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Pharmacokinetics of intravenous theophylline in mutant Nagase analbuminemic rats

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Abstract

It was obtained from our laboratories that the expression of hepatic microsomal cytochrome P450 (CYP) 1A2 increased approximately 3.5 times in mutant Nagase analbuminemic rats (NARs, an animal model for human familial analbuminemia), and theophylline was reported to be metabolized to 1,3-dimethyluric acid (1,3-DMU) and 1-methylxanthine (which was further metabolized to 1-methyluric acid, 1-MU, via xanthine oxidase) via CYP1A2 in rats. Hence, the pharmacokinetic parameters of theophylline, 1,3-DMU and 1-MU were compared after intravenous administration of aminophylline, 5 mg/kg as theophylline, to control Sprague-Dawley rats and NARs. In NARs, the total area under the plasma concentration-time curve from time zero to time infinity (AUC) of theophylline was significantly smaller (1040 versus 1750 μg min/ml) than that in control rats and this could be due to significantly faster renal clearance (CL_R, 1.39 versus 0.571 ml/min/kg, due to inhibition of renal reabsorption of unchanged theophylline) and nonrenal clearance (CL_{NR}, 3.36 versus 2.25 ml/min/kg, due to 3.5-fold increase in CYP1A2) than those in control rats. Based on in vitro hepatic microsomal studies, the intrinsic 1,3-DMU formation clearance was significantly faster in NARs than that in control rats (267 versus 180×10^{-6} ml/min). After intravenous administration of 1,3-DMU, the renal secretion of 1,3-DMU was inhibited in NARs. Inhibition of renal secretion or reabsorption of various compounds in NARs was also discussed.

Keywords: Theophylline; 1,3-DMU; Pharmacokinetics; Mutant Nagase analbuminemic rats; CYP1A2

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Introduction

Role of plasma protein binding in circulating blood in renal excretion of compounds (or drugs) such as mercapturic acid, phenolsulfonphthalein, and furosemide in mutant Nagase analbuminemic rats (NARs, an animal model for human familial analbuminemia, [1]) was reported [2–5]. In NARs, the percentages of intravenous dose excreted in urine as unchanged compounds (A_e) were significantly smaller and this could be due to significantly smaller plasma protein binding values than those in control rats [2–5]. For example, after 5-s intravenous administration of mercapturic acid, 10 μmol/kg, to NARs, the plasma protein binding value was 18.4% at mercapturic acid concentration of 50 μM (the value in control Sprague-Dawley rats was 80.1%) using an ultrafiltration method, and $A_{e,\ 0 \rightarrow 20\ min}$ was 25.6% of intravenous dose of the compound (the value in control rats was 57.6%) [2]. After 5-s intravenous administration of phenolsulfonphthalein, 10 μmol/kg, to NARs, the serum protein binding value was 32.0% at phenolsulfonphthalein concentration of 0.1 mmol/l (the value in control rats was 94.4%) using an ultrafiltration method, and $A_{e,\ 0 \rightarrow 6\ hr}$ was 22.4% of intravenous dose of the compound (the value in control rats was 49.2%) [3]. Similar results were also obtained from other phenolsulfonphthalein studies [4]. After 10-s intravenous administration of furosemide, 0.5 mg/kg, to NARs, the plasma protein binding value was 12.0% at furosemide concentration of 20 μg/ml (the value in control rats was 98.9%), and $A_{e,\ 0 \rightarrow 30\ min}$ was 7% of intravenous dose of the diuretic (the value in control rats was 26%) [5].

However, in NARs, the A_e values of warfarin, bumetanide, and salicylic acid were significantly or tended to be greater than those in control rats [6–8]. For example, after intravenous bolus injection of [^{14}C]warfarin, 1 mg/kg, to NARs, the plasma protein binding value was 63.6% (the value in control rats was 98.9%) when the blood was collected at 30 min after intravenous administration of [^{14}C]warfarin using an ultrafiltration method, and $A_{e,\ 0 \rightarrow 24\ hr}$ was 4.6% of intravenous dose of the anticoagulant (the value in control rat was 1.6%) [6]. After 1-min intravenous administration of bumetanide, 10 mg/kg, to NARs, the plasma protein binding value was 36.8% at bumetanide concentration of 10 μg/ml (the value in control rats was 97.6%) using an equilibrium dialysis method, and $A_{e,\ 0 \rightarrow 24\ hr}$ was 16.0% of intravenous dose of the diuretic (the value in control rats was 7.96%) [7]. After intravenous bolus injection of [^{14}C]salicylic acid, 10 mg/kg, to NARs, the plasma protein binding value was 10.9% (the value in control rats was 93.7%) when the blood was collected at 30 min after intravenous administration of the drug using an ultrafiltration method, and A_e was 59.9 $\pm$ 9.8% (the value in control rats was 45.7 $\pm$ 10.1%; they were not significantly different and this may be due to limited numbers of rats used, n = 3) [8].

