BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **28**: 445–454 (2007)
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/bdd.573

Effects of Water Deprivation on the Pharmacokinetics of Theophylline and One of Its Metabolites, 1,3-Dimethyluric Acid, after Intravenous and Oral Administration of Aminophylline to Rats

Hye D. Choi[a], Hee E. Kang[a], Hye J. Chung[a], Soo K. Bae[a], Karen N. Shin[b,†] and Myung G. Lee[a,*]
[a] *College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Republic of Korea*
[b] *New York University, 7 E 12th St., New York, NY 10003-4475, USA*

ABSTRACT: It has been reported that the expressions of hepatic microsomal cytochrome P450 (CYP) 1A1/2, 2B1/2 and 3A1/2 were not changed in rats with water deprivation for 72 h (rat model of dehydration) compared with the controls. It has been also reported that 1,3-dimethyluric acid (1,3-DMU) was formed from theophylline via CYP1A1/2 in rats. Hence, it could be expected that the formation of 1,3-DMU could be comparable between the two groups of rats. As expected, after both intravenous and oral administration of theophylline at a dose of 5 mg/kg to the rat model of dehydration, the *AUC* of 1,3-DMU was comparable to the controls. After both intravenous and oral administration of theophylline to the rat model of dehydration, the Cl_r of both theophylline and 1,3-DMU was significantly slower than the controls. This could be due to significantly smaller urinary excretions of both theophylline and 1,3-DMU since the *AUC* of both theophylline and 1,3-DMU were comparable between the two groups of rats. The smaller urinary excretion of both theophylline and 1,3-DMU could be due to urine flow rate-dependent timed-interval renal clearance of both theophylline and 1,3-DMU in rats. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: theophylline; 1,3-DMU; pharmacokinetics; water deprivation for 72 h; CYP1A1/2,2B1/2 and 3A1/2; rats

Introduction

Human liver microsomal inhibition studies and the use of recombinant hepatic microsomal cytochrome P450 (CYP) isoforms have demonstrated that CYP1A2 is responsible for the formation of 3-methylxanthine (3-MX) and 1-methylxanthine (1-MX) from theophylline [1], a bronchodilator. CYP1A2 is also the major catalyst for the formation of 1,3-dimethyluric acid (1,3-DMU) with an additional contribution from CYP2E1 and possibly CYP3A4 [1]. In the rats, the metabolism of theophylline to form 1-MX, 3-MX and 1,3-DMU were all significantly increased in Aroclor 1254-, dexamethasone-, phenobarbital- and 3-methylcholanthrene-treated microsomes (inducers of CYP1A1, 3A2, 2B1/2 and 1A1/2, respectively) [2]. In the rats, the formation of 1-MX and 1,3-DMU was catalysed via CYP1A2 and 3A2 [3]. Recently, it was found in our laboratories that theophylline was metabolized via CYP1A1/2, 2B1/2 and 3A1/2, and 1,3-DMU was formed mainly via CYP1A1/2 after intravenous administration of aminophylline, 5 mg/kg as theophylline,

*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
†Undergraduate student.

Received 11 December 2006
Revised 29 May 2007
Accepted 30 May 2007

to male Sprague–Dawley rats pretreated with various inducers and inhibitors of CYP isozymes (our unpublished data).

Dehydration occurs by excessive sweating, polyuria, severe diarrhea and hyperthermia [4]. Kim *et al*. [5] have reported changes in the expressions and/or mRNA levels of CYP isozymes in male Sprague–Dawley rats with water deprivation for 72 h (rat model of dehydration). For example, the expressions of CYP1A2, 2B1/2, 2C11 and 3A1/2 were not changed, however, the expression and mRNA level of CYP2E1 markedly increased in the rat model of dehydration compared with the controls. Hence, it could be expected that the formation of 1,3-DMU would not be changed considerably in the rat model of dehydration compared with the controls. It has been reported that the timed-interval renal clearance of theophylline was dependent on urine flow rate in children with asthma [6] and healthy adults [7] and of 1,3-DMU in pediatric patients [8]. Urine output was significantly smaller in the rat model of dehydration [4,5,9]. Therefore, it could be expected that the time-averaged renal clearance (Cl_r) of theophylline could be slower in the rat model of dehydration. Although pharmacokinetic changes of drugs in the rat model of dehydration have been reported [9], studies on theophylline with respect to CYP isozyme changes in rat model of dehydration [5] do not seem to have been reported. Moreover, it has been reported that hypoxia, dehydration, acidosis and hypokalemia render the severe asthmatic patient vulnerable to cardiac dysrhythmia and cardiorespiratory arrest [10]. Therefore, theophylline was chosen in this study using rats with dehydration as an animal model.

The aim of this study is to report the comparable formation of 1,3-DMU after both intravenous and oral administration of aminophylline, 5 mg/kg as theophylline, to control rats and the rat model of dehydration with respect to CYP isozyme changes [5]. The significantly smaller urinary excretions of both theophylline and 1,3-DMU after both intravenous and oral administration of theophylline due to urine flow rate-dependent timed-interval renal clearance of theophylline and 1,3-DMU were also reported.

