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Faster Clearance of Omeprazole in Mutant Nagase Analbuminemic Rats: Possible Roles of Increased Protein Expression of Hepatic CYP1A2 and Lower Plasma Protein Binding

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ABSTRACT: It is well known that there are various changes in the expression of hepatic and intestinal CYPs in mutant Nagase analbuminemic rats (NARs). It has been reported that the protein expression of hepatic CYP1A2 was increased, whereas that of hepatic CYP3A1 was not altered, and it was also found that the protein expression of the intestinal CYP1A subfamily significantly increased in NARs from our other study. In addition, in this study additional information about CYP changes in NARs was obtained; the protein expression of the hepatic CYP2D subfamily was not altered, but that of the intestinal CYP3A subfamily increased in NARs. Because omeprazole is metabolized via hepatic CYP1A1/2, 2D1, 3A1/2 in rats, it could be expected that the pharmacokinetics of omeprazole would be altered in NARs. After intravenous administration of omeprazole to NARs, the Cl_{nr} was significantly faster than in the controls (110 versus 46.6 ml/min/kg), and this could be due to an increase in hepatic metabolism caused by a greater hepatic CYP1A2 level in addition to greater free fractions of the drug in NARs. After oral administration of omeprazole to NARs, the AUC was also significantly smaller (80.1% decrease) and *F* was decreased in NARs. This could be primarily due to increased hepatic and intestinal metabolism caused by greater hepatic CYP1A2 and intestinal CYP1A and 3A levels. In particular, the smaller *F* could mainly result from greater hepatic and intestinal first-pass effect in NARs than in the controls.
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Key words: omeprazole; NARs; pharmacokinetics; hepatic CYP1A2 and intestinal CYP1A and 3A subfamilies

Introduction

Omeprazole, 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl sulfoxide]-1*H*-benzimidazole, is a gastric parietal cell proton pump inhibitor. The drug has greater antisecretory activity than histamine H_2-receptor antagonists and has been used widely in the treatment of peptic ulcer, efflux oesophagitis and Zollinger––Ellison syndrome [1]. Lee *et al.* [2] reported that omeprazole is metabolized primarily via hepatic microsomal cytochrome P450 (CYP) 1A1/2, 2D1 and 3A1/2 (not via CYP2B1/2, 2C11 and 2E1) in male Sprague–Dawley rats.

Kim *et al.* [3] reported that in mutant Nagase analbuminemic rats (NARs; an animal model for human familial analbuminemia), the protein expression and mRNA level of hepatic CYP3A1, and the mRNA level of hepatic CYP3A2 were not

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altered, but the protein expression and mRNA level of hepatic CYP1A2 increased compared with controls. The protein expression of the intestinal CYP1A subfamily was found to be increased significantly (98.2% increase) in NARs compared with the controls (our unpublished data). However, no studies on the changes in the protein expression of hepatic CYP2D subfamily and intestinal CYP3A subfamily in NARs have yet been reported. Kaminsky and Fasco [4] reported that CYP1A and 3A subfamilies are abundantly expressed but CYP2D subfamily is expressed at a very low level in the rats' intestine.

Watanabe *et al*. [5] reported that omeprazole is primarily metabolized in the liver and is a drug with an intermediate hepatic extraction ratio (hepatic first-pass effect of approximately 59%) in rats. Thus, the hepatic clearance of omeprazole depends on the hepatic intrinsic clearance (Cl_{int}) for the disappearance of omeprazole, free (unbound to plasma proteins) fractions of omeprazole in plasma and hepatic blood flow rate ($Q_{\mathrm{H,B}}$) [6]. In the preliminary study, the plasma protein binding of omeprazole in NARs was significantly smaller than in the controls. Therefore, it could be expected that the pharmacokinetic parameters of omeprazole would be changed in NARs due to an increase in the protein expression and the mRNA level of hepatic CYP1A2 [3], an increase in protein expression of intestinal CYP1A subfamily and an increase in the free fractions (decrease in plasma protein binding) of the drug in plasma compared with the controls.

Lamers *et al*. [7] reported that hypoalbuminemia is developed in patients with Zollinger–Ellison syndrome, and low serum albumin concentrations in peptic ulcer patients may be a clue to the diagnosis of Zollinger–Ellison syndrome. It has also been reported that patients with non-steroidal antiinflammatory drug (NSAID)-induced enteropathy or gastric ulcer are susceptible to hypoalbuminemia caused by massive bleeding from the inflammatory or ulcer site [8,9]. Although the pharmacokinetic changes of some drugs in NARs have been reported [10], the changes with respect to hepatic and intestinal CYP isozymes and plasma protein binding have received little attention. To our knowledge, no pharmacokinetic studies on omeprazole in NARs have yet been reported, likely because the changes in hepatic or intestinal CYP isozymes [3] in NARs and hepatic CYP isozymes responsible for the metabolism of omeprazole [2] have only been reported recently.

This study reports changes in the protein expression of the hepatic CYP2D subfamily and intestinal CYP3A subfamily in NARs based on the western blot analysis, and the pharmacokinetic changes of omeprazole after its intravenous (20 mg/kg) or oral (40 mg/kg) administration to NARs with respect to changes in hepatic CYP1A2 and 2D subfamily and intestinal CYP1A and 3A subfamilies compared with the controls.