It was obtained recently from our laboratories (our unpublished data) that the expression of hepatic microsomal cytochrome P450 (CYP) 1A2 was approximately 3.5-fold increased in NARs compared to control rats based on Western blot analysis. The mRNA level of CYP1A2 was also 3.5- to 4-fold increased in NARs compared to control rats based on Northern blot analysis. However, the expression of CYP2E1 and CYP3A23 was not changed in NARs. It was also reported [9] that theophylline was metabolized to 1,3-dimethyluric acid (1,3-DMU) via CYP1A2 and CYP2E1 and to 1-methylxanthine via CYP1A2 which was further metabolized to 1-methyluric acid (1-MU) via xanthine oxidase in rats. Hence, it could be expected that the pharmacokinetic parameters of theophylline and its metabolites, 1,3-DMU and 1-MU, could be changed in NARs.

The above mentioned six compounds have high plasma protein binding values in control rats: 80.1% for mercapturic acid [2], 94.4% for phenolsulfonphthalein [3], 98.9% for furosemide [5] and

warfarin [6], 97.6% for bumetanide [7] and 93.7% for salicylic acid [8]. However, the role of plasma protein binding in circulating blood in renal excretion of compounds having low plasma protein binding values in NARs has not been published to date. The plasma protein binding values of theophylline and 1,3-DMU in control rats were 26.9 and 20.7%, respectively, using an equilibrium dialysis technique. Although the above mentioned compounds are secreted [2–4] or reabsorbed [6] in rat renal tubules, the changes in renal excretion of compounds which are secreted or reabsorbed from renal tubules have not been thoroughly studied in NARs. The pharmacokinetic parameters of the compounds were reported to be changed in NARs [2–8], however, such changes could not be explained by the changes in CYP isozymes in rats. The purpose of this study was to report the pharmacokinetic changes of theophylline and its metabolites, 1,3-DMU and 1-MU, after intravenous administration of theophylline to NARs with respect to changes in CYP1A2 in rats. The inhibition of renal tubular secretion or renal tubular reabsorption of the compounds in NARs was also discussed.

Materials and methods

Chemicals

Aminophylline intravenous solution (250 mg/10 ml ampoule) was a product from Daewon Pharmaceutical Company (Seoul, Korea). 1,3-DMU, 1-MU, β-hydroxyethyltheophylline [the internal standard of high-performance liquid chromatographic (HPLC) assay] and reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) were supplied by Sigma Chemical Company (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade and, therefore, were used without further purification.

Rats

Male Sprague-Dawley rats (control rats, weighing 290 – 320 g) and NARs (weighing 280 – 320 g) of 9 weeks of age were purchased from Japan SLC Inc. (Hamamatsu, Japan). The protocol of animal study was approved by Animal Care and Use Committee of College of Pharmacy, Seoul National University (Seoul, Korea). Animals were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at temperature between 20 and 23 °C with 12 hr light and dark cycles and a relative humidity of 50%. Animals were individually housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air and water ad libitum.

Isolation of hepatic microsomal protein

Hepatic microsomal fractions of control rats (n = 6) and NARs (n = 6) were prepared by differential centrifugation, washed in pyrophosphate buffer (pH 7.4) and stored in 100 mM Tris-acetate buffer (pH 7.4) containing 1 mM sodium ethylenediamine tetraacetate (EDTA). Microsomal preparations were stored in a − 70 °C freezer until use. Protein content was determined according to the reported methods [10].

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

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 formation of 1,3-DMU from theophylline were determined after incubating the above microsomal fraction (equivalent to 1 mg protein) in 100 mM phosphate buffer (pH 7.4). Theophylline was added to the microsomal fraction having theophylline concentrations of 0.5, 1, 2, 5, 7.5 and 10 mM. The reaction was initiated by adding 1.2 mM NADPH and was conducted in a water-bath shaker kept at 37°C and at a rate of 50 oscillations per min (opm) for 30 min. The reaction was terminated by adding 0.1 ml of ice-cold 8% $ZnSO_4$. The precipitated proteins were removed by centrifugation at 15000 *g* for 10 min and the supernatant was stored in a −70°C freezer until HPLC analysis of 1,3-DMU [11]. The kinetic constants (K_m and V_{max}) for the formation of 1,3-DMU from theophylline were calculated using the Lineweaver-Burk plot [12] by linear regression and the method of least squares. Intrinsic 1,3-DMU formation clearance (CL_{int}) was calculated by dividing V_{max} by K_m. The same experiment was again performed (n = 5 for each rat).

Pretreatment of rats

In the early morning, the carotid artery and the jugular vein of each control rat and NAR were cannulated with polyethylene tubing (Clay Adams, Parsippany, NJ) while the animals were under light ether anesthesia. Both cannulas were exteriorized to the dorsal side of the neck and terminated with long silastic tubing (Dow Corning, Midland, MI). Both silastic tubings were inserted into a wire sheath to allow free movement of the rat. The exposed areas were surgically sutured. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, Korea) and was allowed to recover from the anesthesia for 4 – 5 hr before commencement of the experiment. They were not restrained during the study. Heparinized 0.9% NaCl-injectable solution (20 units/ml), 0.3 ml, was used to flush each cannula to prevent blood from clotting.