Materials and Methods

Chemicals

Aminophylline intravenous solution (200 mg as theophylline/a 10 ml ampoule) was a product from Daewon Pharmaceutical Company (Seoul, Republic of Korea). Theophylline, 1,3-DMU, β-hydroxyethyltheophylline [an internal standard of high-performance liquid chromatographic (HPLC) analysis of theophylline and 1,3-DMU], reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), ethylenediamine tetraacetatic acid (EDTA) and tri(hydroxymethyl)aminomethane (Tris®)-buffer were products from Sigma–Aldrich Corporation (St Louis, MO, USA). Other chemicals were of reagent grade or HPLC grade.

Rats

Male Sprague–Dawley rats of 7–9 weeks of age (weighing 240–330 g) purchased from Charles River Company Korea (Orient, Seoul, Republic of Korea) were randomly divided into two groups; control rats and rat model of dehydration. For control rats, water and food (Sam Yang Company, Pyeongtaek, Republic of Korea) were supplied for 72 h; for the rat model of dehydration, water was deprived for 72 h with free access to food. The rats were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, Republic of Korea) at a temperature between 20 and 23 °C with 12 h light (0700–1900) and dark (1900–0700) cycles and a relative humidity of $50 \pm 5\%$. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under a supply of filtered pathogen-free air. The Animal Care and Use Committee of College of Pharmacy of Seoul National University approved this animal study protocol.

Measurement of V_{max}, K_m, and Cl_{int} for the disappearance of theophylline and the formation of 1,3-DMU in hepatic microsomal fractions

The procedures were similar to the previously reported methods [11]. On day 4, the livers of control rats ($n = 5$) and rat model of dehydration ($n = 5$) were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni,

DOI: 10.1002/bdd

Germany) in 4 volumes of 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*g* for 30 min and the supernatant fraction was further centrifuged at 100 000*g* for 90 min. The microsomal pellet was resuspended in a buffer of 0.154 M KCl/50 mM Tris-HCl in 1 mM EDTA, pH 7.4. The protein content was measured using the reported method [12]. The microsomal fraction (equivalent to 1 mg protein) was incubated with a 10 µl aliquot of phosphate buffer of pH 7.4 that contained 0.1, 0.2, 0.5, 1, 2, 5, 7.5 and 10 mM of theophylline and a 50 µl aliquot of phosphate buffer of pH 7.4 that contained 1.2 mM of NADPH in a final volume of 500 µl by the addition of 100 mM phosphate buffer of pH 7.4 in a thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany) kept at 37 °C and at a rate of 500 oscillations per min (opm). The above incubation concentrations were linear. The reaction was terminated by the addition of a 0.5 ml aliquot of acetonitrile that contained 20 µg/ml of an internal standard (*β*-hydroxyethyltheophylline) after 30 min incubation. The concentrations of theophylline and 1,3-DMU were analysed by the reported HPLC method [13]. The kinetic constants, 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 theophylline and the formation of 1,3-DMU, were calculated using the nonlinear regression method [14]. The intrinsic clearance (Cl_{int}) for the disappearance of theophylline and the formation of 1,3-DMU were calculated by dividing the respective V_{max} by the respective K_m.

Intravenous and oral administration of theophylline to control rats and rat model of dehydration

The pretreatment and surgical procedures for intravenous and oral administration of theophylline were similar to the previously reported methods [15]. In the early morning on day 4, the jugular vein (for drug administration only for intravenous study) and the carotid artery (for blood sampling) of each rat was cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ, USA) while each rat was under light ether anesthesia. Each cannula was exteriorized to the dorsal side of the neck, where each cannula was terminated with a long silastic tube (Dow Corning, Midland, MI, USA). The silastic tube was covered with a wire sheath to allow free movement of the rats. After the exposed areas were surgically sutured, each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, Republic of Korea) and allowed to recover from the anesthesia for 4–5 h before the study began. They were not restrained during the whole experimental period.

Aminophylline (aminophylline injectable solution was diluted with phosphate buffer of pH 7.4) at a dose of 5 mg/kg as theophylline was infused intravenously (total infusion volume of approximately 0.6 ml) over 1 min via the jugular vein of control rats ($n = 9$) and rat model of dehydration ($n = 9$). 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 the infusion), 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after the start of the intravenous infusion of theophylline. An approximately 0.25 ml aliquot of heparinized 0.9% NaCl injectable solution (20 units/ml) was used to flush the cannula after each blood sampling to prevent blood clotting. After centrifugation of blood sample, a 50 µl aliquot of plasma sample was stored in a −70 °C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC, USA) until HPLC analysis of theophylline and 1,3-DMU [13]. At the end of 24 h, each metabolic cage was rinsed with 10 ml of distilled water and the rinsed material was combined with the 24 h urine sample. After measuring the exact volume of the 24 h urine and combined urine samples, two 50 µl aliquots of the combined urine sample were stored in a −70 °C freezer until HPLC analysis of theophylline and 1,3-DMU [13].

