is possible that this unchanged metformin, 5.68% and 4.23%,

DOI: 10.1002/bdd

might be partly attributed to the gastrointestinal (including biliary) excretion of the absorbed drug. Based on the linear pharmacokinetics [16], the 'mean' true fractions of oral dose unabsorbed (F_{unabs}) in this study could be estimated by the following equations [33]:

$$0.0568 = F_{unabs} + (0.262 \times 0.0153) \quad \text{for control rats} \tag{1}$$

$$0.0423 = F_{unabs} + (0.373 \times 0.00771) \quad \text{for rat model of dehydration} \tag{2}$$

where 0.262 (0.374) and 0.0153 (0.00771) are the F (Table 4) and $GI_{24\,h}$ values after intravenous administration of metformin to control rats (rat model of dehydration) (Table 3), respectively. The F_{unabs} values thus calculated were 5.28% and 3.94% for the control rats and the rat model of dehydration, respectively. Hence, approximately 95–96% of the oral dose was absorbed from the gastrointestinal tract for both groups of rats. The significantly greater *AUC* of metformin after oral administration of the drug to the rat model of dehydration could also be due to a significant decrease in $Ae_{0-24\,h}$ compared with the controls (Table 4).

In the rat model of dehydration, the hematocrit was significantly greater than the controls (Tables 1, 3 and 4). The binding of metformin to blood cells was considerable; the equilibrium mean plasma-to-blood cells concentration ratio of metformin in rats was 1.25–1.37 [16]. The bound fractions of drugs to red blood cells were reported to act as barriers for the elimination of doxorubicin [34] and propranolol [35]. Hence, the significantly greater hematocrit in the rat model of dehydration could influence at least partly the significantly slower *Cl* in rats (Table 3).

In conclusion, after intravenous administration of metformin to the rat model of dehydration, the $Ae_{0-24\,h}$ was significantly smaller than the controls (Table 3). This could contribute to the significantly slower Cl_r than the controls (Table 3). The smaller $Ae_{0-24\,h}$ in the rat model of dehydration could be due to the urine flow rate-dependent timed-interval renal clearance of metformin in rats. The Cl_{nr} values were comparable between the control rats and the rat model of dehydration (Table 3). This could be due to comparable expressions of hepatic CYP2C11 and 3A1/2 between the two groups of rats [6]. The comparable Cl_{nr} values could be supported by comparable *in vitro* Cl_{int} values for both groups of rats (Table 2). After oral administration of metformin to the rat model of dehydration, the *AUC* of the drug was also significantly greater than the controls. This was not due to an increase in the absorption of metformin in the rat model of dehydration. The significantly greater *AUC* of metformin after intravenous and oral administration of the drug could be due to significantly greater $Ae_{0-24\,h}$ than in the controls.

Acknowledgements

This work was supported in part by a grant from the Seoul City Collaborative Project to the Industry, Academy, and Research Institute, Korea.

References

1. Scheen AJ. Clinical pharmacokinetics of metformin. *Clin Pharmacokinet* 1996; **30**: 359–371.
2. Sirtori CR, Franceschini G, Galli-Kienle M, *et al*. Disposition of metformin (*N*,*N*-dimethylbiguanide) in man. *Clin Pharmacol Ther* 1978; **24**: 683–693.
3. Tucker GT, Casey C, Phillips PJ, Connor H, Ward JD, Woods HF. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. *Br J Clin Pharmacol* 1981; **12**: 235–246.
4. Choi YH, Lee MG. Effects of enzyme inducers and inhibitors on the pharmacokinetics of metformin in rats: involvement of CYP2C11, 2D1, and 3A1/2 for the metabolism of metformin. *Br J Pharmacol* 2006; **149**: 424–430.
5. Bakar SK, Niazi S. Effect of water deprivation on aspirin disposition kinetics. *J Pharm Sci* 1983; **72**: 1030–1034.
6. Kim SG, Kim EJ, Kim YG, Lee MG. Expression of cytochrome P-450s and glutathione S-transferases in the rat liver during water deprivation: effects of glucose supplementation. *J Appl Toxicol* 2001; **21**: 123–129.
7. 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.
8. Milionis HJ, Elisaf MS. Therapeutic management of hyperglycaemic hyperosmolar syndrome. *Expert Opin Pharmacother* 2005; **6**: 1841–1849.
9. Stoner GD. Hyperosmolar hyperglycemic state. *Am Fam Physician* 2005; **71**: 1723–1730.