Intravenous study

Aminophylline, 5 mg/kg as theophylline diluted in 0.9% NaCl-injectable solution, was infused over 1-min via the jugular vein of control rats (n = 13) and NARs (n = 13). The injection volume was approximately 1 ml. Approximately, a 0.12-ml aliquot of blood 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 and 300 min after intravenous administration. The blood samples were centrifuged immediately and a 50-μl aliquot of each plasma sample was stored in a −70°C freezer until HPLC analysis of theophylline and 1,3-DMU [13]. The heparinized 0.9% NaCl-injectable solution, 0.3 ml, was used to flush the cannula immediately after each blood sampling. At the end of 24 hr, as much blood as possible was collected and plasma was stored in a −70°C freezer until the measurement of protein binding. Each rat was exsanguinated and sacrificed by cervical dislocation. At the same time, the metabolic cage was rinsed with 10 ml of distilled water. The rinsings were combined with the 24-hr urine sample. After measuring the exact volume of combined urine, an aliquot of each urine sample was stored in a −70°C freezer until the analysis of theophylline, 1,3-DMU and 1-MU [13]. Similar experiment was also performed with 1,3-DMU. 1,3-DMU (the 1,3-DMU powder was dissolved in distilled water with minimum amount of NaOH), 5 mg/

kg, was infused over 1-min to control rats (n = 5) and NARs (n = 3). Total injection volume was approximately 1 ml.

Plasma protein binding study

The binding of theophylline to plasma protein was determined using an equilibrium dialysis technique [14]. One ml of plasma was dialyzed against 1 ml of isotonic Sørensen phosphate buffer (pH 7.4) containing 3.0% dextran ('the buffer') to minimize volume shift with a 1 ml dialysis cell (Fisher Scientific, Fair Lawn, NJ) and Spectral/Por 4 membrane (molecular weight cutoff of 12000 – 14000; Spectrum Medical Industries Inc., Los Angeles, CA). Theophylline was spiked into the plasma side at theophylline concentration of 5 μg/ml. The spiked dialysis cell was incubated for 24-hr in a water-bath shaker kept at 37°C and at a rate of 50 opm [14]. Similar experiment was also performed with 1,3-DMU at 1,3-DMU concentration of 1 μg/ml. Equilibrium of theophylline between rat plasma and 'the buffer' in the dialysis cell was found to be established after 4 hr incubation and the binding value was not influenced for up to 24 hr incubation at an initial theophylline concentration of 5 μg/ml.

Biliary excretion study

In the early morning, the jugular vein and the bile duct of each control rat and NAR were catheterized with polyethylene tubing (Clay Adams) while each rat was lightly anesthetized with ether. Aminophylline, 5 mg/kg as theophylline diluted in 0.9% NaCl-injectable solution, was infused over 1-min via the jugular vein of control rats (n = 4) and NARs (n = 4). The injection volume was approximately 1 ml. Each rat was kept in supine position during the entire experiment by tying four feet on a plate. Bile juice was collected between 0–8 hr. After measuring the exact volume of bile juice, an aliquot of bile sample was stored in a −70°C freezer until HPLC analysis of theophylline and 1,3-DMU [13].

HPLC analysis of theophylline, 1,3-DMU and/or 1-MU

The concentrations of theophylline and its metabolites in the above biological samples were analyzed by the reported HPLC method [13]; a 300-μl aliquot of acetonitrile (containing 2 μg/ml of the internal standard, β-hydroxyethyl theophylline) was added to a 50-μl aliquot of biological sample. After vortex-mixing and centrifugation, a 300-μl aliquot of supernatant was evaporated under N_2 gas. The residue was reconstituted with a 100-μl aliquot of the mobile phase and a 50-μl aliquot of the supernatant was injected directly onto the 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 monitered by a UV detector set at 280 nm. The detection limits of theophylline and 1,3-DMU in rat plasma were 100 and 50 ng/ml, respectively, and the detection limits of theophylline, 1,3-DMU and 1-MU in rat urine were 100, 100 and 200 ng/ml, respectively. The coefficients of variation of the assay (within- and between-day) were generally low (below 8.90%).

The concentrations of 1.3-DMU in the microsomal samples (for the measurement of V_{max}, K_m and CL_{int}) were analyzed by a slight modification of the reported method [11]; a 50-μl aliquot of the supernatant was injected directly onto a reversed-phase (C_{18}) column (4.6 mm, i.d. × 150 mm, *l.*; particle size, 5 μm; YMC, Kyoto, Japan). The mobile phase, 10 mM acetate buffer (pH 4.5): acetonitrile

(95:5, v/v), was run at a flow rate of 1.0 ml/min and the column effluent was monitered by a UV detector set at 280 nm. The detection limit of 1,3-DMU was 100 ng/ml. The coefficients of variation of the assay (within-day) were generally low (below 3.56%).

Pharmacokinetic analysis

The total area under the plasma concentration-time curve from time zero to time infinity (AUC) was calculated using the trapezoidal rule-extrapolation method; this method employed the logarithmic trapezoidal rule [15] for the calculation of area during the declining plasma-level phase and the linear trapezoidal rule for the rising plasma-level phase. The area from the last data point to time infinity was estimated by dividing the last measured concentration by the terminal rate constant.