The following experiment was also performed to control rats ($n = 4$) to find whether the timed-interval renal clearance of theophylline or 1,3-DMU is dependent on the urine flow rate or not. A priming dose of theophylline (3 mg/kg) was administered via an intravenous bolus followed by intravenous infusion for 8 h (6 mg/kg) with the assistance of an infusion pump (Model 2400-006; Harvard Instrument, Southnatick, MA) to reach a plateau plasma concentration of theophylline of approximately 5 µg/ml. Priming and maintenance doses of theophylline were estimated based on the

DOI: 10.1002/bdd

intravenous data. The 0.9% NaCl-injectable solution was infused at rates of 0.3–1.5 ml/min to control the urine flow rate. Note that this experiment could not be performed in the rat model of dehydration, because rehydration of 48 h water-deprived rats for the next 24 h caused partial restoration of the total area under the plasma concentration–time curve from time zero to time infinity (*AUC*) of intravenous chlorzoxazone (metabolized to 6-hydroxychlorzoxazone via CYP2E1 in rats) in rat model of water deprivation to the controls [16].

Aminophylline (the same solution that was used in the intravenous study) at a dose of 5 mg/kg as theophylline was administered orally (total oral volume of approximately 1.2 ml) using a feeding tube to control rats ($n = 9$) and rat model of dehydration ($n = 8$). Blood samples were collected at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 360, 480 and 600 min after oral administration of theophylline. Other procedures were similar to those in the intravenous study.

Measurement of plasma protein binding of theophylline in control rats and rat model of dehydration using the equilibrium dialysis technique

Plasma protein binding of theophylline at concentrations of 2 and 10 μg/ml was measured in control rats ($n = 5$) and rat model of dehydration ($n = 5$) using the equilibrium dialysis technique [17]. One milliliter of plasma was dialysed against 1 ml of an isotonic Sørensen phosphate buffer (pH 7.4) that contained 3% (w/v) dextran to minimize volume shift [18] using a 1 ml dialysis cell (Fisher Scientific, Fair Lawn, NJ, USA) and Spectra/Por 4 membrane (mol. wt cut off 12 000–14 000 Da; Spectrum Medical Industries Inc., Los Angeles, CA, USA). To reduce equilibrium time, the drug was spiked into the plasma side [19]. The spiked dialysis cell was incubated in a water-bath shaker kept at 37 °C and at a rate of 50 opm. After 24 h incubation, two 100 μl aliquots were removed from each compartment and stored in a −70 °C freezer until HPLC analysis of theophylline [13].

HPLC analysis of theophylline and 1,3-DMU

The concentrations of theophylline and 1,3-DMU were analysed by the reported HPLC method [13]. A 300 μl aliquot of acetonitrile that contained 2 μg/ml of the internal standard (β-hydroxyethyltheophylline) was added to a 50 μl aliquot of sample. After vortex-mixing and centrifugation, a 300 μl aliquot of the supernatant was evaporated under a gentle stream of nitrogen gas at 30 °C. The residue was reconstituted with a 100 μl aliquot of the mobile phase. After centrifugation, a 50 μl aliquot of the supernatant was injected directly onto the reversed-phase (C_{18}) HPLC column. The mobile phase, 10 mM acetate buffer (pH 5.0): acetonitrile: tetrahydrofuran (94: 5: 1; v/v/v), was run at a flow rate of 1.0 ml/min, and the column effluent was monitored using an ultraviolet detector set at 280 nm. The retention times of 1,3-DMU, theophylline and the internal standard (β-hydroxyethyltheophylline) were approximately 9, 16 and 19 min, respectively. The detection limits of theophylline and 1,3-DMU in rat plasma were 100 and 30 ng/ml, respectively. The coefficients of variation of the assay (within- and between-day) were below 8.90%.

Pharmacokinetic analysis

The *AUC* was calculated using the trapezoidal rule–extrapolated method; this method uses the logarithmic trapezoidal rule [20] for the calculation of the area during the declining plasma-level phase 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 concentration in plasma by the terminal-phase rate constant.

Standard methods [21] were used to calculate the following pharmacokinetic parameters using the noncompartmental analysis (WinNonlin®; Professional edition version 2.1; Pharsight, Mountain View, CA, USA); 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 extent of absolute oral bioavailability (*F*) [15]. The timed-interval renal clearance was calculated by dividing the total amount excreted in the urine during the time interval by the *AUC* during the time interval. The peak plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were read directly from the experimental data.