DOI: 10.1002/bdd

10. Kim JY, Kim YC, Lee MG, Kwon JW, Yoo M. Effects of water deprivation on the pharmacokinetics of DA-8159, a new erectogenic, in rats. *J Pharm Pharm Sci* 2006; **9**: 10–21.
11. 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.
12. 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.
13. Duggleby RG. Analysis of enzyme progress curves by nonlinear regression. *Methods Enzymol* 1995; **249**: 61–90.
14. Kim SH, Choi YM, Lee MG. Pharmacokinetics and pharmacodynamics of furosemide in protein–calorie malnutrition. *J Pharmacokinet Biopharm* 1993; **21**: 1–17.
15. Hale TW, Kristensen JH, Hackett LP, Kohan R, Ilett KF. Transfer of metformin into human milk. *Diabetologia* 2002; **45**: 1509–1514.
16. Choi YH, Kim SG, Lee MG. Dose-independent pharmacokinetics of metformin after intravenous and oral administration in rats: hepatic and gastrointestinal first-pass effects. *J Pharm Sci* 2006; **95**: 2543–2552.
17. Chiou WL. 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* 1978; **6**: 539–546.
18. Gibaldi M, Perrier D. *Pharmacokinetics* (2nd edn). Marcel–Dekker: New York, 1982.
19. Chiou WL. New calculation method for mean apparent drug volume of distribution and application to rational dosage regimens. *J Pharm Sci* 1979; **68**: 1067–1069.
20. 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.
21. Chiou WL. New calculation method of mean total body clearance of drugs and its application to dosage regimens. *J Pharm Sci* 1980; **69**: 90–91.
22. Mitruka BM, Rawnsley HM. *Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals and Normal Humans* (2nd edn). Masson Publishing, USA: New York, 1981; 160.
23. Osman SA, Smith JD, Hanson LE, Meade RJ. The conservation of water by young adult rats with restricted water supplies. *J Nutr* 1966; **90**: 268–274.
24. Tanaka E, Kurata N, Kuroiwa Y, Misawa S. Influence of short-term water deprivation on kinetics of trimethadione and its metabolite in rats. *Jpn J Pharmacol* 1986; **42**: 269–274.
25. Wilkinson GR, Shand DG. A physiological approach to hepatic drug clearance. *Clin Pharmacol Ther* 1975; **18**: 377–390.
26. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993; **10**: 1093–1095.
27. 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.
28. Kimura N, Masuda S, Tanihara Y, *et al*. Metformin is a superior substrate for renal organic cation transporter OCT2 rather than hepatic OCT1. *Drug Metab Pharmacokinet* 2005; **20**: 379–386.
29. Chiou WL. A new simple approach to study the effect of changes in urine flow and/or urine pH on renal clearance and its applications. *Int J Clin Pharmacol Ther Toxicol* 1986; **24**: 519–527.
30. Wallace WM, Golstein K, Taylor A, Teree TM. Thermal dehydration of the rat: distribution of losses among tissues. *Am J Physiol* 1970; **219**: 1544–1548.
31. Elsheikh HA, Osman IAM, Asdullah AS. The effect of water deprivation on the pharmacokinetics of antipyrine and sulphadimidine following intravenous administration in Nubian goats. *Vet Res Commun* 1997; **21**: 587−597.
32. Elsheikh HA, Osman IAM, Eltayeb IB, Asdullah AS. Effect of dehydration on the pharmacokinetics of oxytetracycline hydrochloride administered intravenously in goats (*Capra hircus*). *Gen Pharmacol* 1998; **31**: 455−458.
33. Lee MG, Chiou WL. Evaluation of potential causes for the incomplete bioavailability of furosemide: Gastric first-pass metabolism. *J Pharmacokinet Biopharm* 1983; **11**: 623–640.
34. Lee HJ, Chiou WL. Erythrocytes as barriers for drug elimination in the isolated rat liver. I. Doxorubicin. *Pharm Res* 1989; **6**: 840–843.
35. Lee HJ, Chiou WL. Erythrocytes as barriers for drug elimination in the isolated rat liver. II. Propranolol. *Pharm Res* 1989; **6**: 833–839.

DOI: 10.1002/bdd