Standard methods [16] were used to calculate the following pharmacokinetic parameters: the time-averaged total body clearance (CL), area under the first moment of plasma concentration-time curve (AUMC), mean residence time (MRT), apparent volume of distribution at steady-state (Vss), and time-averaged renal (CL_R) and nonrenal (CL_{NR}) clearances [17];

$$CL = Dose/AUC \tag{1}$$

$$AUMC = \int_0^\infty t \times C_p dt \tag{2}$$

$$MRT = AUMC/AUC \tag{3}$$

$$V_{SS} = CL \times MRT \tag{4}$$

$$CL_R = Ae_{0\rightarrow 24\ hr}/AUC \tag{5}$$

$$CL_{NR} = CL - CL_R \tag{6}$$

where C_p is the plasma concentration of theophylline or 1,3-DMU at time t and $Ae_{0\rightarrow 24\ hr}$ is the total amount of unchanged theophylline or 1,3-DMU excreted in urine for up to 24 hr. The AUC instead of $AUC_{0\rightarrow 24\ hr}$ was employed in the calculation of CL_R of theophylline or 1,3-DMU, since, the contribution of $AUC_{24\ hr\rightarrow\infty}$ to AUC were less than 0.191% for both theophylline and 1,3-DMU in both rats.

The mean values of each clearance [18], Vss [19] and terminal half-life [20] were calculated using the harmonic mean method.

Statistical analysis

The differences between the two means for unpaired data were assessed by t-test. A P value of less than 0.05 was considered to be statistically significant. All results are expressed as mean $\pm$ SD.

Results

Measurement of V_{max}, K_m and CL_{int} for the formation of 1,3-DMU from theophylline in hepatic microsomal fraction

The V_{max}, K_m and CL_{int} for the formation of 1,3-DMU from theophylline in hepatic microsomal fraction in control rats and NARs are listed in Table 1. The V_{max} values were not significantly different between two groups of rats; however, the K_m in NARs was significantly lower (23.4% decrease) than that in control rats. Hence, the CL_{int} in NARs was significantly faster (48.3% increase) than that in control rats suggesting that the clearance of theophylline to form 1,3-DMU could be faster in NARs than that in control rats. This experiment was performed again (n = 5 for each rat) and the results were very similar to those in Table 1. The V_{max}, K_m and CL_{int} values were 450 ± 24.9 versus 473 ± 14.3 pmol/mg/min (P = 0.116429), 3.21 ± 0.702 versus 2.52 ± 0.301 mM (P = 0.077264) and 144 ± 23.0 versus 190 ± 18.9 10^{-6} ml/min (P = 0.009229), respectively. The above data indicated that the affinity of theophylline for the enzyme could increase in NARs.

Intravenous study

Many investigators have reported the dose-dependent metabolic disposition of theophylline in humans [21,22] and in rats [9]. Therefore, theophylline dose, 5 mg/kg, which has been reported to be in the dosing range demonstrating linear pharmacokinetics in rats [9], was administered intravenously to rats. The mean arterial plasma concentration-time profiles of theophylline and 1,3-DMU after intravenous administration of theophylline, 5 mg/kg, to control rats (n = 13) and NARs (n = 13) are shown in Fig. 1, and relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration of theophylline, the plasma concentrations of theophylline declined in a polyexponential fashion for both groups of rats (Fig. 1) with mean terminal half-lives of 140 min for control rats and 153 min for NARs (Table 2). The half-lives were not significantly different between two groups of rats. The MRTs were also not significantly different between two groups of rats (Table 2). However, the AUC of theophylline in NARs was significantly smaller (40.6% decrease) than that in control rats due to significantly faster CL of theophylline (68.2% increase) in the rats (Table 2). The faster CL of theophylline in NARs was due to significantly faster CL_R (143% increase) and CL_{NR} (49.3% increase) of theophylline than those in control rats (Table 2).

The faster CL_R in NARs could be due to significantly greater 24-hr urinary excretion of unchanged theophylline ($A_{e,\ Theo,\ 0 \rightarrow 24\ hr}$, 36.2% increase) and significantly smaller AUC of theophylline than

Table 1
Mean (± SD) V_{max}, K_m and CL_{int} for the formation of 1,3-DMU from theophylline in hepatic microsomes of control rats and NARs

Parameters	Control rats (n = 6)	NARs (n = 6)
V_{max} (pmol/mg/min)	610 ± 142	688 ± 84.2
K_m (mM)	3.37 ± 0.571	2.58 ± 0.190*
CL_{int} (10^{-6} ml/min)	180 ± 22.2	267 ± 37.5**

* P < 0.01.
** P < 0.001 compared with control.

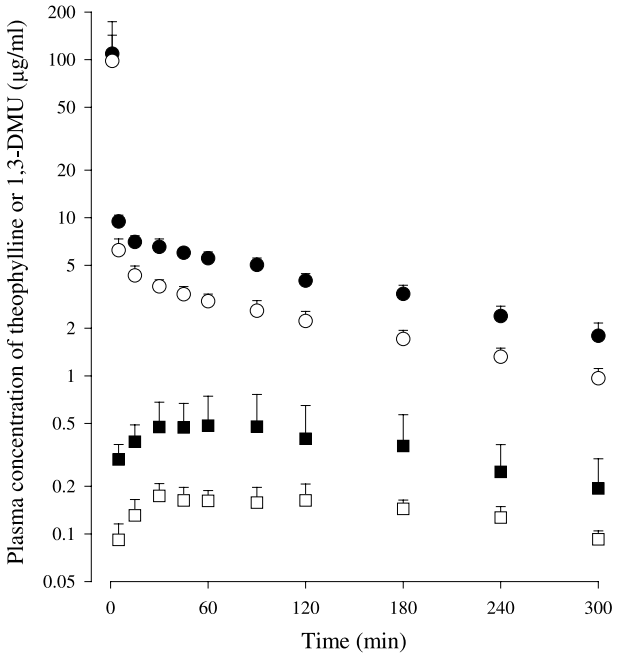

Fig. 1. Mean arterial plasma concentration-time profiles of theophylline (circle) or 1,3-DMU (rectangle) after 1-min intravenous infusion of aminophylline, 5 mg/kg as theophylline, to control rats (closed symbol, n = 13) and mutant Nagase analbuminemic rats (open symbol, n = 13). Vertical bars represent SD.