DOI: 10.1002/bdd

Statistical analysis

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

Results

Measurement of V_{max}, K_m and Cl_{int} for the disappearance of theophylline and the formation of 1,3-DMU in hepatic microsomal fractions

The V_{max}, K_m and Cl_{int} for the disappearance of theophylline and the formation of 1,3-DMU in microsomes prepared from the livers of control rats and rat model of dehydration are listed in Table 1. In the rat model of dehydration, the V_{max}, K_m and Cl_{int} for the disappearance of theophylline were not significantly different compared with the controls. This suggests that the maximum velocity for the disappearance (mainly due to formation of metabolites) of theophylline, affinity of theophylline to the enzyme(s) and formation of metabolites were not affected considerably by the dehydration state. In the rat model of dehydration, the V_{max} for the formation of 1,3-DMU was significantly faster (27.9% increase) than the controls, however, the K_m and Cl_{int} for the formation of 1,3-DMU were comparable between the control rats and the rat model of dehydration. There were no differences in the protein contents in the liver microsomes between two groups.

Pharmacokinetics of theophylline and 1,3-DMU after intravenous administration of theophylline to control rats and rat model of dehydration

After intravenous administration of theophylline to control rats and rat model of dehydration, the mean arterial plasma concentration–time profiles of theophylline and 1,3-DMU are shown in Figures 1A and B, respectively, and some relevant pharmacokinetic parameters are listed in Table 2. The pharmacokinetic parameters of theophylline and 1,3-DMU listed in Table 2 were not significantly different between control rats and rat model of dehydration except significantly slower Cl_r of theophylline (84.5% decrease) and significantly smaller percentage of intravenous dose of theophylline excreted in 24 h urine as an unchanged drug ($Ae_{0-24\,h}$; 86.3% decrease) and significantly slower Cl_r of 1,3-DMU (55.9% decrease) and significantly smaller $Ae_{0-24\,h}$ of 1,3-DMU (64.1% decrease; expressed in terms of intravenous dose of theophylline) in rat model of dehydration than the controls. The formation of 1,3-DMU was fast; 1,3-DMU was detected from the first blood sampling time (1 min) for both groups of rats and reached its peak (T_{max}) rapidly at 66.7 and 56.3 min for control rats and rat model of dehydration, respectively.

There was a straight line between 1/timed-interval renal clearance of theophylline (Figure 2A) as well as 1,3-DMU (Figure 2B) and 1/urine flow rate in control rats, indicating the urine flow rate-dependent timed-interval renal clearance [22] of theophylline or 1,3-DMU in rats; the less urine flow rate, the less theophylline or 1,3-DMU was excreted in the urine.

Pharmacokinetics of theophylline and 1,3-DMU after oral administration of theophylline to control rats and rat model of dehydration

After oral administration of theophylline to control rats and rat model of dehydration, the mean arterial plasma concentration–time profiles of theophylline and 1,3-DMU are shown in Figures 1C and D, respectively, and some

Table 1. Mean (± standard deviation) V_{max}, K_m and Cl_{int} for the disappearance of theophylline and for the formation of 1,3-DMU in liver microsomes of control rats and rat model of dehydration ($n = 5$, each)

Parameter	Disappearance of theophylline		Formation of 1,3-DMU	
	Control	Dehydration	Control	Dehydration
V_{max} (nmol/min/mg protein)	64.3 ± 15.8	70.3 ± 38.5	0.290 ± 0.0141	0.371 ± 0.0492[a]
K_m (mM)	21.1 ± 5.58	20.8 ± 10.9	2.04 ± 0.415	2.52 ± 0.321
Cl_{int} (10^{-3}ml/min/mg protein)	3.08 ± 0.532	3.32 ± 0.45	0.148 ± 0.0389	0.148 ± 0.0165

[a] Significantly different ($p < 0.01$) from control.