Materials and Methods

Chemicals

Omeprazole and torasemide [internal standard for the high-performance liquid chromatographic (HPLC) analysis of omeprazole] were donated by Yungjin Pharmaceutical Company (Seoul, Republic of Korea) and Roche Pharmaceutical Company (Mannheim, Germany), respectively. The reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), β-actin, primary monoclonal antibody for β-actin, Kodak X-OMAT film, tris(hydroxymethyl)-aminomethane (Tris)-buffer, rat globulin [G4890 (57.5% of β-globulin plus 20.2% of γ-globulin) and G2885 (98% of γ-globulin)] and ethylenediamine tetraacetic acid (EDTA; as a disodium salt) were purchased from Sigma–Aldrich Corporation (St Louis, MO). Polyclonal CYP2D and 3A anti-human antibodies and horseradish peroxidase-conjugated goat anti-rabbit antibody were the products of Detroit R&D (Detroit, MI) and Bio-Rad Laboratories (Hercules, CA), respectively. Enhanced chemiluminescence reagents were products from Amersham Biosciences Corporation (Piscataway, NJ). Other chemicals were of reagent or HPLC grade.

Animals

The protocols for the animal studies were approved by Animal Care and Use Committee

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of College of Pharmacy of Seoul National University, Seoul, Republic of Korea. Male Sprague–Dawley control rats (weighing 250–330 g) and NARs (weighing 240–275 g), 7 weeks old, were purchased from Japan SLC Inc. (Hamamatsu, Japan) and Charles River Company Korea (Orient, Seoul, Republic of Korea), respectively. The procedures used for maintenance and handling of the rats were similar to reported methods [2,3,10].

Preparation of hepatic and intestinal microsomes

The procedures used for the preparation of hepatic ($n = 5$, each) [3,10] and intestinal ($n = 3$, each) [11] microsomes were similar to reported methods. Protein contents in the hepatic and intestinal microsomes were measured using a reported method [12].

Immunoblot analysis of hepatic CYP2D subfamily and intestinal CYP3A subfamily

The procedures used were similar to a reported method [13]. Liver and intestinal microsomes were resolved by sodium dodecyl sulfate (SDS) gel electrophoresis on a 7.5% polyacrylamide gel (10 μg protein per lane). Proteins were transferred to a nitrocellulose membrane (Bio-Rad Laboratories) that was then blocked for 1 h in 5% milk powder in phosphate-buffered 0.9% NaCl-injectable solution containing 0.05% (v/v) Tween 20 (PBS-T). For immunodetection, the blots were incubated overnight at 4°C with rabbit anti-human CYP2D (for the liver) or 3A (for the intestine) antibody (diluted 1:10 000 in PBS-T containing 5% bovine serum albumin), followed by incubation for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (diluted 1:10000 in PBS-T containing 5% milk powder). Hepatic CYP2D subfamily and intestinal CYP3A subfamily were detected by enhanced chemiluminescence on Kodak X-OMAT film and quantitated by densitometry with a microcomputer imaging device (model M1; Imaging Research, St Catharines, Ontario, Canada). The β-actin band was used as a loading control.

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

The maximum velocity (V_{max}) and the apparent Michaelis–Menten constant (concentration at which the rate is one-half of the V_{max}; K_m) for the disappearance of omeprazole were determined after incubating the above hepatic microsomes (equivalent to 0.5 mg protein), 5 μl of 0.1 M carbonate buffer (pH 9.8) containing final omeprazole concentrations of 1, 2.5, 5, 10, 20 and 50 μM, and 50 μl of Tris-HCl buffer (pH 7.4) containing 1 mM NADPH in a final volume of 0.5 ml by adding 0.1 M phosphate buffer (pH 7.4) in a thermomixer [Thermomixer 5436; Eppendorf, Hamburg, Germany; 37°C for 500 oscillations/min (opm)]. All of the above microsomal incubation conditions were linear. The reaction was terminated by addition of 1 ml of diethyl ether after 5 min incubation. Omeprazole was measured by a reported HPLC method [14]. The kinetic constants (K_m and V_{max}) for the disappearance of omeprazole were calculated using a non-linear regression method [15]. The intrinsic clearance (Cl_{int}) for the disappearance of omeprazole was calculated by dividing the V_{max} by the K_m.

In vitro *disappearance of omeprazole in rat gastrointestinal S9 fractions*

The procedures used are similar [16] to a reported method [17]. Approximately 1 g of each stomach, small intestine and large intestine from control rats and NARs ($n = 4$; each) was excised after cervical dislocation, rinsed with cold 0.9% NaCl-injectable solution, blotted dry with tissue paper and weighed. Each tissue was homogenized with 4 volumes of 0.25 M sucrose solution, and the supernatant fractions (S9 fractions) were collected after centrifugation ($9000 \times g$ for 10 min). Metabolic activity was initiated by adding 300 μl of the S9 fraction of each tissue homogenate to an Eppendorf tube containing 280 μl of Tris-buffer, 10 μl (10 μg; the same solution used in the intravenous study) of omeprazole and 10 μl of Tris-HCl buffer (pH 7.4) containing 1 mM NADPH. To terminate the enzyme activity, 1 ml of diethyl ether was added after 30 min incubation in a thermomixer.