those in control rats (Table 2). The role of protein binding in circulating blood in renal excretion of compound having low plasma protein binding, theophylline, in NARs was also observed. Considering the plasma protein binding and CL_R of theophylline (Table 2), the estimated CL_R values for unbound (free in plasma) theophylline were 0.781 and 1.58 ml/min/kg for control rats and NARs, respectively; the values were considerably smaller than the reported glomerular filtration rate (GFR), 5.24 ml/min/kg, in rats [23]. The above data indicated that theophylline was reabsorbed in the renal tubules for both groups of rats. The estimated CL_R value for unbound theophylline in NARs was considerably faster (102% increase) than that in control rats suggesting that the renal reabsorption of theophylline was inhibited in NARs. Renal tubular reabsorption of theophylline was also reported in other rat studies [24]. Considering CL_R of theophylline based on plasma data (Table 2) and reported kidney blood flow of 36.8 ml/min/kg [23] and hematocrit of approximately 45% [25] in rats, the estimated renal extraction ratios (CL_R/renal plasma flow, only for urinary excretion of unchanged drug) of theophylline were 2.82 and 6.87% for control rats and NARs, respectively. The above data indicated that theophylline was extracted poorly via the kidney for both groups of rats and renal extraction of unchanged theophylline increased in NARs. Therefore, the faster CL_R of theophylline in NARs could be due to increase in renal extraction ratio of theophylline mainly because of decrease in renal reabsorption of theophylline. In NARs, the significantly greater $A_{e,\ Theo,\ 0 \rightarrow 24\ hr}$ (% of dose) could be at least partly due to significant increase in free fraction of theophylline in plasma (Table 2).

The CL_{NR} of theophylline was also significantly faster in NARs than that in control rats (Table 2). This could be due to 3.5- to 4-fold increase in CYP1A2 in NARs since theophylline was metabolized

Table 2
Mean (± SD) pharmacokinetic parameters of theophylline and its metabolites after 1-min intravenous infusion of aminophylline, 5 mg/kg as theophylline, to control rats and NARs

Parameters	Control rats (n = 13)	NARs (n = 13)
Theophylline		
Terminal half-life (min)	140 ± 20.3	153 ± 17.8
AUC (μg min/ml)	1750 ± 251	1040 ± 128***
MRT (min)	183 ± 25.6	182 ± 26.0
CL (ml/min/kg)	2.86 ± 0.546	4.81 ± 0.596***
CL_R (ml/min/kg)	0.571 ± 0.343	1.39 ± 0.399***
CL_{NR} (ml/min/kg)	2.25 ± 0.351	3.36 ± 0.363***
V_{SS} (ml/kg)	523 ± 60.5	860 ± 152***
$A_{e,\ Theo,\ 0 \rightarrow 24\ hr}$ (μg)	337 ± 111	444 ± 78.2**
$A_{e,\ Theo,\ 0 \rightarrow 24\ hr}$ (% of dose)	22.1 ± 7.09	30.1 ± 5.28**
Protein binding (%)	26.9 ± 5.24	12.0 ± 1.83***
1,3-DMU		
Terminal half-life (min)	144 ± 48.5	174 ± 33.9
AUC (μg min/ml)	152 ± 71.9	66.1 ± 8.84**
$A_{e,\ 1,3\text{-}DMU,\ 0 \rightarrow 24\ hr}$ (μg)[a]	324 ± 87.4	228 ± 94.8*
$A_{e,\ 1,3\text{-}DMU,\ 0 \rightarrow 24\ hr}$ (% of dose)[a]	21.2 ± 5.73	15.5 ± 6.76*
1-MU		
$A_{e,\ 1\text{-}MU,\ 0 \rightarrow 24\ hr}$ (μg)[a]	226 ± 59.3	125 ± 48.0***
$A_{e,\ 1\text{-}MU,\ 0 \rightarrow 24\ hr}$ (% of dose)[a]	14.8 ± 3.95	8.47 ± 3.36***

[a] Expressed in terms of theophylline.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$ compared with control.

to 1,3-DMU via CYP1A2 and CYP2E1 and to 1-MU via CYP1A2 in rats [9]. CYP2E1 was not changed in NARs. The contribution of biliary excretion of unchanged theophylline to CL_{NR} of theophylline was almost negligible; the percentages of intravenous dose of theophylline excreted in 8-hr bile juice as unchanged theophylline were 2.29 ± 0.292 and 2.77 ± 1.13% for control rats and NARs, respectively (the corresponding values for 1,3-DMU were 7.42 ± 3.44 and 7.78 ± 2.65%). The above data suggested that the CL_{NR} values of theophylline listed in Table 2 could represent metabolic clearances of theophylline. Hence, it could be concluded that the nonrenal metabolism of theophylline was faster in NARs than that in control rats. The faster CL_{NR} of theophylline in NARs could be supported by significantly faster intrinsic 1,3-DMU formation clearance (CL_{int}) in NARs (Table 1). The contributions of CL_R to CL of theophylline were 20.0 and 28.9% for control rats and NARs, respectively (Table 2).