DOI: 10.1002/bdd

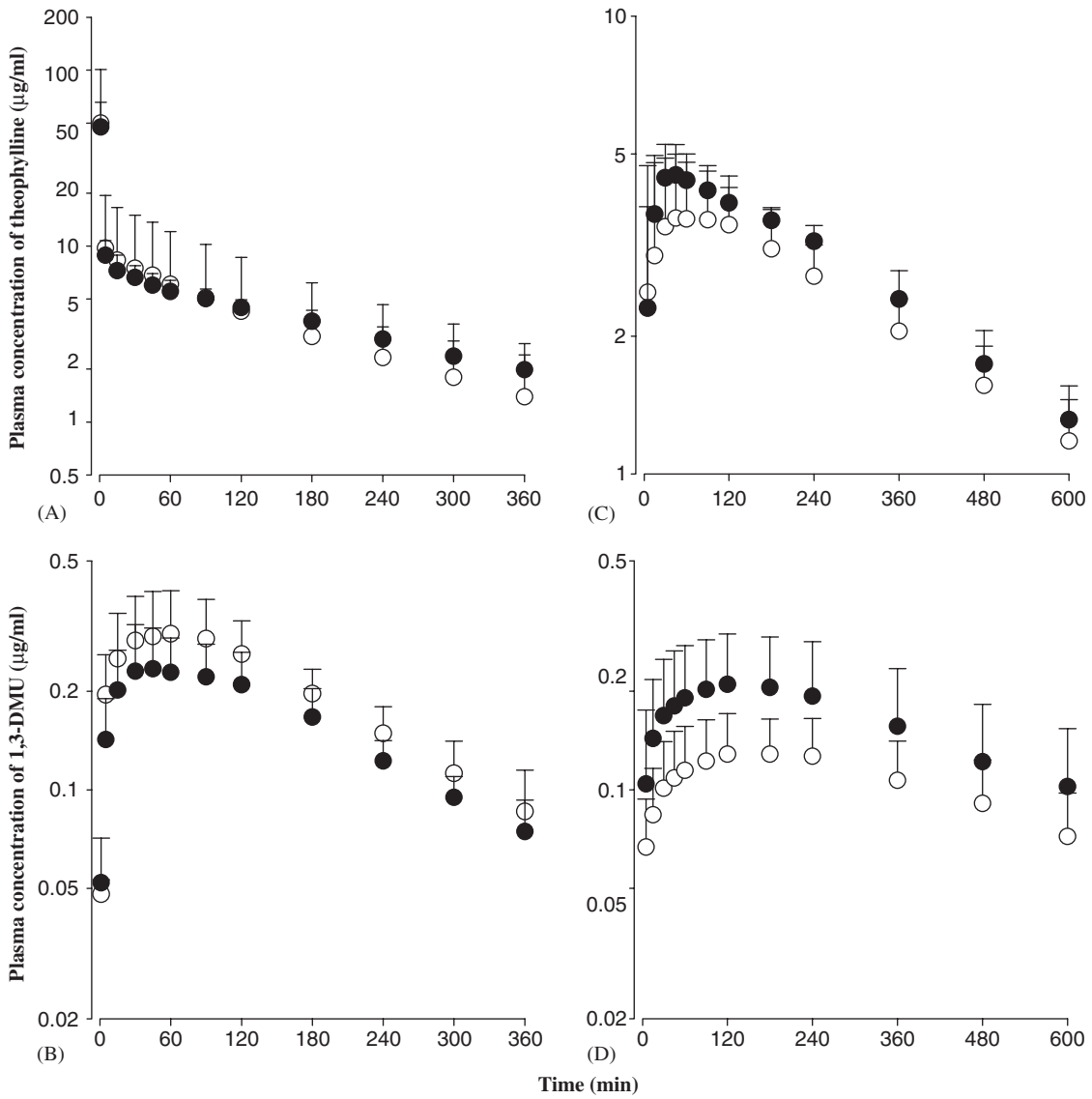

Figure 1. Mean arterial plasma concentration–time profiles of theophylline (top panel) and 1,3-DMU (bottom panel) after 1 min intravenous infusion (left panel) and oral administration (right panel) of theophylline at a dose of 5 mg/kg to control rats (○; $n = 9$ for both intravenous and oral administration) and rat model of dehydration (●; $n = 9$ and 8 for intravenous and oral administration, respectively). Vertical bars represent standard deviation

relevant pharmacokinetic parameters are also listed in Table 2. Absorption of theophylline from the rat gastrointestinal tract was rapid; theophylline was detected in plasma from the first blood sampling time (5 min) for both groups of rats and reached its peak (T_{max}) rapidly at 75.6 and 33.4 min for control rat and rat model of dehydration, respectively. The formation of 1,3-DMU was also rapid for both groups of rats; 1,3-DMU was detected from the first blood sampling time (5 min) for both groups of rats and reached its peak (T_{max}) at 157 and 143 min for control rats and rat model of dehydration, respectively. The pharmacokinetic parameters of theophylline and 1,3-DMU listed in Table 2 were also not significantly different between two groups of rats

DOI: 10.1002/bdd

Table 2. Mean (± standard deviation) pharmacokinetic parameters of theophylline and 1,3-DMU after intravenous and oral administration of theophylline at a dose of 5 mg/kg to control rats and rat model of dehydration