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Measurement of plasma protein binding of omeprazole using equilibrium dialysis

Protein binding of omeprazole to fresh plasma from NARs ($n = 4$) and control rats ($n = 5$) was determined using equilibrium dialysis [18]. Plasma (1 ml) was dialysed against 1 ml of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran ('the buffer') to reduce volume shift [19] in a 1 ml dialysis cell (Spectrum Medical Industries, Los Angeles, CA) using a Spectra/Por 4 membrane (mol. wt cutoff 12–14 KDa; Spectrum Medical Industries). Omeprazole [dissolved in 0.1 M carbonate buffer (pH 9.8)] was spiked into the plasma compartment to reduce the equilibration time [20] at an omeprazole concentration of 10 μg/ml. After 6 h incubation, two 100 μl samples were collected from each compartment and stored at –70°C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until used for the HPLC analysis of omeprazole [14]. In the preliminary study, it took approximately 6 h to reach an equilibrium between plasma and 'the buffer' compartments, and the binding value of omeprazole to 4% human serum albumin (HSA) was constant, $91.7 \pm 0.785\%$, at omeprazole concentrations ranging from 1 to 200 μg/ml. Thus, 6 h incubation and an omeprazole concentration of 10 μg/ml were chosen for this plasma protein binding study. Protein binding of omeprazole was also performed using rat globulins. Experiments were carried out using 1.8% β-globulin (which contained 0.63% γ-globulin as an impurity), 0.63% γ-globulin and 1.3% γ-globulin. Protein binding of omeprazole to 0.0042% HSA was also measured.

Intravenous and oral administration of omeprazole to rats

The procedures used for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) were similar to a reported method [21]. Watanabe *et al.* [22] reported that immobilization stress could change the pharmacokinetics of omeprazole in rats, so the rats were not restrained in the present study.

Omeprazole (the same solution used in the plasma protein binding study) at a dose of 20 mg (2 ml)/kg was infused over 1 min via the jugular vein of NARs ($n = 8$) and control rats ($n = 9$). A blood sample (approximately 0.22 ml) was collected via the carotid artery at 0 (control), 1 (end of the infusion), 3, 7, 15, 30, 45, 60, 70, 80 and 90 min after the start of the intravenous infusion of omeprazole. A heparinized 0.9% NaCl-injectable solution (0.3 ml) was used to flush the cannula immediately after each blood sampling. A blood sample was immediately centrifuged and 100 μl of each plasma sample was stored at −70°C until used for the HPLC analysis of omeprazole [14]. The procedures used for the preparation and handling of the 24 h urine ($Ae_{0-24\,h}$) sample and gastrointestinal tract (including its contents and feces) sample at 24 h ($GI_{24\,h}$) were similar to reported methods [2,3,10,21].

Omeprazole (the same solution used in the intravenous study) at a dose of 40 mg (5 ml)/kg was administered orally using gastric gavage to NARs ($n = 9$) and control rats ($n = 8$). Blood samples were collected at 0, 5, 15, 30, 60, 90, 105, 120, 135, 150, 180 and 240 min after oral administration of omeprazole. Other procedures were similar to those for the intravenous study.

HPLC analysis of omeprazole

Concentrations of omeprazole in the samples were determined using a slight modification of a reported HPLC method [14]; torasemide instead of lansoprazole was used as the internal standard. Briefly, in a 2.2 ml microfuge tube containing 100 μl of sample, 50 μl of methanol containing torasemide (internal standard; 50 μg/ml) and 50 μl of 0.2 M phosphate buffer (pH 7.0) were added. The mixture was then extracted with 1 ml of ether. The organic layer was transferred into a clean Eppendorf tube and evaporated under a gentle stream of nitrogen gas at 50°C. The residue was reconstituted in 125 μl of the mobile phase and 50 μl was directly injected onto a reversed-phase HPLC column (C_8; 150 mm, $l \times 4.6$ mm, i.d.; particle size, 5 μm; Waters, Milford, MA). The mobile phase, phosphate buffer [0.2 M KH_2PO_4 (pH 7.0)]: acetonitrile (77: 23, v/v), was run at a flow-rate of 1.4 ml/min, and the column eluent was monitored using an ultraviolet detector at 302 nm at room temperature. The retention

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times of omeprazole and torasemide (internal standard) were approximately 10.2 and 8.1 min, respectively. The detection limits of omeprazole in rat plasma and urine samples were 20 and 50 ng/ml, respectively. The coefficients of variation of omeprazole in rat plasma and urine samples were below 5.34% and 7.90%, respectively.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to time infinity (*AUC*) was calculated using the trapezoidal rule–extrapolation method [23]. The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal-phase rate constant.

Standard methods [24] were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, CA); the time-averaged total body, renal and non-renal clearances (Cl, Cl_r and Cl_{nr}, respectively), the terminal half-life, the first moment of *AUC* (*AUMC*), the mean residence time (*MRT*), the apparent volume of distribution at steady state (V_{ss}) and the extent of absolute oral bioavailability (F) [21]. The peak plasma concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the experimental data.