In NARs, the AUC of theophylline was significantly smaller (40.6% decrease) and hence the CL of theophylline was significantly faster (68.2% increase) than those in control rats (Table 2). Similar results were also reported [26] in spontaneously hyperlipidemic rats with hypoalbuminemia (HLRs, which had approximately one half the serum albumin concentration of control Sprague-Dawley rats). After administration of theophylline, 10 mg/kg, to HLRs, the AUC of theophylline was significantly smaller (36.0% decrease) and the CL of theophylline was significantly faster (57.1% increase) than those in

control rats [26]. Although the changes in AUC and CL of theophylline were similar between HLRs [26] and NARs (Table 2), the explanation for the difference was not the same. In HLRs, the enzymatic activities and apoprotein expression levels of CYP1A2 decreased significantly compared with the control [26]. Hence, the changes of AUC and CL of theophylline in HLRs were explained by significantly larger volume of distribution of theopylline due to significant increase in unbound fraction of theophylline in HLRs [26].

The V_{SS} of theophylline was significantly larger (64.4% increase) in NARs and this could be due to significant increase in unbound fraction of theophylline in NARs (Table 2). It was also reported that in NARs, tissue distribution (volume of distribution) of mercapturic acid [2], phenolsulfonphthalein [3,4], furosemide [5], salicylic acid [8], taurocholic acid (plasma protein binding values were 76 and 24% for control rats and NARs, respectively) [27], phenytoin (plasma protein binding values were 92.8 and 60.3% for control rats and NARs, respectively) [28] and warfarin [6] increased significantly compared with those in control rats due to significant increase in unbound fraction in the rats. However, tissue distribution of bumetanide was comparable between two groups of rats although the unbound fraction increased significantly in NARs [7]. The tissue distribution and protein binding of cisplatin were reported to be comparable between two groups of rats [29].

The mean arterial plasma concentration-time profiles of 1,3-DMU after intravenous administration of 1,3-DMU, 5 mg/kg, to control rats (n = 5) and NARs (n = 3) are shown in Fig. 2, and relevant pharmacokinetic parameters are listed in Table 3. After intravenous administration of 1,3-DMU, the plasma concentrations of 1,3-DMU declined in a polyexponential fashion for both groups of rats (Fig.

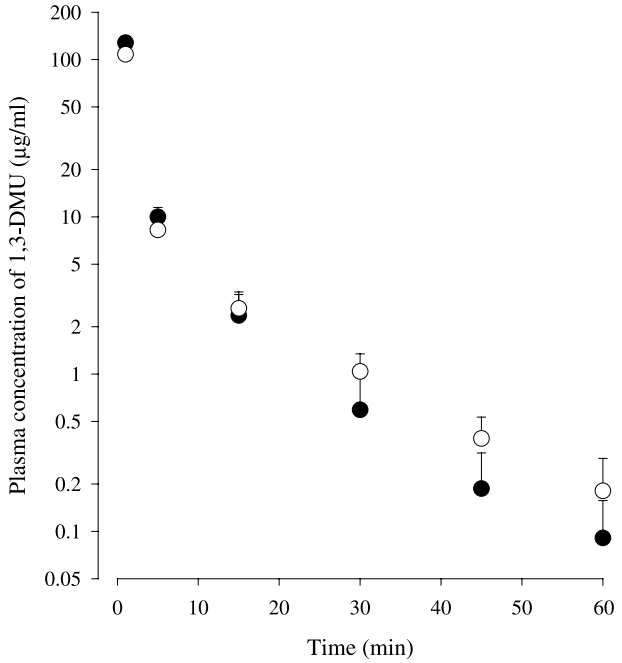

Fig. 2. Mean arterial plasma concentration-time profiles of 1,3-DMU after 1-min intravenous infusion of 1,3-DMU, 5 mg/kg, to control rats (closed symbol, n = 5) and mutant Nagase analbuminemic rats (open symbol, n = 3). Vertical bars represent SD.

Table 3
Mean (± SD) pharmacokinetic parameters of 1,3-DMU after 1-min intravenous infusion of 1,3-DMU, 5 mg/kg, to control rats and NARs

Parameters	Control rats (n = 5)	NARs (n = 3)
Terminal half-life (min)	10.5 ± 3.83	12.8 ± 0.656
AUC (μg min/ml)	329 ± 23.2	301 ± 36.6
MRT (min)	5.15 ± 1.47	7.38 ± 2.11
CL (ml/min/kg)	15.2 ± 1.08	16.6 ± 1.90
CL_R (ml/min/kg)	12.9 ± 1.38	9.89 ± 2.89
CL_{NR} (ml/min/kg)	1.95 ± 0.969	5.16 ± 4.05
V_{SS} (ml/kg)	75.0 ± 16.9	116 ± 46.3
$A_{e,\ 1,3\text{-DMU},\ 0 \rightarrow 24\ hr}$ (μg)[a]	1330 ± 115	802 ± 257**
$A_{e,\ 1,3\text{-DMU},\ 0 \rightarrow 24\ hr}$ (% of dose)[a]	77.6 ± 5.19	57.7 ± 17.6*
Protein binding (%)	20.7 ± 6.03	21.0 ± 1.61

[a] Expressed in terms of theophylline.
* $P < 0.05$.
** $P < 0.01$ compared with control.