Parameter	Intravenous administration		Oral administration	
	Control (n=9)	Dehydration (n=9)	Control (n=9)	Dehydration (n=8)
Body weight (g)				
Initial[a]	255 ± 7.82	257 ± 7.50	293 ± 27.7	301 ± 34.0
Final[b]	281 ± 10.3	253 ± 14.2[c]	313 ± 31.2	279 ± 24.8[d]
Urine output (ml/24 h)	23.1 ± 5.81	2.77 ± 1.54[c]	20.9 ± 9.91	1.17 ± 0.995[c]
Food intake (g/day)	20.3 ± 3.84	8.94 ± 4.30[c]	17.7 ± 5.57	10.0 ± 5.22[d]
Theophylline				
AUC (μg min/ml)	1840 ± 295	2090 ± 327	1950 ± 384	2190 ± 296
Terminal half-life (min)	151 ± 26.4	183 ± 51.3	284 ± 40.9	269 ± 33.9
C_{max} (μg/ml)			4.43 ± 1.81	4.69 ± 0.647
T_{max} (min)			75.6 ± 55.8	34.4 ± 14.0
MRT (min)	178 ± 52.6	271 ± 71.4		
V_{ss} (ml/kg)	551 ± 70.7	625 ± 143		
Cl (ml/min/kg)	2.73 ± 0.354	2.39 ± 0.496		
Cl_r (ml/min/kg)	0.363 ± 0.323	0.0562 ± 0.107[c]	0.195 ± 0.344	0.0143 ± 0.0230[d]
Cl_{nr} (ml/min/kg)	2.20 ± 0.472	2.33 ± 0.494		
$Ae_{0-24\ h}$ (% of theophylline dose)	17.5 ± 8.84	2.40 ± 0.473[c]	11.4 ± 8.10	1.18 ± 0.865[d]
F (%)			106	105
1,3-DMU				
AUC (μg min/ml)	92.1 ± 24.5	71.6 ± 7.74	115 ± 33.9	156 ± 77.7
Terminal half-life (min)	143 ± 63.0	159 ± 61.7	404 ± 119	371 ± 90.4
C_{max} (μg/ml)	0.306 ± 0.106	0.243 ± 0.0830	0.161 ± 0.0588	0.212 ± 0.0867
T_{max} (min)	66.7 ± 24.6	56.3 ± 17.5	157 ± 66.7	143 ± 55.0
Cl_r (ml/min/kg)	0.217 ± 0.0267	0.0956 ± 0.0209[c]	0.195 ± 0.155	0.0198 ± 0.0197[c]
$Ae_{0-24\,h}$(% of theophylline dose)	15.6 ± 2.32	5.60 ± 1.72[c]	8.24 ± 4.31	1.32 ± 0.797[c]

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

except significantly slower Cl_r of theophylline (92.7% decrease) and significantly smaller $Ae_{0-24\,h}$ of theophylline (89.6% decrease), and significantly slower Cl_r of 1,3-DMU (89.9% decrease) and significantly smaller $Ae_{0-24\,h}$ of 1,3-DMU (84.0% decrease; expressed in terms of theophylline dose) in rat model of dehydration than the controls.

Plasma protein binding of theophylline in control rats and rat model of dehydration

The plasma protein binding values of theophylline at 2 μg/ml were 25.2 ± 2.39% and 26.2 ± 3.47% for control rats and rat model of dehydration, respectively; they were not significantly different between two groups of rats. The corresponding values at 10 μg/ml were 21.0 ± 2.71% and 23.3 ± 6.65%; they were also not significantly different between two groups of rats.

Discussion

Many investigators have reported the dose-dependent metabolic disposition of theophylline in rats [23]. Therefore, theophylline at a dose of 5 mg/kg which has been reported to be in the range of linear pharmacokinetics in rats [23] was administered intravenously and orally in the present study.

Induction of dehydration was evident based on the significantly smaller 24 h urine output (88.0% and 94.4% decrease for intravenous and oral administration, respectively) and significant

DOI: 10.1002/bdd

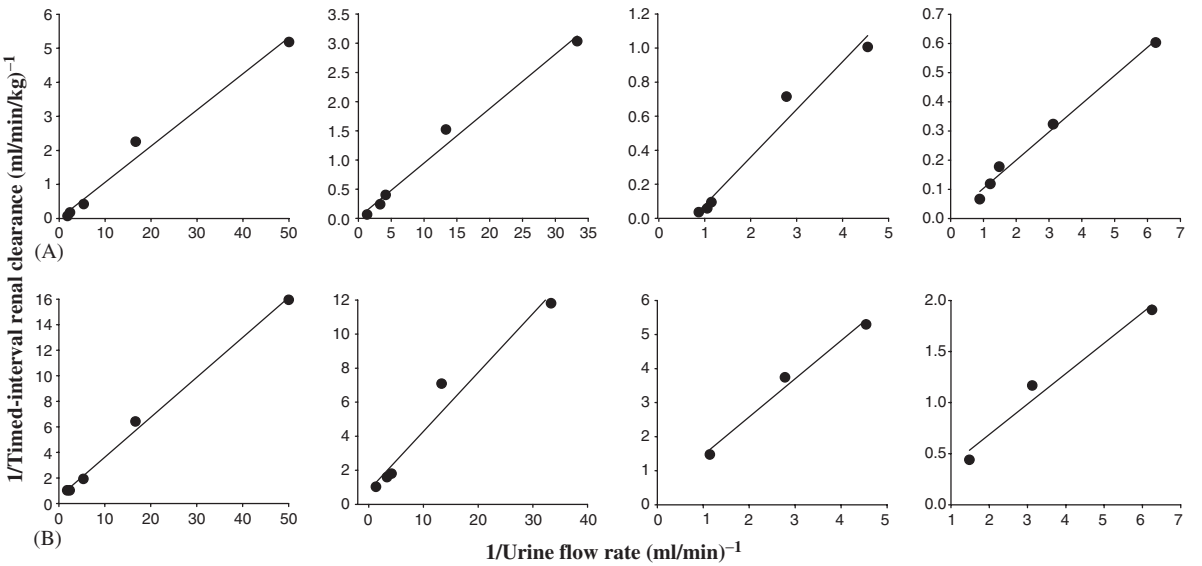

Figure 2. Relationship between 1/timed-interval renal clearance of theophylline (A) as well as 1,3-DMU (B) and 1/U (urine flow rate) in control rats ($n = 4$, each)