Statistical analysis

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

Results

Protein expression of hepatic CYP2D subfamily and intestinal CYP3A subfamily

The levels of hepatic CYP2D subfamily and intestinal CYP3A subfamily measured by the western blot analysis in two groups of rats are shown in Figure 1. The protein expression of hepatic CYP2D subfamily was comparable between the two groups of rats. However, compared with the controls, the protein expression of intestinal CYP3A subfamily was increased significantly (110% increase) in NARs.

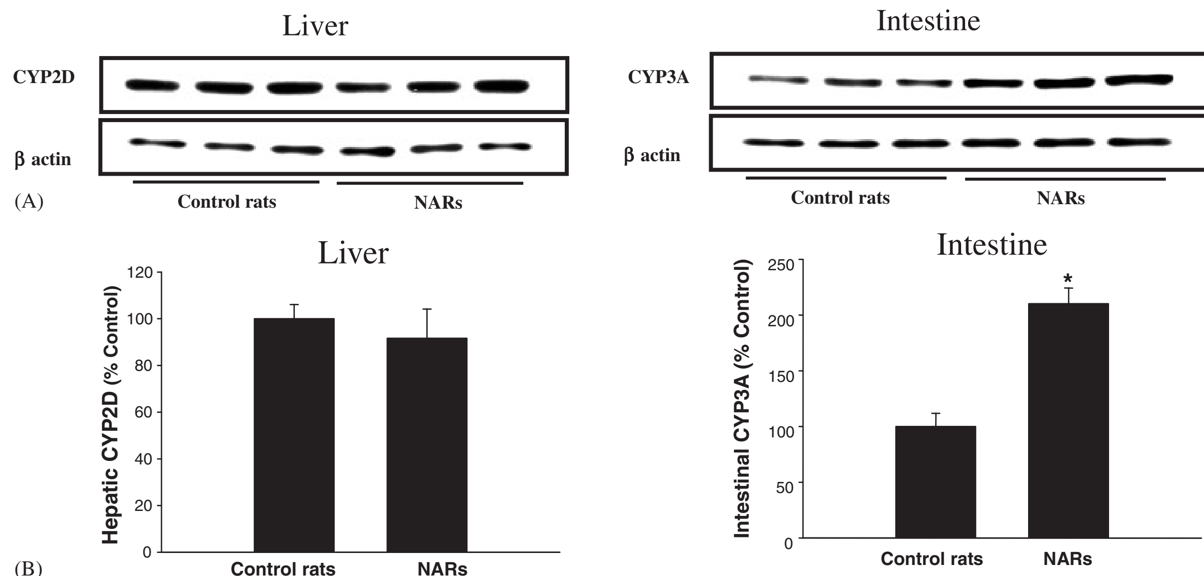

Figure 1. Immunoblotting of hepatic CYP2D subfamily and intestinal CYP3A subfamily in NARs and control rats (A). Expressed in terms of % of control rats, 100% (B). The β-actin was used as a loading control. Bars represent standard deviation. $^{*}p<0.05$ compared with the controls

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V_{max}, K_m and Cl_{int} for the disappearance of omeprazole in hepatic microsomes of NARs and control rats

The $V_{\max}$, K_{m} and Cl_{int} for the disappearance of omeprazole in hepatic microsomes of NARs and control rats are listed in Table 1. In NARs, the $V_{\max}$ was significantly faster (128% increase) than in the controls, suggesting that the maximum velocity for the disappearance (primarily metabolism) of omeprazole was significantly faster in NARs than in the controls. However, the K_{m} values were not significantly different between the two groups of rats, suggesting that the affinity of the enzyme(s) (primarily CYP1A2) for omeprazole was not altered in NARs. As a result, the hepatic Cl_{int} was significantly faster (37.0% increase) in NARs than in the controls, suggesting that the metabolism of omeprazole increased in NARs. Because the protein content in hepatic microsomes in NARs was significantly greater (29.9% increase) than in the controls, the Cl_{int} was also calculated based on grams liver. The Cl_{int} based on grams liver was also significantly faster (75.4% increase) in NARs than in the controls.

In vitro disappearance of omeprazole in rat gastrointestinal S9 fractions

The *in vitro* disappearance of omeprazole after spiking the drug into the S9 fractions of each stomach, small intestine and large intestine homogenates is listed in Table 3. The stomach and small intestine of both control rats and NARs, and the large intestine of NARs had some metabolic activities for omeprazole, however, the large intestine of the control rats had almost negligible metabolic activities for omeprazole. The percentages of the spiked amounts of omeprazole disappeared per gram stomach, small intestine and large intestine were 12.5%, 15.5% and 0%, respectively, for control rats, and 22.4%, 25.8% and 16.8%, respectively, for NARs. Note that in NARs, the metabolic activities of small and large intestines increased significantly compared with the controls.