2). The terminal half-lives of 1,3-DMU were very short; the mean values were 10.5 min for control rats and 12.8 min for NARs (Table 3). As expected from very short terminal half-life, 1,3-DMU was eliminated fast from rats; the CLs of 1,3-DMU were 5.31 and 3.45 times faster (Table 3) than those of theophylline (Table 2) in control rats and NARs, respectively. Considering the plasma protein binding and CL_R of 1,3-DMU (Table 3), the estimated CL_R values for unbound 1,3-DMU were 16.3 and 12.5 ml/min/kg for control rats and NARs, respectively; the values were considerably greater than the reported GFR, 5.24 ml/min/kg, in rats [23]. The above data indicated that 1,3-DMU was secreted in the renal tubules for both groups of rats, but the renal secretion of 1,3-DMU was inhibited in NARs. Renal tubular secretion of 1,3-DMU was also reported in other rat studies [24]. Considering the CL_R of 1,3-DMU based on plasma data (Table 3) and reported kidney blood flow of 36.8 ml/min/kg [23] and hematocrit of approximately 45% [25] in rats, the estimated renal extraction ratios (only for urinary excretion of unchanged drug) of 1,3-DMU were 63.7 and 48.9% for control rats and NARs, respectively. The values were considerably greater than those of theophylline as mentioned earlier. The above data indicated that 1,3-DMU was extracted considerably via kidney for both groups of rats. In NARs, $A_{e,\ 1,3\text{-DMU},\ 0 \rightarrow 24\ hr}$ was significantly smaller (25.6% decrease) and this could be due to inhibition of renal tubular secretion and decrease in renal extraction ratio of 1,3-DMU. In NARs, the significant decrease in $A_{e,\ 1,3\text{-DMU},\ 0 \rightarrow 24\ hr}$ (% of dose) was not due to decrease in free fraction of 1,3-DMU in plasma; the unbound fractions in plasma were 79.3 and 79.0% for control rats and NARs, respectively (Table 3).

Unexpectedly, the AUCs of 1,3-DMU were comparable between control rats and NARs after intravenous administration of 1,3-DMU (Table 3). Similar results were also reported for cisplatin (protein binding values were 52.9–59.6 and 56.8–68.0% for control rats and NARs, respectively) [29], bilirubin [30], inulin (negligible protein binding) [2] and [^{14}C]antipyrine (negligible protein binding) and [^{14}C]urea [8]. However, in NARs, the AUC (or $AUC_{0\text{-}t}$) values of mercapturic acid (44.9% decrease) [2], phenolsulfonphthalein (91.3% decrease [3] and 81.4% decrease [4]), sulfobromophthalein (40.6% decrease) [31], taurocholic acid (56.4% decrease) [27], furosemide (61.5% decrease) [5], warfarin (98.4 and 95.4% decrease at warfarin doses of 1 and 40 mg/kg, respectively) [6], bumetanide (59.1%

decrease) [7], phenytoin (50% decrease) [28], salicylic acid (83.0 and 17.6% decrease at salicylic acid doses of 10 and 173 mg/kg, respectively [8], and 68.9% decrease at salicylic acid dose of 100 μCi/kg [32]), azosemide (81.9% decrease, the protein binding values were 97.9 and 84.6% for control rats and NARs, respectively) (our unpublished data) and torasemide (88.8% decrease, the protein binding values were 94.1 and 23.3% for control rats and NARs, respectively) [33] decreased significantly compared with those in control rats. Although the exact reasons are not clear, the differences in AUC (or CL) of compounds between control rats and NARs could not be due to the differences in acidity or basicity of compounds or in pharmacokinetic parameters (such as volume of distribution, fraction of dose excreted via kidney, protein binding, etc). The AUC differences could depend on compounds and at least partly due to doses of compounds; the AUC difference increased with decreasing doses. For example, the AUCs of bilirubin were not significantly different between two groups of rats at trace amount, but were significantly different at loading amount [30]. The AUC differences of warfarin [6] and salicylic acid [8] were greater at 1 mg/kg than those at 40 mg/kg [6] and at 10 mg/kg than those at 173 mg/kg [8], respectively. The contributions of CL_R to CL of 1,3-DMU were 84.9 and 59.6% for control rats and NARs, respectively (Table 3).