decrease in body weight gain (from 257 to 253 g versus from 255 to 281 g and from 301 to 279 g versus from 293 to 313 g for intravenous and oral administration, respectively) in the rat model of dehydration (Table 2). Similar results have also been reported from other rat studies [9]. Food intake also decreased significantly in rat model of dehydration (56.0% and 43.5% decrease for intravenous and oral administration, respectively) (Table 2). Similar results have also been reported from other rat studies [5,9,24,25]. Hence, the decrease in body weight gain could be due to less food intake to prevent elevations in extracellular fluid osmolarity and sodium concentration [5] in addition to water deprivation in rat model of dehydration.

Theophylline was metabolized via CYP1A1/2, 2B1/2 and 3A1/2, and 1,3-DMU was formed via CYP1A1/2 in rats as mentioned earlier. It has been reported that the expressions of CYP1A1/2, 2B1/2 and 3A1/2 were not changed in the rat model of dehydration compared with the controls [5]. Hence, it could be expected that the *AUC* of theophylline and the formation (*AUC*) of 1,3-DMU in the rat model of dehydration could be comparable to the controls. As expected, the *AUC* values of both theophylline and 1,3-DMU after both intravenous and oral administration of theophylline were not significantly different between the two groups of rats (Table 2). This could be supported by comparable Cl_{int} for the disappearance of theophylline and the formation of 1,3-DMU between the two groups of rats (Table 1). The hepatic extraction ratio (the hepatic first-pass effect) of theophylline was roughly estimated [26] in control rats by dividing the Cl_{nr} of theophylline (Table 2) by the reported hepatic blood flow rate of 55.2 ml/min/kg [27] and hematocrit of approximately 45% [28] in control rats, assuming that the Cl_{nr} of theophylline was attributed solely to the liver [26]. Hence, the estimated hepatic extraction ratio represents the maximal possible value of the liver [26]. The hepatic extraction ratio of theophylline thus estimated in control rats was approximately 7.25%. Since, theophylline is a low hepatic extraction ratio drug, the hepatic clearance of the drug depends more on the intrinsic clearance (Cl_{int}) and free (unbound to plasma proteins) fractions of theophylline in plasma rather than on the hepatic blood flow rate [29]. The comparable Cl_{nr} of theophylline for both groups of rats (Table 2) could be supported by comparable Cl_{int} for the disappearance of theophylline (Table 1) and comparable free fractions of theophylline in plasma as mentioned earlier.

DOI: 10.1002/bdd

Although the contribution of Cl_r to Cl of theophylline was not considerable (less than 13.3%) for both control rats and rat model of dehydration (Table 2), the Cl_r of theophylline in rat model of dehydration was significantly slower than the controls for both after intravenous and oral administration (Table 2). This could be mainly due to significantly smaller $Ae_{0-24\,h}$ in rat model of dehydration, since the *AUC* of theophylline was not significantly different between two groups of rats for both routes of administration (Table 2). The smaller $Ae_{0-24\,h}$ of theophylline in rat model of dehydration (Table 2) could be due to urine flow rate-dependent timed-interval renal clearance of theophylline in control rats (Figure 2A); the less urine output, the less $Ae_{0-24\,h}$ of theophylline was obtained. Urine flow rate-dependent timed-interval renal clearance of theophylline has also been obtained from humans [6,7,22]. The 24 h urine output was significantly smaller in the rat model of dehydration than in the controls in the present (Table 2) and other rat [5,9] studies. Kidney function was reported to be impaired in the rat model of dehydration [30,31]. Hence, this factor could also contribute to the significantly smaller $Ae_{0-24\ h}$ of theophylline in the rat model of dehydration (Table 2). Similar explanations could be applied also to the significantly smaller $Ae_{0-24\,h}$ of 1,3-DMU in rat model of dehydration (Table 2) as shown in Figure 2B. The urine flow rate-dependent Cl_r of 1,3-DMU has also been reported in pediatric patients [8]. The Cl_r of theophylline was significantly slower in the rat model of dehydration than in the controls, however, the Cl was comparable between the two groups of rats (Table 2). This could be due to a lower contribution of Cl_r to Cl of theophylline as mentioned above (Table 2).

After intravenous and oral administration of theophylline, the *AUC* of the drug was comparable for both the control rats and rat model of dehydration (Table 2). This suggests that theophylline is rapidly and almost completely absorbed from the gastrointestinal tract and the first-pass (gastric, intestinal and hepatic) effects of theophylline are almost negligible in rats. Similar results were also reported for antipyrine, tolbutamide and warfarin [29].