Table 1. Mean ($\pm$standard deviation) $V_{\max}$, K_{m} and Cl_{int} for the disappearance of omeprazole in hepatic microsomes from control rats and NARs

Parameter	Control rats ($n=5$)	NARs ($n=5$)
$V_{\max}$ (nmol/min/mg protein)	3.41 ± 1.68	7.77 ± 3.81[a]
K_{m} (μM)	26.4 ± 11.8	45.3 ± 22.9
Cl_{int} (ml/min/mg protein)	0.127 ± 0.0374	0.174 ± 0.0165[a]
Cl_{int} (ml/min/g liver)	1.64 ± 0.598	2.88 ± 0.378[b]
Protein content (mg/g liver)	12.7 ± 1.31	16.5 ± 1.41[b]

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

Plasma protein binding of omeprazole

Protein binding values of omeprazole to fresh plasma from NARs and control rats were $60.7\pm12.6\%$ and $81.5\pm3.03\%$, respectively; they were significantly different. The binding values of omeprazole were $8.14\pm1.77\%$, $46.0\pm5.93\%$, $36.0\pm5.27\%$ and $40.5\pm2.03\%$ for 0.0042% HSA, 1.8% β-globulin plus 0.63% γ-globulin, 0.63% γ-globulin and 1.3% γ-globulin, respectively.

Pharmacokinetics of omeprazole after its intravenous administration to rats

For the intravenous administration of omeprazole to NARs and control rats, the mean arterial plasma concentrations–time profiles of the drug are shown in Figure 2(A). The relevant pharmacokinetic parameters are listed in Table 2. Compared with the controls, the *AUC* was significantly smaller (57.1% decrease), the terminal half-life was significantly longer (46.9% increase), the V_{ss} was significantly larger (101% increase), the Cl, Cl_{r} and Cl_{nr} were significantly faster (135%, 59.0% and 136% increase, respectively) and the percentage of the intravenous dose of omeprazole excreted in the 24 h urine as unchanged drug ($Ae_{0-24\,\mathrm{h}}$) was significantly smaller (31.2% decrease) in NARS. Omeprazole was below the detection limit in the gastrointestinal tract (including its contents and feces) at 24 h ($GI_{24\,\mathrm{h}}$) for both groups of rats.

Pharmacokinetics of omeprazole after its oral administration to rats

For the oral administration of omeprazole to NARs and control rats, the mean arterial plasma concentration–time profiles of the drug are shown in Figure 2B. The relevant pharmacokinetic

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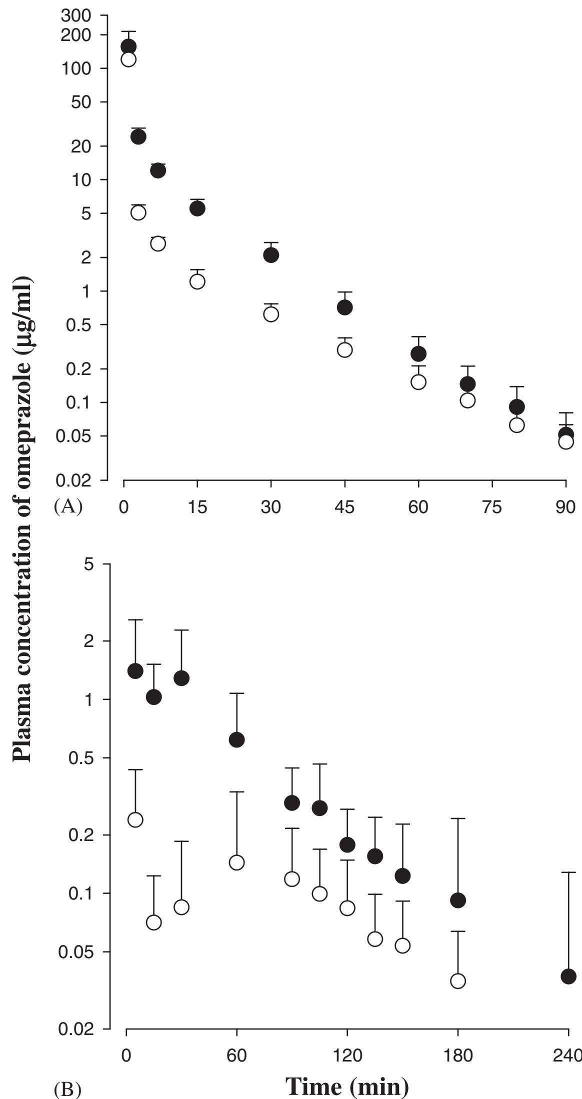

Figure 2. Mean arterial plasma concentration–time profiles of omeprazole after its intravenous administration at a dose of 20 mg/kg to control rats (●; $n=9$) and NARs (○; $n=8$) (A), and after oral administration at a dose of 40 mg/kg to control rats (●; $n=8$) and NARs (○; $n=9$) (B). Bars represent standard deviation

Table 2. Mean (±standard deviation) pharmacokinetic parameters of omeprazole after its intravenous or oral administration at a dose of 20 or 40 mg/kg, respectively, to control rats and NARs