The mean arterial plasma concentration-time profiles of 1,3-DMU after intravenous administration of theophylline, 5 mg/kg, to control rat and NARs are also shown in Fig. 1 and the relevant pharmacokinetic parameters are also listed in Table 2. After intravenous administration of theophylline, the formation of 1,3-DMU was fast; 1,3-DMU was detected in plasma from the first blood sampling time (1 min) as shown in Fig. 1. After intravenous administration of 1,3-DMU to both groups of rats, the plasma concentrations of 1,3-DMU declined fast (due to fast CL) with mean terminal half life of 10.5–12.8 min (Table 3) and 1,3-DMU were detected in plasma only up to 60 min (Fig. 2). However, after intravenous administration of theophylline, plasma concentrations of 1,3-DMU were 'almost' constant during 30–300 min (Fig. 1) and mean terminal half-lives were 140–153 min (Table 2). The above data indicated that 1,3-DMU was continuously formed from theophylline after intravenous administration of theophylline for both groups of rats. After intravenous administration of theophylline to NARs, the AUC of 1,3-DMU (56.5% decrease) and $A_{e,\ 1,3\text{-DMU},\ 0 \rightarrow 24\ hr}$ (26.9% decrease) were significantly smaller than those in control rats (Table 2). The $A_{e,\ 1\text{-MU},\ 0 \rightarrow 24\ hr}$ was also significantly smaller (42.8% decrease) in NARs (Table 2).

Discussion

It has been reported that the expression of CYP1A2 was decreased in HLRs, which had approximately one half the serum albumin concentration of the Sprague-Dawley rats [26]. In the present study, CYP1A2 was induced in NARs. The plasma albumin level in NARs used in the present study is approximately one thousandth of control animals [34]. Hence, CYP1A2 expression in NARs appeared to be completely different from that in HLRs presumably because of the difference in pathophysiology (e.g. metabolic function).

After intravenous administration of theophylline to NARs, the pharmacokinetic parameters of theophylline were changed compared with control rats; while the AUC was significantly smaller, CL, CL_R and CL_{NR} were significantly faster and V_{SS} and $A_{e,\ Theo,\ 0 \rightarrow 24\ hr}$ (amount and percentages of intravenous dose) were significantly greater than those in control rats (Table 2). In NARs, significantly faster CL_R of theophylline was due to inhibition of renal tubular reabsorption and significantly faster

CL_{NR} of theophylline was due to 3.5- to 4-fold increase in CYP1A2 in the rats. In NARs, the AUC of 1,3-DMU, $A_{e,\ 1,3\text{-DMU},\ 0 \rightarrow 24\ hr}$ and $A_{e,\ 1\text{-MU},\ 0 \rightarrow 24\ hr}$ were significantly smaller than those in control rats (Table 2).

In the present study, the renal reabsorption of theophylline and renal secretion of 1,3-DMU were inhibited in NARs. This provoked us to elucidate the renal excretion mechanism of compounds (or drugs) in NARs and the following results were obtained. The renal secretion of the following compounds was inhibited in NARs. First, the estimated renal clearances of mercapturic acid based on unbound (free) fraction in plasma were 72.9 and 14.3 ml/min/kg for control rats and NARs, respectively [2]. The values for both control rats and NARs were greater than the reported GFR value, 5.24 ml/min/kg, in control rats [23], indicating that mercapturic acid is secreted in the renal tubule for both groups of rats. However, the value in NARs was considerably slower (80.4% decrease) than that in control rats. Second, the estimated unbound renal clearances of phenolsulfonphthalein were 41.3 and 17.8 ml/min/kg for control rats and NARs, respectively, using protein binding value at phenolsulfonphthalein concentration of 0.1 mmol/l [3]. The values in both control rats and NARs were also greater than the reported GFR value [23], indicating that phenolsulfonphthalein is secreted in the renal tubule for both groups of rats. However, the value in NARs was considerably slower (56.9% decrease) than that in control rats. Third, the estimated unbound renal clearances of furosemde were 222 and 1.94 ml/min/kg for control rats and NARs, respectively [5]. The value in NARs was considerably slower (99.1% decrease) than that in control rats. Above data indicated that furosemide is mainly secreted in renal tubule for control rats and reabsorbed in the renal tubule for NARs. It was reported [35] that furosemide is reabsorbed at distal tubule and collecting duct in rats. Fourth, the estimated unbound renal clearance values of bumetanide were 11.5 and 2.36 ml/min/kg for control rats and NARs, respectively [7]. The value in NARs was considerably slower (79.5% decrease) in control rats. Above data indicated that bumetanide is mainly secreted in renal tubule for control rats and reabsorbed in renal tubule for NARs. It was reported [36] that bumetanide was reabsorbed in renal tubule in the rabbits, and the renal clearance of bumetanide was dependent on urine flow rate. Fifth, the estimated unbound renal clearances of salicylic acid were 8.70 and 4.73 ml/min/kg for control rats and NARs, respectively [8]. The value in NARs was considerably slower (45.6% decrease) than that in control rats. Above data indicated that salicylic acid is mainly secreted for control rats and filtered by glomerulus for NARs. Finally, in the present 1,3-DMU study (Table 3), the renal secretion of 1,3-DMU was inhibited in NARs as mentioned earlier.

Interestingly, the renal reabsorption of the following compounds was also inhibited in NARs. First, the estimated unbound renal clearance values of warfarin were 0.308 and 1.63 ml/min/kg for control rats and NARs, respectively [6]. The above data indicated that warfarin is reabsorbed in renal tubule for both groups of rats. However, the value in NARs was considerably faster (429% increase) than that in control rats indicating that reabsorption of warfarin was inhibited in NARs. Second, in the present study, the renal reabsorption of theophylline was inhibited in NARs as mentioned earlier. More studies are required which renal transport system(s) could be inhibited in NARs.

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