In conclusion, after both intravenous and oral administration of theophylline to control rats and rat model of dehydration, the *AUC* of both theophylline and 1,3-DMU (intravenous and oral administration) and Cl_{nr} of theophylline (after intravenous administration) were comparable between the two groups of rats (Table 2). The modification of the dosage regimen of theophylline in patients with dehydration seemed not to be required, if the present rat data could be extrapolated to humans. Human studies are required to prove the above hypothesis.

Acknowledgements

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

References

1. Minors JO, McKinnon RA. CYP1A. In *Metabolic Drug Interactions*, Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M (eds). Lippincott Williams & Wilkins, A Wolters Kluwer Company: Philadelphia, PA, 2000; 61–74.
2. McManus ME, Miners JO, Gregor D, Stupans I, Birkett DJ. Theophylline metabolism by human, rabbit and rat liver microsomes and by purified forms of cytochrome P450. *J Pharm Pharmacol* 1988; **40**: 388–391.
3. Nosaka H, Nadai M, Kato M, *et al*. Effect of a newly developed ketolide antibiotic, telithromycin, on metabolism of theophylline and expression of cytochrome P450 in rats. *Life Sci* 2006; **79**: 50–56.
4. Bakar SK, Niazi S. Effect of water deprivation on aspirin disposition kinetics. *J Pharm Sci* 1983; **72**: 1030–1034.
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: Effect of glucose supplementation. *J Appl Toxicol* 2001; **21**: 123–139.
6. Agbaba D, Pokrajac M, Varagic VM, Pesic V. Dependence of the renal excretion of theophylline on its plasma concentrations and urine flow rate in asthmatic children. *J Pharm Pharmacol* 1990; **42**: 827–830.
7. Tang-Liu DD, Tozer TN, Riegelman S. Urine flow-dependence of theophylline renal clearance in man. *J Pharmacokinet Biopharm* 1982; **10**: 351–364.
8. Bonnacker I, Berdel D, Suverkrup R, von Berg A. Renal clearance of theophylline and its major metabolites: age and urine flow dependency in paediatric patients. *Eur J Clin Pharmacol* 1989; **36**: 145–150.
9. Bae SK, Kim YC, Lee JK, *et al*. Effects of water deprivation for 72 h on the pharmacokinetics of DA-7867, a new

DOI: 10.1002/bdd

oxazolidinone, in rats. *Biopharm Drug Dispos* 2006; **27**: 147–156.
10. Phipps P, Garrard CS. The pulmonary physician care. 12: Acute severe asthma in the intensive care unit. *Thorax* 2003; **58**: 81–88.
11. Kim EJ, Suh OK, Lee MG. Pharmacokinetics of intravenous theophylline in mutant Nagase analbuminemic rats. *Life Sci* 2003; **72**: 1231–1245.
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. Kwaskatsu K, Nishmura K, Kawai M, Chikuma M. Separation and determination of theophylline from paraxanthine in human serum by reversed phase high-performance liquid chromatography. *J Pharm Biomed Anal* 1989; **7**: 965–973.
14. Duggleby RG. Analysis of enzyme progress curves by nonlinear regression. *Methods Enzymol* 1995; **249**: 61–90.
15. Kim SH, Choi YM, Lee MG. Pharmacokinetics and pharmacodynamics of furosemide in protein–calorie malnutrition. *J Pharmacokinet Biopharm* 1993; **21**: 1–17.
16. 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.
17. 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.
18. Boudinot FD, Jusko WJ. Fluid shifts and other factors affecting plasma protein binding of prednisolone by equilibrium dialysis. *J Pharm Sci* 1984; **73**: 774–780.
19. Øie S, Guentert TW. Comparison of equilibrium time in dialysis experiments using spiked plasma or spiked buffer. *J Pharm Sci* 1982; **71**: 127–128.
20. 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.
21. Gibaldi M, Perrier D. *Pharmacokinetics* (2nd edn). Marcel–Dekker: New York, 1982.
22. 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.
23. Teunissen MW, Brorens IO, Geerlings JM, Breimer DD. Dose-dependent elimination of theophylline in rats. *Xenobiotica* 1985; **15**: 165–171.
24. 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.
25. 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.
26. 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.
27. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993; **10**: 1093–1095.
28. Mitruka BM, Rawnsley HM. *Clinical, Biochemical and Hematological Reference Values in Normal Experimental Animals and Normal Humans* (2nd edn). Masson: New York, 1981; 67.
29. Wilkinson GR, Shand DG. A physiological approach to hepatic drug clearance. *Clin Pharmacol Ther* 1975; **18**: 377–390.
30. Kim SH, Kwon JW, Kim WB, Lee I, Lee MG. Effects of water deprivation for 72 h on the pharmacokinetics of a new carbapenem, DA-1131, in rats. *Life Sci* 2002; **71**: 2291–2298.
31. Son IJ, Moon YJ, Lee MG, Shon YT. No effect of water deprivation for 48 h on the pharmacokinetics of intravenous tacrolimus in rats. *Res Commun Mol Pathol Pharmacol* 2000; **107**: 279–289.

DOI: 10.1002/bdd