Parameter	Control rats	NARs
Intravenous	($n=9$)	($n=8$)
AUC (μg min/ml)	438±70.9	188±24.7[a]
Terminal half-life (min)	13.0±1.95	19.1±4.33
MRT (min)	8.54±2.19	7.35±1.60
V_{ss} (ml/kg)	406±149	817±263
Cl (ml/min/kg)	46.8±8.05	110±20.7[a]
Cl_r (ml/min/kg)	0.183±0.0696	0.291±0.0882[b]
Cl_{nr} (ml/min/kg)	46.6±8.00	110±20.7[a]
$Ae_{0–24\,h}$ (% of dose)	0.385±0.106	0.265±0.0749[b]
$GI_{24\,h}$ (% of dose)	BD[c]	BD[c]
Oral	($n=8$)	($n=9$)
AUC (μg min/ml)	94.2±32.1	18.7±11.1[a]
C_{max} (μg/ml)	2.11±0.987	0.276±0.174[a]
T_{max} (min)	17.5±13.4	26.7±39.8
Terminal half-life (min)	33.6±18.2	40.2±20.5
Cl_r (ml/min/kg)	1.44±0.759	3.28±1.37[d]
$Ae_{0–24\,h}$ (% of dose)	0.384±0.201	0.127±0.0313[d]
$GI_{24\,h}$ (% of dose)	1.80±0.973	1.10±0.439
F (%)	10.7	4.98

[a]Significantly different ($p<0.001$) from control rats.
[b]Significantly different ($p<0.05$) from control rats.
[c]Below the detection limit.
[d]Significantly different ($p<0.01$) from control rats.

Table 3. Mean (±standard deviation) omeprazole remaining (% of the spiked amount) after 30 min incubation in S9 fractions of each stomach, small intestine and large intestine homogenates from control rats and NARs

Tissue	Control rats ($n=4$)	NARs ($n=4$)
Stomach	87.5±5.60	77.6±9.50
Small intestine	84.5±5.81	74.2±5.37[a]
Large intestine	100±3.61	83.2±5.75[b]

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

parameters are also listed in Table 2. After oral administration, absorption of omeprazole from the gastrointestinal tract was rapid for both groups of rats; omeprazole was detected in plasma at the first blood sampling time (5 min) and rapidly reached T_{max} (17.5–26.7 min for both groups of rats). Compared with the controls, the *AUC* was significantly smaller (80.1% decrease), the C_{max} was significantly lower (86.9% decrease), the Cl_r was significantly faster (128% increase) and the $Ae_{0–24\,h}$ was significantly smaller (66.9% decrease) in NARs.

Discussion

The *AUC*s of omeprazole were dose-proportional after its intravenous (at doses of 2.5–10 mg/kg) or oral (at doses of 10–40 mg/kg) administration to rats [5]. In the preliminary study, the $AUC_{0–2\,h}$ of omeprazole after its intravenous administration at a dose of 20 mg/kg to control rats was

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approximately 2-times that of $AUC_{0-2\,h}$ obtained from its intravenous administration at a dose of 10 mg/kg to rats [5]. Thus, the intravenous and oral doses of 20 and 40 mg/kg, respectively, were chosen for this study.

After intravenous administration, the contribution of the Cl_r to the Cl was almost negligible; the values were less than 0.385% for both groups of rats (Table 2). This suggests that almost all of the intravenous omeprazole was eliminated via a non-renal route (Cl_{nr}). The contribution of the biliary excretion of omeprazole to the Cl_{nr} was also negligible; Lee *et al.* [2] reported that only $0.0436 \pm 0.0159\%$ of the intravenous dose of omeprazole at a dose of 20 mg/kg was excreted in the 24 h bile samples in ten control rats with bile duct cannulation. Watanabe *et al.* [5] reported that the liver is the main metabolizing organ for omeprazole in rats. This suggests that omeprazole is almost completely metabolized in rat liver. Two major metabolites of omeprazole, omeprazole sulfone and omeprazole sulfide, were formed in rats [25]. Thus, the Cl_{nr} of omeprazole listed in Table 2 could represent the metabolic clearance of the drug in rats and the changes in the Cl_{nr} could be due to changes in the metabolism of the drug in rat liver.

After intravenous administration of omeprazole to NARs, the *AUC* was significantly smaller than controls, possibly as a result of the significantly faster Cl than in the controls (Table 2). The faster Cl was attributable to the significantly faster Cl_{nr} and Cl_r in NARs than in the controls (Table 2). However, the contribution of the faster Cl_r to the faster Cl of omeprazole seemed to be almost negligible because the Cl_r/Cl ratio was only 0.385% (Table 2). Since omeprazole is a drug with an intermediate hepatic extraction ratio in rats [5], its hepatic clearance depends on the hepatic Cl_{int} for the disappearance of omeprazole, the free fractions of the drug in plasma and $Q_{H,B}$ in rats [6]. The significantly faster Cl_{nr} of omeprazole in NARs (Table 2) could be supported by significantly faster *in vitro* hepatic Cl_{int} (Table 1), resulting from an increase in the protein expression and mRNA level of hepatic CYP1A2 [3] and the significantly greater free fraction of the drug in plasma (112% increase) than in the controls. However, the contribution of $Q_{H,B}$ to the faster Cl_{nr} of omeprazole in NARs did not seem to be considerable, because the liver and kidney function did not seem to be altered in NARs [3].

After intravenous administration of omeprazole to NARs, the V_{ss} was significantly larger than in the controls (Table 2). This could have been due to the significantly greater free fractions of omeprazole in plasma from NARs than in controls. Similar results have also been reported for other drugs in NARs; the V_{ss} of azosemide [3], bumetanide [26], torasemide [27] and oltipraz [10] were significantly larger in NARs due to an increase in free fractions of the drugs in plasma. The plasma levels of albumin, and α-, β- and γ-globulins in NARs were found to be approximately 0.0042%, 3.1%, 1.8% and 1.3%, respectively [28]. The protein binding value of omeprazole to plasma from NARs was 60.7%, and this value was somewhat unexpected, because the value to 0.0042% HSA was only 8.14%. Thus, the difference, approximately 50%, could have been due to binding of omeprazole to globulins in plasma from NARs. The binding values of omeprazole to rat globulins at omeprazole concentration of 10 μg/ml were 46.0%, 36.0% and 40.5% for 1.8% β-globulin plus 0.63% γ-globulin, 0.63% γ-globulin and 1.3% γ-globulin, respectively. The total value (the total protein binding value to 0.0042% human serum albumin, 1.8% β-globulin, plus 1.3% γ-globulin) of 58.6% was very close to 60.7%, the value to plasma from NARs. Considerable binding of drugs to globulins in plasma from NARs have also been reported in other studies, such as azosemide [3], bumetanide [21], torasemide [27], cisplatin [29], oltipraz [10] and methotrexate [30].

After intravenous administration of omeprazole to NARs, the Cl_{nr}s based on plasma and blood concentrations were 110 ml/min/kg (Table 2) and 183 ml/min/kg, respectively. The Cl_{nr} based on blood concentrations was calculated from the reported blood and plasma ratio of the drug, about 0.6 [31]. This value, 183 ml/min/kg, was considerably greater than the reported $Q_{H,B}$ of 55.2 ml/min/kg in control rats [32]. This could support the extrahepatic metabolism of omeprazole in NARs. The $Q_{H,B}$ do not seem to have been reported in NARs.

After oral administration of omeprazole to NARs, the *AUC* was also significantly smaller

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(80.1% decrease) than in the controls (Table 2). The value, 80.1% decrease, was greater than the 57.1% decrease after intravenous administration (Table 2) and this resulted in a considerably smaller (53.5% decrease) F in NARs than controls. Generally, a decrease in F can result primarily for two reasons. The first is a decrease in absorption and the second is an increase in first-pass effect. However, in the case of omeprazole, a decrease in absorption could not be the cause of a smaller F, because omeprazole is almost completely absorbed in rats [5]. Thus, the decrease in F of omeprazole in NARs could be due to an increase in the first-pass effect. It has been estimated that the hepatic and intestinal first-pass effects of omeprazole were approximately 15.9% and 72.4% of the total oral dose, respectively, in rats [5]. An increase in the hepatic and/or intestinal first-pass effect could be possibly due to an increase in the hepatic and/or intestinal metabolism of omeprazole in NARs. These were sufficiently proved by the *in vitro* disappearance study using hepatic microsomes and intestinal S9 fractions (containing both microsome and cytosol) (Tables 1, 3), although a relationship between *in vitro* intestinal metabolism and intestinal first-pass effect was not shown kinetically. Although the S9 fractions were only 20% to 25% of the CYP activity of microsomal fractions, the intestinal S9 fractions of NARs showed significantly greater omeprazole metabolic activity via CYP than the controls. Therefore, this result could more strongly support the change in intestinal metabolism of omeprazole via CYP in NARs. The greater intestinal metabolic activity of omeprazole in NARs could be caused by a significant increase in the protein expression of the intestinal CYP1A subfamily (our unpublished data) and the 3A subfamily (Figure 1). Although the exact values of hepatic and intestinal first-pass effects were not measured separately in NARs, the total percentages of both first-pass effects were supposed to be increased in NARs and this could result in a smaller F in NARs. Similarly, it has been also reported that hepatic and intestinal first-pass effects of several drugs were subjected to significant changes in various disease states in accordance with their specific CYP isozyme changes [33,34].

In conclusion, after intravenous administration of omeprazole to NARs, the Cl_{nr} (AUC) was significantly faster (smaller) than in the controls (Table 2). This could have been due to the significantly faster hepatic Cl_{int} for the disappearance of omeprazole (Table 1) (increase in hepatic metabolism of omeprazole caused by increase in the protein expression of hepatic CYP1A2 [3]) and the significantly greater free fraction of the drug in plasma in NARs. After oral administration of omeprazole to NARs, the AUC of the drug decreased more than that after intravenous administration (Table 2). This could have been due primarily to an increase in the hepatic and intestinal first-pass effects of omeprazole.

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References

1. Im W, Blakeman DP, Davis JP. Irreversible inactivation of rat gastric (H^+–K^+)-ATPase *in vivo* by omeprazole. *Biochem Biophys Res Commun* 1985; **126**: 78.
2. Lee DY, Shin HS, Bae SK, Lee MG. Effects of enzyme inducers and inhibitors on the pharmacokinetics of intravenous omeprazole in rats. *Biopharm Drug Dispos* 2006; **27**: 209–218.
3. Kim EJ, Lee AK, Kim SH, Kim SG, Lee MG. Pharmacokinetics and pharmacodynamics of intravenous azosemide in mutant Nagase analbuminemic rats. *Drug Metab Dispos* 2003; **31**: 194–201.
4. Kaminsky LS, Fasco MJ. Small intestinal cytochromes P450. *Crit Rev Toxicol* 1991; **21**: 407–422.
5. Watanabe K, Furuno K, Eto K, Oishi R, Gomita Y. First-pass metabolism of omeprazole in rats. *J Pharm Sci* 1994; **83**: 1131–1134.
6. Wilkinson GR, Shand DG. A physiological approach to hepatic drug clearance. *Clin Pharmacol Ther* 1975; **18**: 377–390.
7. Lamers CB, Hafkenscheid JC, van Tongeren JH. Serum albumin levels in patients with the Zollinger–Ellison syndrome. *Gastroenterology* 1977; **73**: 975–979.
8. Bjarnason I, Macpherson AJ. Intestinal toxicity of non-steroidal anti-inflammatory drugs. *Pharmacol Ther* 1994; **62**: 145–157.

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9. Guji A, Nishiya H, Haga T, *et al*. A case of spontaneous bacterial peritonitis with ascites caused by hypoproteinemia after a massive bleeding from a gastric ulcer. *Kansen Zasshi* 1993; **67**: 71–75.
10. Bae SK, Kang HE, Kang MK, Kim JW, Kim T, Lee MG. Pharmacokinetics of oltipraz in mutant Nagase analbuminemic rats. *J Pharm Sci* 2006; **95**: 998–1005.
11. Lee JH, Lee MG. Telithromycin pharmacokinetics in rat model of diabetes mellitus induced by alloxan or streptozotocin. *Pharm Res* 2008; **25**: 1915–1924.
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. 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.
14. Kang WK, Kim DS, Kwon KI. Advanced method for determination of omeprazole in plasma by HPLC. *Arch Pharm Res* 1999; **22**: 86–88.
15. Duggleby RG. Analysis of enzyme progress curves by nonlinear regression. *Method Enzymol* 1995; **249**: 61–90.
16. Yu SY, Bae SK, Kim EJ, *et al*. Dose-independent pharmacokinetics of a new reversible proton pump inhibitor, KR-60436, after intravenous and oral administration to rats: gastrointestinal first-pass effect. *J Pharm Sci* 2003; **92**: 1592–1603.
17. Litterst CL, Mimnaugh EG, Regan RL, Gram TE. Comparison of *in vitro* drug metabolism by lung, liver, and kidney of several common laboratory species. *Drug Metab Dispos* 1975; **3**: 259–265.
18. 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.
19. 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.
20. Øie S, Guentert TW. Comparison of equilibrium time in dialysis experiments using spiked plasma or spiked buffer. *J Pharm Sci* 1982; **71**: 127–128.
21. Kim SH, Choi YM, Lee MG. Pharmacokinetics and pharmacodynamics of furosemide in protein–calorie malnutrition. *J Pharmacokinet Biopharm* 1993; **21**: 1–17.
22. Watanabe K, Matsuka N, Okazaki M, Hashimoto Y, Araki H, Gomita Y. The effect of immobilization stress on the pharmacokinetics of omeprazole in rats. *Acta Med Oka* 2002; **56**: 19–23.
23. Chiou WL. Critical evaluation of potential error in pharmacokinetics studies using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. *J Pharmacokinet Biopharm* 1978; **6**: 539–549.
24. GibaldiM, PerrierD. *Pharmacokinetics*, 2nd edn. Marcel Dekker: New York, 1982.
25. Webster LK, Jones DB, Mihaly GW, Morgan DJ, Smallwood RA. Effect of hypoxia on oxidative and reductive pathways of omeprazole metabolism by the isolated perfused rat liver. *Biochem Pharmacol* 1985; **34**: 1239–1245.
26. Kim EJ, Lee MG. Pharmacokinetics and pharmacodynamics of intravenous bumetanide in mutant Nagase analbuminemic rats: importance of globulin binding for the pharmacodynamic effects. *Biopharm Drug Dispos* 2001; **22**: 147–156.
27. Kim EJ, Lee MG. Pharmacokinetics and pharmacodynamics of intravenous torasemide in mutant Nagase analbuminemic rats. *Biopharm Drug Dispos* 2003; **24**: 27–35.
28. Nagase S, Shimamune K, Shumiya S. Albumin-deficient rat mutant: an animal model for analbuminemia. *Exp Anim* 1980; **29**: 33–38.
29. Takada K, Kawamura T, Inai M, *et al*. Pharmacokinetics of cisplatin in analbuminemic rats. *Biopharm Drug Dispos* 1999; **20**: 421–428.
30. Choi YH, Bae SK, Oh JM, Kim SO, Lee MG. Pharmacokinetics of intravenous methotrexate in mutant Nagase analbuminemic rats. *Biopharm Drug Dispos* 2007; **28**: 385–392.
31. Regårdh CG. Pharmacokinetics and metabolism of omeprazole in man. *Scand J Gastroenterol* 1986; **118** (Suppl): 99–104.
32. Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993; **10**: 1093–1095.
33. Lee DY, Lee MG, Shin HS, Lee I. Changes in omeprazole pharmacokinetics in rats with diabetes induced by alloxan or streptozotocin: faster clearance of omeprazole due to induction of hepatic CYP1A2 and 3A1. *J Pharm Pharm Sci* 2007; **10**: 420–433.
34. Lee JH, Lee MG. Effects of acute renal failure on the pharmacokinetics of telithromycin in rats: negligible effects of increase in CYP3A1 on the metabolism of telithromycin. *Biopharm Drug Dispos* 2007; **28**: 157–166.

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